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Original Article

The CCL28 levels are elevated in the serum of patients with irritable bowel syndrome and associated with the clinical symptoms

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Abstract: Background: Inflammation and inflammatory mediators have been proposed to be key players in the pathobiology of Irritable bowel syndrome (IBS. The chemokine CCL28 plays a role in the trafficking of inflammatory cells into mucosal tissues. However, its levels in patients with IBS has not been yet elucidated. Method: In this study, the levels of CCL28 were measured in the serum of 41 patients with IBS and 41 age- and gender-matched normal individuals using Elisa. Then, the receiver operating characteristic (ROC) curve was conducted to assess the diagnostic value of CCL28. Results: Our data showed that the levels of CCL28 are significantly elevated in patients with IBS compared to the control donors. Moreover, we observed that the level of CCL28 is associated with many clinical symptoms such as constipation, diarrhea, and abdominal pain. The area under the ROC curve was 0.71 (95% confidential interval, 0.598-0.823), the sensitivity and specificity of CCL28 for the diagnosis of IBS patients were 68.3% and 70.7%, respectively with a cut off of 278.9 ng/mL. Conclusions: We demonstrated that CCL28 is elevated in patients with IBS and correlates with clinical findings, indicating that CCL28 might be an appropriate biomarker for the diagnosis of IBS; however, further studies are necessary.

Keywords: CCL28, inflammation, irritable bowel syndrome

Introduction

Irritable bowel syndrome (IBS) has been regarded as a chronic functional impairment of the gastrointestinal system. Epidemiological studies have revealed that IBS's incidence is 9-22% of the population and is more common amongst women than men. IBS is characterized by abdominal pain or discomfort and is associated with altered bowel habits, with the absence of any pathophysiological and biochemical abnormalities [1, 2]. It can cause many problems in the quality of life and the functionality of patients. It also has economic consequences; the following describes the economic costs of IBS for different countries: the USA $742-$7547, UK £90-£316, France €567-€862, Canada $259, Germany €791, Norway NOK 2098 (€262) and Iran $92 [3].

Although many studies indicate that various factors such as the abnormality of intestinal movement and psychological factors, may be involved in the pathophysiology of IBS, the exact mechanisms underlying the pathogenesis of IBS are still unknown [4]. Accumulating evidence indicates that low-grade inflammation plays a role in this disease. Reported evidence from patients with post-infection (PI)-IBS have demonstrated an elevation in the number of colonic lamina propria T lymphocytes and upregulation of interleukin-1β (IL-1β) in the mucosa of patients with PI-IBS compared to patients who did not develop IBS after gastroenteritis [5, 6]. It also has been recently shown that the number of T lymphocytes in the colon of patients with IBS have increased. Furthermore, the numbers of mast cells adjacent

to the neural terminal of the ilium and the colon of the patients with IBS have remarkably elevated and positively correlated with clinical symptoms such as abdominal discomfort and diarrhea [7-9]. On the other hand, other studies reported a similar or even lower number of mast cells in the colonic or rectal mucosa of these patients [10, 11]. Similarly, the levels of many cytokines in the serum of IBS patients have been studied and the reported evidence indicates that the IL-6, TNF-α and IL-1β have been elevated, while the levels of IL-10 have been decreased [12-14], suggesting a subclinical inflammatory component for the mechanisms underlying the etiologic of IBS.

It has been recently demonstrated that mucosal-associated epithelial chemokine (MEC) or CCL28 is expressed and secreted by the colon and intestinal epithelial cells and chemoattracts immune cells, primarily T lymphocytes and IgA-producing plasma cells in the intestinal epithelium [15, 16]. Moreover, a recent study has revealed that CCL28 is upregulated in the colonic cells when these cells were treated with either inflammatory cytokines or flagellin of bacteria [17]. In addition, as recently reported, the amount of CCL28 expression is much higher at both protein and mRNA levels in gastric mucosa of the patients infected with helicobacter-pylori than non-infected individuals [15]. Even though IBS has been recently regarded as an inflammatory disease the possible role of CCL28 in IBS has not been yet elucidated. In the current study, we aimed to determine the serum levels of CCL28 in patients with IBS in composition to normal individuals. Our data shows that CCL28 is significantly higher in IBS patients than normal donors and shows sensitivity and specificity of 68.3% and 70.7%, respectively for the diagnosis of IBS.

Material and methods

Patients

The protocol was approved by the Ethical Research Committee of Kurdistan University of Medical Sciences (KUMS) according to the criteria set by the declaration of Helsinki. 41 patients with IBS and 41 normal donors were enrolled in this study from November 2012-November 2013. The sample size was calculated based on the assumption of 1% type one error, power of 95%, and 20 (SD 21) ng/mL different in the mean of CCL28 between the two groups [18]. Patients were diagnosed at a gastrointestinal ward (Toheed hospital, Sanandaj, Iran) based on Rome II criteria [19]. IBS was diagnosed in the presence of abdominal pain or discomfort for at least 12 weeks in the preceding 12 months associated with two of the following: relief after a bowel movement and/or association with a change in frequency of bowel movements; and/or an association with a change in consistency of motions. Patients with any other chronic disease, malignancies, or patients who had taken any anti-inflammatory or immunosuppressive medications were excluded from the study. Inclusion criteria: new diagnostic patients with IBS according to Rom II; willing to precipitate in the study. Exclusion criteria: any malignancies; any other inflammatory disease; having any immune-suppressive or anti-inflammatory medications.

Individuals in the control group did not have any symptoms of IBS or any other inflammatory diseases. The patients and the control group were age-matched (±5) and gender-matched.

The enzyme-linked immunosorbent (ELISA) assay

After obtaining written informed consent from each participant, 5 ml of blood samples were taken from them. Serum samples were kept at -80°C until used. The concentration of CCL28 levels were examined using solid-phase sandwich ELISA kits according to the manufacturer's instructions (Cat# DY717, R & D Systems, San Diego, CA). Briefly, the 96 well-microplates (R & D) were coated with mouse anti-CCL28 capture overnight. The microplates were washed three times and blocked with BSA (1%) for 1 hour. Next, 100 mL of either standards or serum was added to each well and incubated for 2 hours at room temperature (RT). The microplates were then washed, incubated with detection antibody for 2 hours at RT, and washed again. 100 mL of Streptavidin-HRP was added to each well and after incubating for 20 minutes at RT. The substrate solution was then added, and after incubating for 20 min in a dark place, the reaction was stopped by stop solution. Finally, the optical density (OD) of the sample was determined by a plate reader (Stat Fax® 4700, Winooski, VT).

Table 1. Demographic characteristics of the IBS patients and normal individuals

Groups	Patients	Healthy individuals
Gender		
Female	51.2% (21*)	51.2% (21*)
Male	48.8% (20*)	48.8% (20*)
Age (year)	36.56 (±11.28)	37.17 (±11.71)
Duration of IBS (year)	4.6 (±5.72)	N/A
Abdominal pain		
Frequency (day/month)	14.87 (±12.14)	N/A
Intensity	6.00 (±3.5)	
Constipation		N/A
Frequency (day/month)	7.95 (±9.89)	
Intensity	4.00 (±3.21)	
Diarrhea		N/A
Frequency (day/month)	6.9 (±8.19)	
Intensity	4.2 (±5.2)	
Bloating		N/A
Frequency (day/month)	20.39 (±11.62)	
Intensity	6.97 (±1.7)	
Tenesmus		N/A
Frequency (day/month)	8.25 (±10.89)	
Intensity	4.46 (±3.88)	

*Shows the number of patients or healthy individuals. NA, Not applicable.

Table 2. Clinical symptoms in patients with IBS

Clinical symptoms	Presence	Not presence
Abdominal pain	85.4	14.6
Constipation	65.9	34.1
Diarrhea	53.7	46.3
Bloating	97.6	2.4
Tenesmus	61	39
Burping	53.7	46.3
Dyspepsia	58.5	41.5

Statistical analysis

Serum concentrations of CCL28 were compared between the patients and healthy donors by using, independent t-test, or in case of no normal distribution the Mann-Whitney U test. Numeral variations were calculated by average ± standard deviation (SD). The normality of the data distributions was assessed using histograms with normal distribution curves. The anti-vinculin distribution was normalized by a square root transformation. Homogeneity of variance was assessed by Bartlett's test. Pearson correlation coefficient and Spearman correlation were used to evaluate the relationship between CCL28 levels and the duration of symptoms. Chi-square and Fisher tests were also used to compare qualitative variables between the two groups. The Receiver operating characteristic (ROC) curve was derived from the levels of CCL28 in the serum of patients with IBS. Using the ROC curve, sensitivity, and specificity were calculated by combining the optimal CCL28 cut-off values. All statistical analyses were performed using SPSS 12.0 software (SPSS Inc, Chicago, Illinois). The 0.05 significance level was used throughout.

Results

Demographical features of patients

41 patients with IBS and 41 control individuals (21 males and 20 females from each group) were enrolled in the current study. The average age for the IBS patients was 36.5±11.7 years, whereas it was 37.1±11.7 for the control group. All the demographic characteristics of the patients including age, sex, and clinical symptoms are shown in **Table 1**.

Clinical features of patients with IBS

All clinical characteristics of the patients are demonstrated in **Table 2**. 85.4% of patients with IBS showed to have abdominal pain (*P value* <0.001) and only 14.6% did not have any abdominal pain. Moreover, most of the patients had constipation (65.9%), diarrhea (66.7%), bloating (97.6%), Tenesmus (61%), burping (53.7), and dyspepsia (58.8). However, none of the patients or healthy individuals showed any other diseases such as anemia, stone kidney, and neurological or thyroid disorders (data not shown).

CCL28 levels in serum of patients and healthy individuals

Next, we analyzed the levels of CCL28 in the serum of the patients with IBS and the control group and observed that the amount of this chemokine is significantly higher in IBS patients

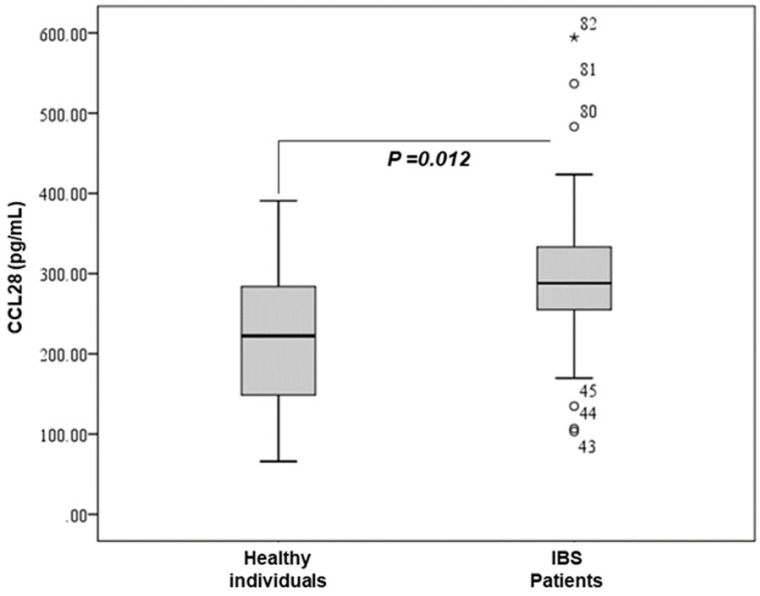

Figure 1. The levels of CCL28 is higher in patients than in the control group. The levels of CCL28 was detected in the serum of 41 patients and 41 normal individuals by Elisa. The average of CCL28 in patients and normal individuals were 293±113 and 228±114, respectively.

Table 3. Association of clinical data with the CCL28 in the serum of patients with IBS

Symptoms	Association with the levels of CCL28	*P value*
Age	0.076	0.496
Duration of IBS (years)	0.322	0.006
Abdominal pain		
Frequency (day/month)	0.360	0.001
Intensity	0.318	0.004
Constipation		
Frequency (day/month)	0.343	0.002
Intensity	0.297	0.007
Diarrhea		
Frequency (day/month)	0.348	0.002
Intensity	0.330	0.022
Bloating		
Frequency (day/month)	0.294	0.007
Intensity	0.309	0.005
Burping		
Frequency (day/month)	0.275	0.012
Intensity	0.265	0.16
Dyspepsia		
Frequency (day/month)	0.182	0.102
Intensit	0.204	0.06

than in healthy individuals (**Figure 1**). Interestingly, we found that the levels of CCL28 are associated with many clinical features of IBS including abdominal pain, constipation, diarrhea, bloating, burping, and the duration of the disease. However, there was no association between the levels of CCL28 and dyspepsia (**Table 3**).

Cut off values and sensitivity and specificity of CCL28 in the diagnosis of IBS

To evaluate the performance of CCL28 as a diagnostic marker for IBS, the ROC curve was obtained by plotting sensitivity versus specificity. As shown in **Figure 2**, the area under the curve was 0.71 (95% confidential interval, 0.598-0.823). The cut-off for CCL28 was 278.7 ng/mL and the sensitivity and specificity were 68.3% and 70.7%, respectively.

Discussion

IBS is one of the most frequent gastrointestinal disorders which not only affects many patients in the world but also causes many financial problems for the health sectors of governments. Although the precise molecular pathways underlying the ethology of IBS are still unknown, recent studies and documents imply that inflammation is involved in the pathology of this disease. In the current study, we investigated the levels of CCL28 in the serum of IBS patients and age- and sex-matched normal individuals and found that not only the levels of CCL28 are significantly higher in the IBS patients but also it is associated with the clinical symptoms of the disease. Moreover, CCL28 exhibits a sensitivity and

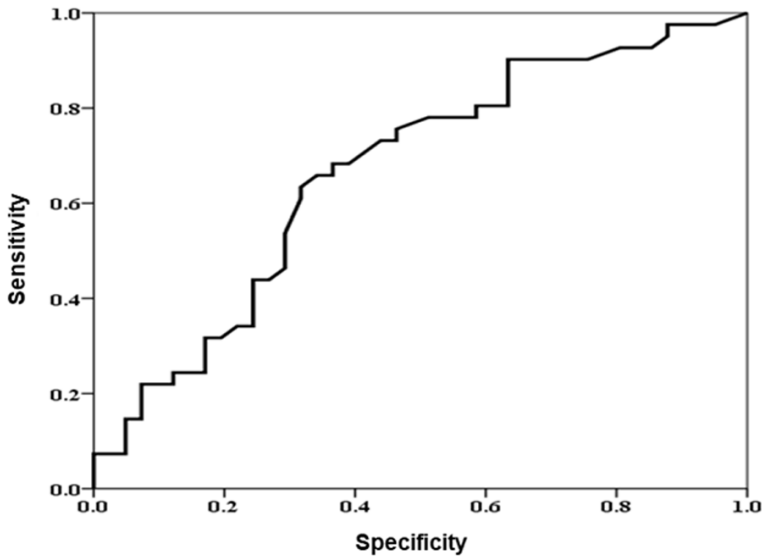

Figure 2. ROC curve of CCL28 as a biomarker for the diagnosis of IBS. Calculated sensitivity (y-axis) is plotted against 1-specificity formula (x-axis) for CCL28. The sensitivity and specificity of CCL28 were 68.3% and 70.7%, respectively and the area under ROC was 0.71.

specificity of 68.3% and 70.7%, respectively, for the diagnosis of IBS.

Although some studies have clearly shown that levels of many proinflammatory cytokines such as TNF-α, IL-6, and IL-1β are higher in patients with IBS than the normal control [13, 20], a few other studies showed decreased or normal expression of these inflammatory cytokines in patients with IBS [21], indicating that due to the heterogenicity of the samples the role of inflammation, if any a low grade, in the pathogenesis of IBS is still controversial. Thus, further précised and well-designed studies are required to conclude.

In the current study we enrolled 41 patients with IBS and compared the level of CCL28 in 41 normal individuals age- and sex-matched. Our data showed that CCL28 is increased in the serum of patients with IBS, indicating that CCL may play a role in the pathogenesis of IBS. However, we have not examined the CCL28 at mRNA and protein levels within the mucosa of the patients and it needs to be further examined in future studies. CCL28 is predominately expressed by epithelial cells within the colon and to a lower extent in other mucosal tissues such as the small intestine. It binds to its receptor CCR10 which is mainly expressed on IgA-secreting B cells and chemoattracts these cells into the gut mucosal tissues [16, 22]. Consistently, we have recently reported that CCL28 is elevated in the serum of patients with celiac disease and decreased after a gluten-free diet [23]. In line with our observations, a recent study has demonstrated that CCL28 is constitutively expressed in keratinocytes and it has upregulated in atopic dermatitis (AD). Serum levels of CCL28 were significantly higher in patients with severe AD than mild, moderate, and normal individuals [24], indicating that CCL28 could be a biomarker for the diagnosis of inflammation in the epithelial tissues. Moreover, Del Valle-Pinero AY et al, have recently examined the expressed levels of 84 inflammatory molecules that are involved in the immune responses in the peripheral blood of patients with IBS and reported that CCL16 was upregulated 7.46 times than the normal controls [25]. However, CCL28 was not investigated in this later study. In contrast to the recent study which reported that expression levels of CCL16 in constipation-dominant IBS patients were 199.7 folds higher than normal individuals [25]. In the current work we exhibit that the levels of CCL28 in the serum of diarrhea-dominant IBS patients were slightly higher (312 vs. 256 ng/mL; *P* value = 0.14) than constipation-dominant patients (data not shown), implying that CCL28 and CCL16 may play a different role in the pathogenesis of IBS. Moreover, evidence from other laboratories have demonstrated the serum levels of IL-6 and TNF-α, but not IL-10, were significantly elevated in patients with diarrhea-dominant IBS [12, 13]. Macsharry J et al have demonstrated; however, IL-10 is downregulated in the mucosa of patients with IBS [21], indicating that although the expression of IL-10 might have been downregulated in the mucosa of the gut, this downregulation might not have been so dramatically to be detected in the peripheral blood.

The current study has several limitations including the small number of patients and focusing

on only one chemokine. Future studies not only should enroll more patients and healthy individuals but also the expression levels of CCL28 should be investigated at both mRNA and protein levels in the gut tissues of patients with IBS. Moreover, for better understanding the mechanistic pathways involved in this disease, it is important to include the role of other chemokines and inflammatory biomarkers in future studies.

In conclusion, herein we show for the first time that CCL28 is increased in the serum of patients with IBS and is associated with the clinical symptoms of the disease. Our data support the notion that inflammation might play a role in the pathogenesis of IBS.

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Disclosure of conflict of interest

None.

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References

[1] El-Salhy M. Recent developments in the pathophysiology of irritable bowel syndrome. World J Gastroenterol 2015; 21: 7621-7636.

[2] Vaiopoulou A, Karamanolis G, Psaltopoulou T, Karatzias G and Gazouli M. Molecular basis of the irritable bowel syndrome. World J Gastroenterol 2014; 20: 376-383.

[3] Canavan C, West J and Card T. Review article: the economic impact of the irritable bowel syndrome. Aliment Pharmacol Ther 2014; 40: 1023-1034.

[4] Lee YJ and Park KS. Irritable bowel syndrome: emerging paradigm in pathophysiology. World J Gastroenterol 2014; 20: 2456-2469.

[5] Ng QX, Soh AYS, Loke W, Lim DY and Yeo WS. The role of inflammation in irritable bowel syndrome (IBS). J Inflamm Res 2018; 11: 345-349.

[6] Gwee K, Collins S, Read N, Rajnakova A, Deng Y, Graham J, McKendrick M and Moochhala S. Increased rectal mucosal expression of interleukin 1β in recently acquired post-infectious irritable bowel syndrome. Gut 2003; 52: 523-526.

[7] Robles A, Perez Ingles D, Myneedu K, Deoker A, Sarosiek I, Zuckerman MJ, Schmulson MJ and Bashashati M. Mast cells are increased in the small intestinal mucosa of patients with irritable bowel syndrome: a systematic review and meta-analysis. Neurogastroenterol Motil 2019; 31: e13718.

[8] Uno Y. Hypothesis: mechanism of irritable bowel syndrome in inflammatory bowel disease. Med Hypotheses 2019; 132: 109324.

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

[10] Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A and Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. Gastroenterology 2002; 122: 1778-1783.

[11] Cenac N, Andrews CN, Holzhausen M, Chapman K, Cottrell G, Andrade-Gordon P, Steinhoff M, Barbara G, Beck P, Bunnett NW, Sharkey KA, Ferraz JG, Shaffer E and Vergnolle N. Role for protease activity in visceral pain in irritable bowel syndrome. J Clin Invest 2007; 117: 636-647.

[12] Patel SR, Singh A, Misra V, Misra SP, Dwivedi M and Trivedi P. Levels of interleukins 2, 6, 8, and 10 in patients with irritable bowel syndrome. Indian J Pathol Microbiol 2017; 60: 385-389.

[13] Rana SV, Sharma S, Sinha SK, Parsad KK, Malik A and Singh K. Pro-inflammatory and anti-inflammatory cytokine response in diarrhoea-predominant irritable bowel syndrome patients. Trop Gastroenterol 2012; 33: 251-256.

[14] Vazquez-Frias R, Gutierrez-Reyes G, Urban-Reyes M, Velazquez-Guadarrama N, Fortoul-van der Goes TI, Reyes-Lopez A and Consuelo-Sanchez A. Proinflammatory and anti-inflammatory cytokine profile in pediatric patients with irritable bowel syndrome. Rev Gastroenterol Mex 2015; 80: 6-12.

[15] Hansson M, Hermansson M, Svensson H, Elfvin A, Hansson LE, Johnsson E, Sjoling A and Quiding-Jarbrink M. CCL28 is increased in human Helicobacter pylori-induced gastritis and mediates recruitment of gastric immunoglobulin A-secreting cells. Infect Immun 2008; 76: 3304-3311.

[16] Miles A, Liaskou E, Eksteen B, Lalor PF and Adams DH. CCL25 and CCL28 promote alpha4 beta7-integrin-dependent adhesion of lympho-

cytes to MAdCAM-1 under shear flow. Am J Physiol Gastrointest Liver Physiol 2008; 294: G1257-1267.
[17] O'Gorman MT, Jatoi NA, Lane SJ and Mahon BP. IL-1beta and TNF-alpha induce increased expression of CCL28 by airway epithelial cells via an NFkappaB-dependent pathway. Cell Immunol 2005; 238: 87-96.
[18] Zeggini E and Morris A. Analysis of complex disease association studies: a practical guide. Academic Press 2010.
[19] Han SH, Lee OY, Bae SC, Lee SH, Chang YK, Yang SY, Yoon BC, Choi HS, Hahm JS and Lee MH. Prevalence of irritable bowel syndrome in Korea: population-based survey using the Rome II criteria. J Gastroenterol Hepatol 2006; 21: 1687-1692.
[20] Chang L, Adeyemo M, Karagiannides I, Videlock EJ, Bowe C, Shih W, Presson AP, Yuan PQ, Cortina G, Gong H, Singh S, Licudine A, Mayer M, Tache Y, Pothoulakis C and Mayer EA. Serum and colonic mucosal immune markers in irritable bowel syndrome. Am J Gastroenterol 2012; 107: 262-272.
[21] Macsharry J, O'Mahony L, Fanning A, Bairead E, Sherlock G, Tiesman J, Fulmer A, Kiely B, Dinan TG, Shanahan F and Quigley EM. Mucosal cytokine imbalance in irritable bowel syndrome. Scand J Gastroenterol 2008; 43: 1467-1476.
[22] Berri M, Virlogeux-Payant I, Chevaleyre C, Melo S, Zanello G, Salmon H and Meurens F. CCL28 involvement in mucosal tissues protection as a chemokine and as an antibacterial peptide. Dev Comp Immunol 2014; 44: 286-290.
[23] Rashidiani S, Jalili A, Babaei E, Sheikhesmaeili F, Fakhari S, Ataee P and Parhizkar B. The chemokine CCL28 is elevated in the serum of patients with celiac disease and decreased after treatment. Am J Clin Exp Immunol 2017; 6: 60-65.
[24] Ezzat MH and Shaheen KY. Serum mucosa-associated epithelial chemokine in atopic dermatitis: a specific marker for severity. Indian J Dermatol 2009; 54: 229-236.
[25] Del Valle-Pinero AY, Martino AC, Taylor TJ, Majors BL, Patel NS, Heitkemper MM and Henderson WA. Pro-inflammatory chemokine C-C motif ligand 16 (CCL-16) dysregulation in irritable bowel syndrome (IBS): a pilot study. Neurogastroenterol Motil 2011; 23: 1092-1097.