Reports of Biochemistry & Molecular Biology
Vol.8, No.1, Apr 2019

Original article

www.RBMB.net

The Serum Levels of *CCL2* and *CCL16* Expression in Patients with Irritable Bowel Syndrome

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Abstract

Background: Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by altered bowel habits and abdominal pain in the absence of a recognizable structural anomaly. The pathogenesis of IBS has been associated with inflammation and the expression of pro-inflammatory chemokines, such as CCL2 and CCL16. Our study aimed to investigate the relationship between the serum levels of CCL2 and CCL16 and IBS. Additionally, we examined how serum levels of these chemokines relate to IBS subtypes.

Methods: Patients with IBS diagnosed according to the Rome III criteria participated in this study (n= 96). Healthy individuals with no history of allergic, autoimmune, chronic or active gastrointestinal infectious diseases were used as controls (n= 44). The serum levels of CCL2 and CCL16 was measured via enzyme-linked immunosorbent assay (ELISA).

Results: A significant decrease in the serum levels of CCL16 and CCL2 was observed in the patients with IBS. Additionally, the serum levels of CCL16 in IBS patients with diarrhea (D-IBS) was significantly higher than those with the mixed IBS (M-IBS) subtype.

Conclusions: The significant increase in the serum levels of CCL-16 in patients with D-IBS compared to patients with M-IBS suggests that CCL-16 may be used as an immunological biomarker to differentiate between these two subtypes.

Keywords: CCL-2, CCL-16, C-IBS, D-IBS, MCP-1, M-IBS, IBS.

Introduction

Irritable Bowel Syndrome (IBS) is one of the most common gastrointestinal (GI) diseases with the capacity to significantly decrease the quality of the effected individual. IBS understood to be a bowel disorder with abnormalities in GI function characterised by a cluster of clinical symptoms including, dyspepsia, bloating, abnormalities in the ability to pass stool (feeling of urgency, incomplete defecation, and/or constipation), the passage of mucus, diarrhea, and abdominal pain. The prevalence of IBS is approximately 10-15% (1).

According to the Rome III criteria, IBS is divided into three subtypes: IBS with diarrhea (D-IBS), IBS with constipation (C-IBS), and IBS with mixed symptoms (M-IBS) (2). Although the etiology of IBS is unknown, several factors have been associated with the development of IBS. These factors include the individual's gut microbiota, depression, anxiety, visceral hypersensitivity, intestinal permeability, metabolism of bile acid, genetics, gut-brain interactions and the dysfunction of gut motility (3-8). Additionally, various studies

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Received: 25 Apr, 2018; Accepted: 17 Jun, 2018

have shown inflammatory factors of the immune system to have significant role in the development of IBS (9, 10). Chemokines are a family of cytokines which have a major role in the homing, regulation and infiltration of leukocytes to the sites of inflammation (11). Monocyte chemoattractant protein-1 (MCP-1), also known as CCL2, is a chemokine produced by a variety of cells including, mononuclear cells, fibroblasts, endothelial cells, smooth muscle cells, and epithelial cells. CCL2 has the ability to induce an inflammatory response and recruit leukocytes to the intestinal membrane and cause inflammation (12). Chemokine (C-C motif) ligand 16 (CCL16) is constitutively produced by parenchymal cells of the liver, thymus and spleen, and is present in human plasma. CCL16 has chemotactic properties for monocytes and lymphocytes (13-15). Alterations in the protein or gene expression levels of CCL2 and CCL16 have been detected in patients with IBS, however, the results have been conflicting (16-19). In the present study, we aimed to investigate the serum levels of CCL2 and CCL16 in patients with IBS. We also aimed to study the correlation between serum levels of CCL2 and CCL16 and IBS subtypes.

Materials and methods

Ethics statements

All patients with IBS were selected from the Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran. All healthy controls were from the Kerman Blood Transfusion Centeer, Kerman, Iran. Written informed consent was provided to all participants and obtained prior to enrollment the study. The research was performed between 2014 and 2017. The research study was approved by the Ethical Committee of the Kerman University of Medical Sciences. The approval number is 678 /94.

Selection of patients and controls

Our study included 96 patients with IBS and 44 healthy individuals from the Kerman Blood Transfusion Center. Patients were selected according to the Rome III criteria by a gastroenterologist. Those with organic GI disorders were not included in this study. Patients with IBS were divided into the three subtypes according to the Rome III criteria: IBS with diarrhea (D-IBS) (n = 32), IBS with constipation IBS (C-IBS) (n = 33), and IBS with mixed symptoms (M-IBS) (n = 31) (2). Healthy controls were excluded if they had any history of allergic, autoimmune, chronic or active GI infections. The demographic data for the controls was as follows: males (frequency = 55.8%, n = 24), females (frequency = 44.2%, n = 20). The mean age for the males and females in the control group was 31.8 ± 10.95 and 32.16 ± 12.82, years, respectively. The duration of the disease in patients with IBS was 4.88±0.36 years. The patients with IBS and controls were age and gender-matched. The demographic and clinical characteristics of patients are summarized in Table 1.

Table 1. Clinical and demographic characteristics of patients with IBS

Variables	Number/ Mean ±SD	Frequency
	Gender	
Male	53	56.2
Female	43	43.8
Age (yr) Mean ±SD		
Male	31.7±11.54	-
Female	65.6±11.32	-
	Marital status	
Married	57	59.4
Single	37	38.5
Divorced/widow	2	2.1
	Education	
Lower secondary education	43	44.7
Upper secondary education	25	26.0
Bachelor or equivalent	28	29.2
	Occupation	
Housekeeping	25	26
Farmer	11	11.5
Laborer	14	6.14
Employee	11	5.11
Collegian	17	7.17
Unemployed	10	4.10
Student	6	3.6
Driver	1	1
Self-employee	1	1
	IBS sub-types*	
D-IBS	32	33.3
C-IBS	33	34.3
M-IBS	31	3.32

* Irritable bowel syndrome was classified into IBS with diarrhea (D-IBS), IBS with constipation IBS (C-IBS), and IBS with mixed symptoms (M-IBS), according to the Rome III criteria.

All medications administered to the patients were divided into 9 separate combinations depending on IBS subtypes; 1: Dimethicone + Fluoxetine, 2: Dimethicone +Pantoprazole, 3: Mebeverine + Belladonna + Metronidazole, 4:

Dimethicone + Pesilium, 5: Nortriptyline + Mebeverine + Colpermin, 6: Nortriptyline + Metronidazole + Pantoprazole, 7: Lactulose + Pantoprazole + Fluoxetine, 8: Domperidone + Dimethicone + Pesilium, 9: Nortriptyline + Pesilium + Colpermin.

Cytokine assay

Serum was separated from 5 ml of whole blood samples and cryopreserved at minus 80 degree centigrade until further analysis. Serum levels of CCL2 and CCL16 were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Abcam®, USA). The assay range of Abcam® ELISA kits were 1.1-800 pg/ml for the detection of CCL2 and 8.23-6000 pg/ml for CCL16. Inter-Assay and Intra-Assay precision had been performed to validate the ELISA Kits according to the declaration of Abcam® Company. The results for CCL2 and CCL16 are available online at the following website, respectively: http://www.abcam.com/human-mcp1-elisa-kit-ccl2-ab179886.html, and http://www.abcam.com/Human-LEC-ELISA-Kit-CCL16-ab100532.html.

Statistics

Statistical analyses were performed by employing the SPSS soft- ware version 17.0. Descriptive statistics, one-way analysis of variance (ANOVA), Chi squared, Tukey and independent *t*-tests were used to determine statistical significance of the data. A *P*-value less than 0.05 was considered as statistically significant. The serum levels of chemokines were presented as mean ± SD.

Results

The serum levels of CCL2 and CCL16 are significantly decreased in patients with IBS

The levels of CCL2 and CCL16 within the serum of patients with IBS were 153.44±65 and 2330.6±1564.7 pg/ml, respectively. Among the healthy controls, the serum levels of CCL16 and CCL2 were 263.39 ±74.07 and 3186.38 ± 1592.03 pg/ml, respectively. There was a significant decrease in the serum levels of CCL2 in patients with IBS compared to controls (*P* = 0.001) (Fig. 1). Additionally, a significant decrease in the serum levels of CCL16 in patients with IBS was observed compared to controls (*P*=0.004) (Fig. 2).

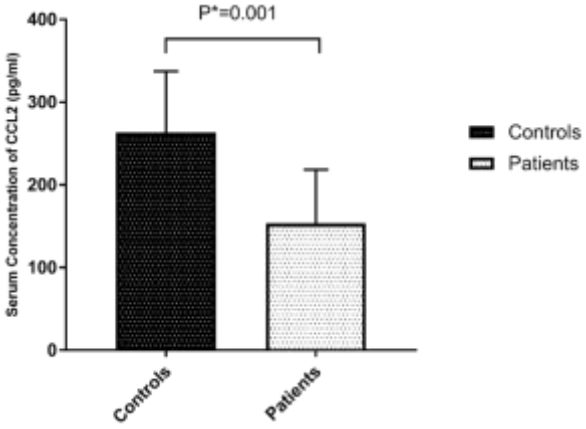

Fig. 1. Serum levels of CCL2 in patients with IBS were significantly lower than healthy controls (p=0.001). Statistically significant differences measured by independent t-test and values are presented as mean ± SD.

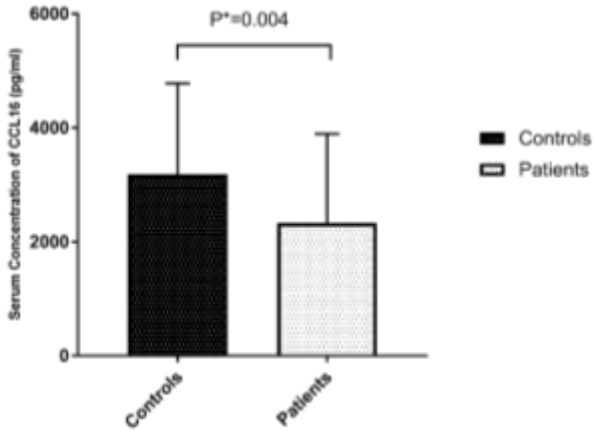

Fig. 2. Serum levels of CCL16 in patients with IBS were significantly lower than healthy controls (P= 0.004). Statistically significant differences measured by independent t-test and values are presented as mean ± SD.

Serum levels of CCL2 and CCL16 in irritable bowel syndrome subtypes

The serum levels of CCL2 and CCL16 in patients with D-IBS were 148.92 ± 64.72 pg/ml and 2802.49 ± 1749.74 pg/ml, respectively. Both chemokines showed a significant decrease in IBS subtypes compared to controls (P=0.001). The serum levels of CCL2 and CCL16 in patients with C-IBS were 160.06 ± 64.67 pg/ml, 2428.89 ± 1582.02 pg/ml, respectively. The serum levels of the CCL2 and CCL16 in C-IBS patients were significantly lower than controls (p= 0.001). The serum levels of CCL2 and CCL16 in patients with M-IBS were 151.42 ± 67.19 and 1726.82 ±

1126.49, respectively. Additionally, the serum levels of the CCL2 and CCL16 in M-IBS patients were significantly decreased compared to controls (P=0.001).

The serum levels of CCL2 and CCL16 were compared between the IBS subtypes. No significant difference between the IBS subtypes regarding the serum levels of CCL2 (P=0.774) were found. In contrast, comparing serum levels of CCL16 between the three subtypes of IBS showed significantly higher levels of CCL16 in the D-IBS patients compared to M-IBS (p=0.011) (Fig. 3).

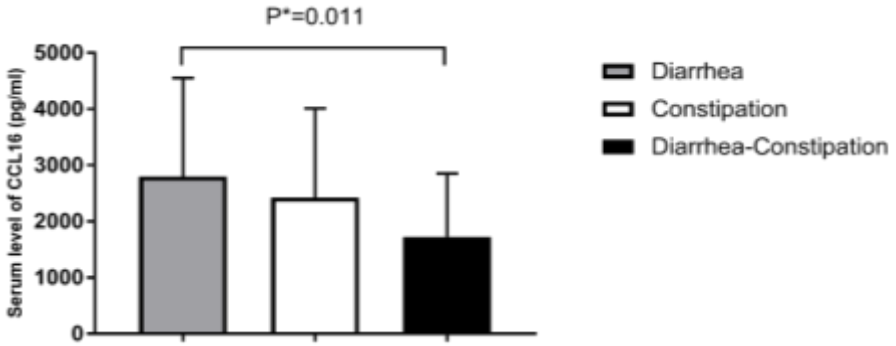

Fig. 3. Comparison of CCL16 between IBS subtypes. Serum levels of CCL16 in diarrhea-IBS were significantly higher than diarrhea-constipation as known to mixed IBS (P=0.011). Statistical analysis was performed by one-way ANOVA test and values are presented as mean ± SD. Statistically significant differences measured by Tukey test.

No correlation was found between levels of CCL1 or CCL16 in patients with IBS and the clinical symptoms (data not shown). Additionally, our results showed no significant correlation between the serum levels of CCL-2 and CCL-16 among the nine various combinations of medicines (Table 2). Moreover, there was no significant difference between males and females with regard to the level of chemokines, in all patients with IBS and also among IBS subtypes (data not shown).

Table 2. Serum levels of CCL-2 and CCL-16 with nine various combinations of medicines

Different combinations of medications*	Serum levels of CCL-2 (pg/ml)		Serum levels of CCL-16 (pg/ml)	
	Mean	SD	Mean	SD
1	149.45	24.28	1641.40	346.53
2	160.72	19.79	2164.37	352.30
3	135.12	16.94	2578.90	463.16
4	164.19	19.41	2235.48	317.45
5	154.93	15.46	2235.48	317.45
6	164.77	30	3269.07	386.53
7	149.61	16.26	2576.07	771.01
8	164.08	23.19	2625.96	473.51
9	144.98	13.96	1412.65	326.89

*1. Dimethicone + Fluoxetine, 2: Dimethicone +Pantoprazole, 3: Mebeverine + Belladonna + Metronidazole, 4: Dimethicone + Pesilium, 5: Nortriptyline + Mebeverine + Colpermin, 6: Nortriptyline + Metronidazole + Pantoprazole, 7: Lactulose + Pantoprazole + Fluoxetine, 8: Domperidone + Dimethicone + Pesilium, 9: Nortriptyline + Pesilium + Colpermin.

Discussion

Our results showed a significant decline in serum levels of CCL2 and CCL16 in our cohort of patients with IBS compared. Inconsistencies exist between our findings and previous reports. Schoepfer et al., has previously shown that the levels of CCL2 in the cultured peripheral blood mononuclear cells (PBMCs) from IBS patients were not significantly different compared to healthy controls (19). In a separate study, significantly higher levels of CCL2 were observed in patients with IBS compared to controls. They concluded that CCL2 may have an important role in IBS due to its role in inflammation (17). Furthermore, Darkoh et al., showed that the levels CCL2 in the serum and stool of patients with either idiopathic or post-

infectious IBS, were significantly increased compared to controls (16). Valle-Pinero et al., showed that the gene expression of CCL16 in patients with IBS was increased by 7.46 fold. They also reported the gene expression of CCL16 to be upregulated by 138.47 fold in C-IBS patients compared with patients with D-IBS and M-IBS (18). Conversely, we observed a significant difference in the serum levels of CCL16 among IBS subtypes. Our results showed a significant increase in the levels of CCL16 in the serum of patients with D-IBS compared to patients with M-IBS. Such differences were not found for the serum levels of CCL2 among the IBS subtypes. Chiba and colleagues has reported that the serum levels of CCL2 was significantly higher in patients with D-IBS compared to those with C-IBS (20). To ensure that the decline in the serum levels of CCL2 and CCL16 were not a result of the medications prescribed for IBS, we analysed the correlation between the various combinations of medications and the serum levels of CCL2 and CCL16 in patients with IBS and among IBS subtypes. However, no correlation was found.

Very little is known about the changes of chemokines in IBS. According to majority of studies, the serum levels of CCL2 and gene expression of CCL16 in IBS patients were significantly increased, however our data showed the serum levels of CCL2 and CCL16 in IBS patients to be significantly decreased. Therefore, to eliminate the role of confounding factors, such as the effect of medications, the use of patients with IBS who have not yet received medications may allow for more accurate and trustworthy results. Additionally, examining the levels of chemokines in patients with post-infectious IBS and idiopathic IBS may offer more insight into the role of chemokines in IBS. One of our critical observations was that there was a significant difference in serum levels of CCL16 among D-IBS and M-IBS subtypes. This finding suggests that examining serum levels of CCL16 may offer a novel immunological biomarker to confirm the diagnosis of D-IBS from M-IBS subtypes. Further investigation is required to examine this potential role.

Acknowledgment

These data have been extracted from the thesis of Dr. Saeed Pishgouyi, Resident of Gastroenterology in Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran. Special acknowledgement should be dedicated to the staff members of Kerman Blood TransfusionCenter, who helped us with blood collection from healthy volunteers.

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