ORIGINAL ARTICLE

Serum levels of chemokines MCP-1, GRO-alpha and E-selectin correlates with familial Mediterranean fever

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ABSTRACT

Background: MCP-1(CCL-2) and GRO-α (CXCL-1) are chemokines that play an essential role in human body homeostatic and pathological processes. Both chemokines are critical in the pathogenesis of familial Mediterranean fever, an inflammatory disorder characterized by chronic inflammation attacks, neutrophil migration, and disruption of the process of apoptosis in damaged areas. E-selectin, one of the molecules that mediate leukocyte-endothelial adhesion, is also expressed through activated endothelial cells and is shed from these cells, its serum levels may indicate endothelial dysfunction in conditions such as familial Mediterranean fever disease.

Aim: In this study, we estimated the serum levels of MCP-1, CXCL-1, and E-selectin as diagnostic markers in familial Mediterranean fever patients, as compared to control subjects.

Patient and methods: 50 subjects were studied, 30 familial Mediterranean fever patients and 20 healthy controls, aged 1 to 18 years. Serum chemokines, namely, MCP-1, GRO-α, and E-selectin, were measured with Luminex® LabScan100.

Results: Serum concentrations of GRO-α was significantly increased in patients with familial Mediterranean fever, compared to healthy controls. MCP-1 and E-selectin values were also elevated in familial Mediterranean fever patients, but this was not statistically significant.

Conclusion: Our findings indicate that GRO-α may play a significant role in the development of familial Mediterranean fever. GRO-α may be a marker of familial Mediterranean fever disease development, as well as a possible useful therapeutic target. MCP-1 and E-selectin were non-significantly elevated in familial Mediterranean fever.

Keywords: Familial Mediterranean fever, MCP-1, CCL-2, GRO-α, CXCL-1, E-selectin, Luminex.

N Z J Med Lab Sci 2020; 74: 21-25

INTRODUCTION

Familial Mediterranean fever is an inherited autoinflammatory disease frequently sharing similar clinical signs and symptoms with inflammatory bowel disease. The two diseases are characterized by neutrophil migration and chronic inflammation attacks, and in both conditions, disrupted apoptosis mechanisms are present in the damaged areas (1,2). Many inflammatory diseases, such as inflammatory bowel disease, are associated with familial Mediterranean fever in an increasing frequency. Moreover, in conditions where inflammatory bowel disease accompanies familial Mediterranean fever, the frequency of other inflammatory diseases, including juvenile rheumatoid arthritis, also increases (3,4).

Chemokines are a superfamily of small, secreted proteins that have been essential to many homeostatic and pathological human body processes. Chemokines have many roles in intercellular signalling and are especially important in the recruitment of leukocytes to inflammatory sites (5). The monocyte chemoattractant protein-1 (MCP-1/CCL-2) is part of the family of chemokines and a critical monocyte chemotactic factor (6). MCP-1 is activated either by oxidant stress, cytokines, or growth factors constitutively and after motivation. It is formed by various types of cells: epithelial, mesangial, smooth, astrocyte, monocyte, and microcellular (7,8). MCP-1 controls monocyte migration and invasion, natural killer cells (NK), and memory-T lymphocytes. MCP-1 recruits monocytes into active inflammatory concentration and has been shown to be the primary chemokine to recruit monocytes (9). MCP-1 uses the CCR2 receptor to mediate its effects, and, unlike MCP-1, CCR2 expression is relatively limited to certain types of cells. MCP-1 and its receptor CCR2 are induced during different diseases, including inflammatory bowel disease (10). In inflammatory bowel disease, the mucosa shows ulcerative lesions that are in the company of a prominent infiltrate of inflammatory cells. In many clinical and experimental studies on inflammatory bowel disease, various chemokines, including MCP-1, have been shown to be upregulated in mucosal tissues (11).

GRO-α (or CXCL1), a participant of the CXC family, is a 73-amino acid with growth-related properties and is produced by multiple cell lines. Its growth stimulating activity on malignant melanoma cells is well known. Still, many new functions and features of GRO-α were later discovered and associated with inflammation and cardiovascular disease (12). Among many other functions, the GRO family was shown to induce neutrophil chemotaxis, and respiratory burst (13), T-lymphocyte chemotaxis (14), and also, monocytes were shown to be a probable leukocyte target for GRO-α (15).

Endothelial adhesion of leukocytes is primarily regulated by the contact of adhesion molecules and their ligands on specific cells. Several molecules have been identified which mediate leukocyte-endothelial adhesion, including E-selectin intracellular adhesion. Activated endothelial cells express the E-selectin (16). Due to the shedding of selectin adhesion molecules from activated cells, these proteins can be found in soluble forms then released into circulation and may be used as endothelium activation markers and may also indicate endothelial dysfunction by their increased serum levels (17).

The objective of our study was to estimate the role of MCP-1, CXCL-1, and E-selectin in familial Mediterranean fever patients.

METHODS

Ethics

This research was accepted by the National Research Centre's ethics committee in Egypt and written informed consent was obtained from the control cases and the patient's guardian of all children involved in our study before their enrollment. Ethics number (13/146).

Subjects

Thirty familial Mediterranean fever patients and 20 healthy controls aged 1 to 18 years were recruited. Patients enrolled in the study were attending the hospital of the National Research Centre, Cairo, Egypt, from March 2015 to June 2016. All familial Mediterranean fever patients were free of any other diseases or medicines that could impair complement activity. Clinical manifestations and therapies of the familial Mediterranean fever patients are shown in Table 1. All tests were performed mainly for research purposes with no instant clinical benefit, and the patients were informed previously. All Familial Mediterranean. The control subjects had at least one month before blood sampling been free of any medication. In the case of patients, sampling was conducted on the second day of hospitalisation. Serum was separated after one hour of coagulation by centrifugation and serum aliquots were preserved at −30 ° C before cytokine analysis. Total leucocytic count was done using a Medonic m20 3-part differential CBC analyser according to the manufacturer's instructions (Boul Medical AB Domnarvsgaten 4, Spanga, Sweden).

Table 1. Clinical characteristics of patients and controls.

Characteristic	Familial Mediterranean Fever patients	Healthy controls
Number of subjects	30	20
Gender (number of male/female)	14/16	8/12
Age range (years)	1 - 34	3 - 35
TLC (× 10^3/mm3; mean ±SD)	7.8 ±2.6	8.6 ±2.2
Medications (colchicine*)	30/30	0/20

*The dose of colchicine ranged from 0.5-1.5 mg/day. TLC: total leucocytic count.

Luminex cytokine assays

The serum chemokines, MCP-1, GRO-alpha, and E-selectin, were assayed using the Premixed Human Multi-Analyte Assay Kit from R&D Systems (USA & Canada) and measured on a Luminex® LabScan100 analyser. According to the manufacturer instructions analyte-specific antibodies were pre-coated onto color-coded micro-particles. Micro-particles, samples, and standards were pipetted into wells, then washed and Streptavidin-PE added, which binds the biotinylated detection antibodies, then the micro-particles were resuspended in buffer and read using the Luminex® analyser.

Statistical analysis

Statistically analyzed data performed by using SPSS version 16.0 software. A nonparametric Mann-Whitney U test was used to compare concentrations of cytokines between FMF patients and healthy controls. Association between cytokine expressions with age, gender, and total leucocytic count of patients was tested using Spearman rank correlation. Data were presented as median except for total leukocyte count which are presented as mean±SD.A *P*-value <0.05 was deemed statistically significant. For each cytokine, the receiver operating characteristic (ROC) curve was designed to evaluate the effectiveness of these cytokines as biomarkers for familial Mediterranean fever patients against healthy controls. The values of the area under the curve (AUC) and 95% confidence intervals were determined for each cytokine.

RESULTS

Serum level of MCP-1 showed no statistically significant elevation in familial Mediterranean fever patients compared with healthy controls (Table 2). Concentrations of CXCL-1 showed a statistically significant increase in patients with familial Mediterranean fever compared to healthy controls. Moreover, there were insignificant higher levels (P<0.01) of E-selectin in the serum of familial Mediterranean fever patients.

Our results showed that the E-selectin level was inversely correlated with age and directly correlated with a total leukocytic count in familial Mediterranean fever patients (P < 0.05, Spearman correlation) (Table 3). Furthermore, our study indicated that there was a significant negative correlation between MCP-1 level and age of Familial Mediterranean fever patients (P < 0.01, Spearman correlation).

Our results indicated that a ROC curve of CXCL-1 showed a good significant AUC value of 0.830, an excellent sensitivity of 80%, and a moderate specificity of 71% (P < 0.001). Also, the ROC curve of MCP-1 had a non-significant AUC value of 0.649, a low sensitivity of 60%, and a moderate specificity of 68.8%. Moreover, E-selectin showed a ROC curve having a non-significant AUC value of 0.632, an average sensitivity of 66.7%, and a low specificity of 50% (Table 4 & Fig.1).

Table 2. Serum CXCL-1, MCP-1 and E-selectin in familial Mediterranean fever patients and controls.

Parameter	Groups	Median	Minimum	Maximum	*P*-value*
CXCL-1	Familial Mediterranean Fever patients	149.67	57.04	867.07	0.001*
	Healthy controls	45.23	9.07	334.88	
CCL-2	Familial Mediterranean Fever patients	286.04	93.69	1679.08	0.112
	Healthy controls	244.15	35.25	787.07	
E-selectin	Familial Mediterranean Fever patients	82937	28367	113000	0.176
	Healthy controls	58517	142	113000	

*P<0.05 by non-parametric Mann-Whitney U test

Table 3. Association of the three cytokines with clinical parameters of familial Mediterranean Fever patients.

Parameters	R (Spearman correlation)	*P*-value
CXCL-1 level - age	-0.286	0.150
CXCL-1 level - gender	0.016	0.478
CXCL-1 level - TLC	-0.007	0.490
Parameters	**R (Spearman correlation)**	***P*-value**
E-selectin - age	-0.388*	0.023
E-selectin - gender	0.268	0.089
E-selectin -TLC	0.370*	0.041
Parameters	**R (Spearman correlation)**	***P*-value**
CCL-2 level - Age	-0.465**	0.010
CCL-2 level - Gender	0.133	0.263
CCL-2 level - TLC	0.247	0.122

*Correlation is significant at the 0.05 level (1-tailed) **Correlation is significant at the 0.01 level (1-tailed)..

Table 4. AUC values of CXCL-1, CCL-2 and E-selectin.

Cytokines	AUC	S.E.	Sig.	Sensitivity	Specificity	95% Confidence interval
CXCL-1	0.830	0.067	0.001*	80%	71%	0.699-961
CCL-2	0.649	0.088	0.112	60%	68.8%	0.477-0.821
E-selectin	0.632	0.103	0.169	66.7%	50%	0.430-0.834

*Correlation is significant at the 0.01 level (1-tailed).

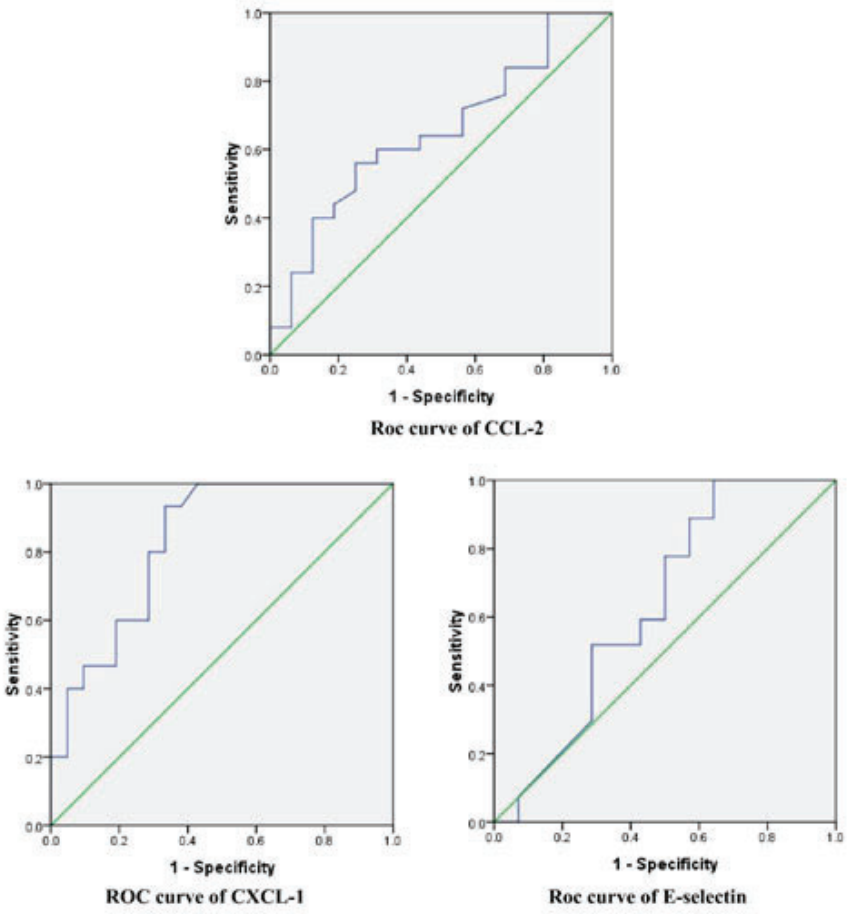

Figure 1. ROC curve of CXCL-1, MCP-1, and E-selectin for familial Mediterranean fever patients versus healthy controls. Diagonal segments are produced by ties.

DISCUSSION

Familial Mediterranean fever is an inherited autoinflammatory disease caused by pyrin mutation, which is involved in inflammasome complex formation (18). Pyrin is encoded by MEFV, the mutated gene found in familial Mediterranean fever patients. Pathogenic variants of MEFV prefer the active pyrin and induce proinflammatory cytokine release and proptosis (19). Accordingly, activated cytokine networks are implicated in the pathogenesis of familial Mediterranean fever (20,21). In our study, we estimated the role of MCP-1 (CCL2), GRO-a (CXCL1), and E-selectin as essential players in the process of chemotaxis and adhesion of recruited neutrophils and monocytes to the site of inflammation. We used the Luminex array system to estimate the serum level of these three cytokines, and we were able to identify some significant differences in levels between familial Mediterranean fever patients and healthy controls.

We found a negligible difference between patients with familial Mediterranean fever and controls in MCP-1 levels. A recent study showed that MCP-1 not only enhances the inflammatory response but can also have anti-inflammatory effects by inhibiting the migration of T cells in experimental colitis models when given under sub-physiological doses (22). Furthermore, MacDermott demonstrated that low-dose of MCP-1 also prevented the inflammation-enhanced carcinoma from the beginning. There is undoubtedly a need for more work to examine these effects in full (23).

Koga *et al*. showed a non-significant increase in MCP-1 serum levels in healthy control subjects compared with familial Mediterranean fever patients (24). Other studies on inflammatory bowel disease also showed contradictory results. Familial Mediterranean fever and inflammatory bowel disease have similar clinical and biological properties. Under both conditions, both are characterised by neutrophil migration and chronic inflammation attacks in the damage areas (1,2). Spoettl *et al.* found that MCP-1 can play a role in the disrupted intestinal differentiation occurring in the mucosa of patients with inflammatory bowel disease (10). Several studies have also identified elevated MCP-1 mRNA and protein expression in patients with inflammatory bowel disease mucosa (25). The small number of subjects involved in the various studies and perhaps the massive difference in diffusion rates of MCP-1 may explain this discrepancy (26).

In our study, the serum levels of GRO-α were significantly increased in patients with familial Mediterranean fever as compared to healthy subjects. Our results agree with Koga et *al.* who showed a significant increase in serum levels of CXCL1 (GRO-α) in familial Mediterranean fever patients (24). In conditions like Crohn's disease and ulcerative colitis, which carry very similar clinical and biological properties with familial Mediterranean fever, Mitsuyama *et al.* found that serum levels of GRO-α are significantly elevated in patients with inflammatory bowel disease and levels thereof perfectly correlate with disease grade. The serum GRO-α in other colitis was only marginally increased compared with familial Mediterranean fever and inflammatory bowel disease. In comparison to more acute colitis, specific inflammatory events that occur in acute and chronic diseases with familial Mediterranean fever and inflammatory bowel disease, distinguished mainly by chronic inflammatory cellular infiltrates that have the potential to produce GRO-α (27).

GRO-α is produced by multiple cell lines, and it has many functions and properties associated with inflammation. In familial Mediterranean fever patients, loss or gain of function mutation of pyrin protein renders it incapable of regulating inflammasome formation or leads to its over-activation (28). This increased inflammasome activity leads to increased activated caspase-1, which split pro-IL-18 and pro-IL-1β into their active matured variants. Proinflammatory cytokines IL-1, IL-18, and TNF-α, induce the expression of a large number of chemokines (29). Such chemokines can be identified in the blood with slightly lower rates than in tissues, thereby providing a gradient that signals cells moving into the tissues. Other studies indicate that monocytes also serve as target cells for members of the CX-C subfamily, suggesting that the classification of biological chemokine activities for different cell types along the lines of the conserved cysteine structural motif is oversimplified (30). This finding is consistent with the demonstration by Quigley *et al.* that in inflammatory bowel disease patients the circulatory GRO-α levels were higher than in healthy subjects (31). Previous studies focused on chemokine activities as soluble proteins, which were thought to serve as chemical factors attracting leukocytes exposed to the gradient of this soluble molecule. The presence of GRO homologs on the endothelial surface can be explained by several possible mechanisms. The protein can directly associate with the cell membrane via a transmembrane region (32).

Selectins are an adherence molecule familiar which controls the initial interaction of adhesion molecules, which regulate the initial interaction between leukocytes and vascular endothelium in acute and chronic inflammation tissues and sites. P- and E-selectin are two members of this family, expressed selectively in inflammation sites by endothelial cells. In particular, E-selectin is involved in migrating neutrophils to sites of acute inflammation and unique lymphocyte subsets into sites of chronic inflammation. The lymphocyte subsets are capable of binding E-selectin are cells that have undergone conversion to a "memory" phenotype, including both alpha/beta and gamma/delta T cells, and show preferential homing to inflammatory extra-lymphoid sites (33). In our study, we found that E-selectin expression with insignificant increases in patients than controls. E-selectin can be secreted into the bloodstream during only acute inflammation stages and can thus be involved in the accumulation of PMNs in the inflammatory areas.

We disagree with Erden *et al*. who demonstrated that genome-wide studies showed associations between single nucleotide polymorphisms within the ABO gene locus and the levels of E-selectin and P-selectin in the plasma, highlighting the possibility for a link between inflammation and ABO blood group (34). There has been previously shown that the soluble E-selectin level was increased in familial Mediterranean fever patients at the time of diagnosis. Many contribute to the possible link between the ABO blood group and colchicine resistance in familial Mediterranean fever patients (35). Also, E-selectin levels in active inflammatory bowel disease is increased significantly, and measurements of E-selectin may be clinically useful in inflammatory bowel disease for long-term monitoring (36).

In our study we found correlations between CXCL-1, MCP-1, and E-selectin with age, gender, and total leukocyte count. We found a negative relationship between MCP-1 & E-selectin with age. Only E-selectin has a positive correlation with the total leukocyte count. CXCL-1 showed no correlation with the three parameters. Our results indicated that a ROC curve of CXCL-1 showed a good significant AUC value of 0.830, a good sensitivity of 80%, and a moderate specificity of 71% ($P < 0.001$). In addition, the ROC curve of CCL-2 had a non-significant AUC value of 0.649, a low sensitivity of 60, and a moderate specificity of 68.8%. Moreover, E-selectin showed a ROC curve having a non-significant AUC value of 0.632, moderate sensitivity of 66.7%, and a low specificity of 50%.

ACKNOWLEDGEMENTS

This research was supported by the National Research Centre of Egypt.

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REFERENCES

1. Lichtenberger GS, Flavell RA, Alexopoulou L. Innate immunity and apoptosis in IBD. *Inflamm Bowel Dis* 2004;10 Suppl 1: S58-S62
2. McDermott MF. A common pathway in periodic fever syndromes. *Trends Immunol* 2004; 25: 457-460.
3. Ayaz NA, Ozen S, Bilginer Y, et al. MEFV mutations in systemic onset juvenile idiopathic arthritis. *Rheumatology (Oxford)* 2009; 48: 23-25.
4. Yurtcu E, Gokcan H, Yilmaz U, Sahin FI. Detection of MEFV gene mutations in patients with inflammatory bowel disease. *Genet Test Mol Biomarkers* 2009; 13: 87-90.
5. Muller WA. Getting leukocytes to the site of inflammation. *Vet Pathol* 2013; 50: 7-22.
6. Van Coillie E, Van Damme J, Opdenakker G. The MCP/eotaxin subfamily of CC chemokines. *Cytokine Growth Factor Rev* 1999; 10: 61–86.
7. Brown Z, Strieter RM, Neild GH, et al. IL-1 receptor antagonist inhibits monocyte chemotactic peptide1 generation by human mesangial cells. *Kidney Int* 1992; 42: 95–101.
8. Barna BP, Pettay J, Barnett GH, et al. Regulation of monocyte chemoattractant protein-1 expression in adult human non-neoplastic astrocytes is sensitive to tumor necrosis factor (TNF) or antibody to the 55-kDa TNF receptor. *J Neuroimmunol*. 1994; 50: 101–107.
9. Palframan RT, Jung S, Cheng G, et.al. Inflammatory chemokine transport and presentation in HEV: a remote-control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. *J Exp Med 2001*; 194: 1361–1373.
10. Spoettl T, Hausmann M, Herlyn M, et al. Monocyte chemoattractant protein-1 (MCP-1) inhibits the intestinal-like differentiation of monocytes. *Clin Exp Immunol* 2006; 145: 190–199.
11. Khan WI, Motomura Y, Wang H, et al. Critical role of MCP-1 in the pathogenesis of experimental colitis in the context of immune and enterochromaffin cells. *Am J Physiol Gastrointest Liver Physiol* 2006; 291: G803–G811.
12. Bechara C, Chai H, Lin PH, Chen C. Growth related oncogene-alpha (GRO-a): Roles in atherosclerosis, angiogenesis and other inflammatory conditions. *Med Sci Monit* 2007; 13(6): RA87-RA90.
13. Geiser T, Dewald B, Ehrengruber MU, et al. The interleukine-8-related chemotactic cytokines GRO alpha, GRO beta, and GRO gamma activate human neutrophil and basophil leukocytes. *J Biol Chem* 1993; 268: 15419–15424.
14. Jinquan T, Frydenberg J, Mukaida N, et al. Recombinant human growth-regulated oncogene-alpha induces T lymphocyte chemotaxis. A process regulated via IL-8 receptors by INF-gamma, TNF-alpha, IL-4, IL-10, and IL-13. *J Immunol* 1995; 155: 5359–5368.
15. Boisvert WA, Santiago R, Curtiss LK, et al. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 1998; 101: 353–363
16. Radi ZA, Kehrli ME Jr, Ackerman MR. Cell adhesion molecules, leukocyte trafficking, and strategies to reduce leukocyte infiltration. *J Vet Intern Med* 2001; 15: 516-529.
17. Videm V, Albrigtsen M. Soluble ICAM - 1 and VCAM - 1 as markers of endothelial activation. *Scand J Immunol* 2008; 67: 523-531.
18. Kholoussi S, Kholoussi N, Zaki ME, et al. Immunological evaluation in patients with familial Mediterranean fever. *Open Access Maced J Med Sci* 2018; 6: 310-313.
19. Schnappauf O, Chae JJ, Kastner DL, Aksentijevich I. The pyrin inflammasome in health and disease. *Front Immunol* 2019; 10: 1745.
20. Manukyan GP, Ghazaryan KA, Ktsoyan ZhA, et al. Cytokine profile of Armenian patients with Familial Mediterranean fever. *Clin Biochem* 2008; 41: 920-922.
21. Ben-Zvi I, Livneh A. Chronic inflammation in FMF: markers, risk factors, outcomes and therapy. *Nat Rev Rheumatol* 2011; 7: 105–112.
22. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; 140: 883-99.
23. MacDermott RP. Chemokines in the inflammatory bowel diseases. *J Clin Immunol* 1999; 19: 266-272.
24. Koga T, Migita K, Sato S, et al. Multiple serum cytokine profiling to identify combinational diagnostic biomarkers in attacks of familial Mediterranean fever. *Medicine (Baltimore)* 2016; 95; e3449.
25. Mazzucchelli L, Hauser C, Zgraggen K, et al. Differential in situ expression of the genes encoding the chemokines MCP-1 and RANTES in human inflammatory bowel disease. *J Pathol* 1996; 178: 201–206.
26. Palmieri O, Latiano A, Salvatori E, et al. The -A2518G polymorphism of monocyte chemoattractant protein-1 is associated with Crohn's disease. *Am J Gastroenterol* 2010; 105: 1586–1594.
27. Mitsuyama K, Tsuruta O, Tomiyasu N, et al. Increased circulating concentrations of growth-related oncogene (GRO)-alpha in patients with inflammatory bowel disease. *Dig Dis Sci* 2006; 51: 173-177.
28. Kanazawa N. Rare hereditary autoinflammatory disorders: towards an understanding of critical in vivo inflammatory pathways. *J Dermatol Sci* 2012; 66: 183–189.
29. Zhang JM, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin* 2007; 45: 27–37.
30. Choi J, Selmi C, Leung PSC, et al. Chemokine and chemokine receptors in autoimmunity: the case of primary biliary cholangitis. *Expert Rev Clin Immunol* 2016; 12: 661-672.
31. Quigley EMM. Overlapping irritable bowel syndrome and inflammatory bowel disease: less to this than meets the eye? *Therap Adv Gastroenterol* 2016; 9: 199-212.
32. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta* 2014; 1843: 2563-2582.
33. http://grantome.com/grant/NIH/R01-AI041671-05.
34. Erden A, Batu ED, Armagan B, et al. Blood group 'A' may have a possible modifier effect on familial Mediterranean fever and blood group '0'may be associated with colchicine resistance. *Biomark Med 2018*; 12: 565-572.
35. Paterson AD, Lopes-Virella MF, Waggott D, et al. Genome-wide association identifies the ABO blood group as a major locus associated with serum levels of soluble E-selectin. *Arterioscler Thromb Vasc Biol* 2009; 29: 1958-1967.
36. Bhatti M, Chapman P, Peters M, et al. Visualising E-selectin in the detection and evaluation of inflammatory bowel disease. *Gut* 1998; 43: 40-47.