Investigation of the Levels of Serum Amyloid A, YKL-40, and Pentraxin-3 in Patients with Familial Mediterranean Fever

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Background: Familial Mediterranean Fever (FMF) is an autosomal recessive form of recurrent episodes of fever and an autoinflammatory disease characterized by inflammation of the serous membranes. The clinical diagnosis is supported by the laboratory findings. This study investigated the relationship of Serum Amyloid A (SAA), YKL-40, and Pentraxin-3 (PTX-3) with the FMF disease. **Methods:** About 50 patients with FMF were enrolled in this study. Patients were divided into three groups according to disease severity score (mild, moderate, and severe). Thirty-seven healthy individuals were included as the control group. Serum SAA, YKL-40, and PTX-3 concentrations were measured using an ELISA kit. **Results:** Serum SAA and YKL-40 levels of FMF patients were significantly higher than in the control ($P < 0.001$).

Key words: Familial Mediterranean Fever; inflammation; PTX-3; Serum Amyloid A; YKL-40

PTX-3 levels were found to be higher in patients even though there was no significant difference ($P = 0.113$). Whereas the positive predictive value was 71.9% for cut-off point of SAA, the positive predictive value was 83.3% for cut-off point of YKL-40. Whereas a significant correlation was detected in SAA and PTX-3 with YKL-40 (respectively; $P = 0.036$, $P < 0.001$), there was no correlation between the PTX-3 with SAA ($P = 0.219$). **Conclusions:** YKL-40 can be used together with SAA to support the diagnosis of FMF and to monitor the severity of the disease. In this study, YKL-40 levels were examined for the first time in FMF patients and further studies are necessary using larger patient samples. J. Clin. Lab. Anal. 30:1158–1163, 2016. © 2016 Wiley Periodicals, Inc.

INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessive disease with recurrent episodes of fever, and inflammation in the peritoneum, synovium, or pleura, accompanied by pain and elevation in acute-phase reactants (1). FMF disease is frequently seen in Turkish, Jewish, Armenian, and Arab populations who belong to East Mediterranean (2). The pathogenesis of FMF disease is not well clarified; however, mutations in MEFV gene might have a critical role by leading to a functional distortion in pyrin protein (3).

Serum Amyloid A (SAA) is a highly conserved, acute-phase protein composed of 104 amino acids which is synthesized predominantly by the liver and monocytes/macrophages, smooth muscle cells, brain, and fibroblasts (4). Prolonged tissue damage and inflammation under the influence of interleukin-1

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Received 22 May 2015; Accepted 16 April 2016

DOI 10.1002/jcla.21997

Published online in Wiley Online Library (wileyonlinelibrary.com).

(IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α) causes an elevation in its plasma membrane within 24–48 h (5). Pirin protein with mutation stimulates interleukin-1 beta (IL-1 β) secretion through activating caspase-1. The increased IL-1 β stimulates the synthesis of C-reactive protein (CRP) and SAA particularly in the liver (6).

YKL-40, also called human cartilage glycoprotein-39 (HC gp-39), is an inflammatory glycoprotein composed of 383 amino acids with approximately 40 kD in size and is synthesized in response to endothelial damage. It is derived from the three N-terminal amino acids: tyrosine (Y), lysine (K), and leucine (L) (7). YKL-40 is secreted by various cell types including neutrophils, monocytes, macrophages, chondrocytes, fibroblast-like synovial cells, and separated in vascular smooth muscle cells particularly during inflammation, fibrosis, and cell destruction (8, 9). However, the exact function of YKL-40 remains unknown and many questions are waiting to be clarified (10). Recently, an ongoing research based on the clinical sense of YKL-40 suggested YKL-40 as a possible promising biomarker in acute and chronic diseases characterized by inflammation, remodeling of the extracellular matrix, and development of fibrosis (11).

Pentraxins are an evolutionarily conserved protein family with a pentraxin protein domain which has a multimeric structure and is involved in acute immunological responses and identified as acute-phase reactant proteins. Since the discovery of the five-subunit structure of CRP, these proteins are called as pentraxin (12). This pentameric superfamily of multifunctional conserved proteins having a carboxy terminal chain composed of 200 amino acids has two groups; the short pentraxins include Serum Amyloid P component (SAP) and C-reactive protein (CRP) and the long pentraxins include pentraxin-3 (PTX3) composed of 381 amino acids and there are several neuronal pentraxins (13). PTX-3 is a cytokine-modulated protein secreted rapidly by mononuclear phagocytes, neutrophils, fibroblasts, epithelial, and endothelial cells in response to inflammatory signals (ex; IL-1 ve TNF-a) (14).

In the current research, our aim is to investigate the relationship between serum SAA, YKL-40, PTX-3, CRP levels and sedimentation rate in FMF patients.

MATERIALS AND METHODS

Patients

About 50 patients with FMF diagnosis who were admitted to Turgut Ozal University Medical Faculty Hospital between May 2014 and November 2014 were included in this study. These patients are diagnosed with

FMF according to Tel-Hashomer criteria (15) and are in non-amyloidosis attack-free period; all of the patients were receiving colchicine at the time of admission.

Exclusion criteria are having diabetes mellitus, hypertension, thyroid disease, rheumatic diseases (rheumatoid arthritis, osteoarthritis, Behcet's disease, and gout), chronic kidney disease, coronary artery disease, cancer, alcohol and/or smoking, and taking any medication other than colchicine. The patients who had next attack within 1-week period were excluded from the study. Control group comprises 37 healthy patients with similar age and gender.

Patients were divided into three groups according to disease severity score of Pras et al. (16). Age of onset, monthly attack frequency, the presence of arthritis, erysipelas-like erythema presence, the presence of amyloidosis, and colchicine dose were evaluated according to this scoring system. Patients are classified as group 1 = mild, group 2 = moderate, and group 3 = severe.

The local ethics committee of our medical faculty approved the study and the study was conducted in accordance with the Helsinki declaration. Informed consent form was received from all the participants.

Laboratory Analysis

Serum CRP and sedimentation rate were studied on the day of blood collection and serum samples were kept frozen at -80°C until SAA, YKL-40, and PTX-3 in the assay. CRP levels and erythrocyte sedimentation rate (ESR) were examined by immunoturbidimetric method (Roche Diagnostics, Mannheim, Germany) and Westergren method (Vital Diagnostics, Forli, Italy), respectively, with available commercial kits. Serum SAA, YKL-40, and PTX-3 concentrations were measured with enzyme-linked immunoassay (ELISA) kits (for SAA: AssayPro, IL; for YKL-40 and PTX-3: Boster Immunoleader, CA).

Statistical Analysis

All data are expressed as mean $\pm$ standard deviation (SD) and the Kolmogorov–Smirnov normality test was applied to determine the distribution pattern of the variables. All results were normally non-distributed and we used non-parametric tests for statistical analysis. For comparison of binary groups Mann–Whitney *U*-test, for comparison of more than two groups Kruskal–Wallis ANOVA, and for the correlations between measurements Spearman correlation test were applied. The predictive diagnostic value of tests was determined by the receiver-operating characteristic (ROC) curve. The best cut-off value was determined as the value with the highest sensitivity and specificity.

The SPSS 15.0 (SPSS, Inc, Chicago, IL) statistical software package was used for statistical analysis. $P < 0.05$ was regarded as statistically significant.

RESULTS

Groups were composed of patients with similar age and gender ($P > 0.05$, for each). Serum CRP, ESR, SAA, and YKL-40 levels showed a remarkable increase in patients with FMF compared to healthy controls ($P < 0.001$). There is no statistically significant difference between patients and controls regarding PTX-3 levels ($P = 0.113$). Table 1 shows the results between patient and controls.

Group 3 patients showed statistically significant elevation in CRP and ESR levels compared to group 1 and group 2. The highest levels of SAA, YKL-40, and PTX-3 were observed in group 3 patients, although there is no statistically significant difference between the groups (Table 2).

There is no correlation between SAA and PTX-3. Other tests showed the presence of significant correlations ($P < 0.05$). Table 3 illustrates the correlation between test results.

The area under (AUC) the ROC curve for SAA is 0.719, and the cut-off value is 478.5 $\mu\text{g/ml}$ ($P < 0.001$, sensitivity 82%, specificity 56.8%, positive predictive value 71.9%). AUC the ROC curve for YKL-40 is 0.854 and the cut-off value is 169.85 pg/ml ($P < 0.001$, sensitivity 80%, specificity 78.4%, positive predictive value 83.3%). AUC of the ROC curve for PTX-3 was not statistically significant by 0.618 ($P = 0.066$). Figure 1 shows the ROC curves of the tests.

DISCUSSION

This study is a novel research which investigates serum YKL-40 and PTX-3 levels in comparison with serum SAA, CRP, and sedimentation rate in FMF

patients. According to our results, the positive predictive value for SAA was found to be 71.9%, whereas for YKL-40 it was found to be higher as 83.3%. In other words, YKL-40 test was suggested more useful than SAA in the diagnosis of FMF. No significant difference was observed in PTX-3 levels in the comparison of the patients with the healthy control subjects.

In an inflammatory disease, biomarkers can be applied to monitor disease activity and treatment intensity. Ideally, every disease entity should have a single biomarker, which is selected from data on threshold values, indicating the long-term risk of the development of disease-associated complications (17).

CRP, which is synthesized during inflammation in the liver with the impact of proinflammatory cytokines, is extensively measured acute-phase reactant in the investigation of FMF. Both CRP levels increased in 30–90% of patients with FMF during attack-free periods, even while they were receiving colchicine treatment (18, 19). In accordance with the CRP levels in this study, the novel outcome will appear in the literature as the statistically significant difference between the mild and moderate group and the severe group. Thereby, we concluded by stating that CRP will be for use in monitoring the inflammatory process, particularly in predicting the severity of the disease and in the follow-up.

The erythrocytes have a higher density than the plasma under normal circumstances. Therefore, erythrocytes sedimentation occurs in a test tube because of the gravity. During inflammation, the elevated acute-phase reactants adhere the erythrocytes and increase the sedimentation rate (20). The sedimentation rate is utilized in the follow-up of FMF patients and implied to be elevated particularly during the attacks and higher in between the attacks compared to healthy subjects (21, 22). During the examination of our sedimentation rate results, we found a statistical significance between the mild and moderate group and the severe group; this result is also very novel in the literature. We predict that the sedimentation rate would be used in monitoring the inflammatory process and particularly in the follow-up regarding the severity of the disease.

SAA is an acute-phase reactant which is produced a thousand fold higher than normal values in response to proinflammatory cytokines (IL-1, IL-6, and TNF- α) during FMF attacks (23, 24). It is known that even during the asymptomatic attack-free period, the subclinical inflammation endures and SAA levels remain high in patients with FMF (25). During inflammation, the elevated serum SAA levels play a crucial role in the progression of amyloidosis. The exact suppression of inflammation and the motorization of SAA levels between the attack periods are suggested to prevent the

TABLE 1. Comparison of results between patients and controls

Parameter	Patients ^a (n = 50)	Controls ^a (n = 37)	P*
Gender (F/M)	41/9	30/7	0.913
Age (years)	36.6 $\pm$ 10.6	36.8 $\pm$ 10.8	0.939
CRP (mg/l)	10.64 $\pm$ 13.16	1.20 $\pm$ 0.96	< 0.001
ESR (mm/h)	21.24 $\pm$ 15.23	6.62 $\pm$ 3.99	< 0.001
SAA ($\mu\text{g/ml}$)	2126.50 $\pm$ 1876.34	1154.78 $\pm$ 1375.15	< 0.001
YKL-40 (pg/ml)	846.36 $\pm$ 1033.81	151.03 $\pm$ 113.95	< 0.001
PTX-3 (pg/ml)	230.13 $\pm$ 78.69	223.43 $\pm$ 132.48	0.113

^aMean $\pm$ standard deviation, *The values in bold are statistically significant at $P < 0.05$, $P > 0.05$ statistically not-significant, CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; SAA, Serum Amyloid A; PTX-3, Pentraxin-3.

TABLE 2. Comparison of results between groups

Parameter	Group 1 ^a (n = 20)	Group 2 ^a (n = 17)	Group 3 ^a (n = 13)	P*	P**	P***
CRP (mg/l)	6.43 ± 9.1	8.01 ± 8.6	20.55 ± 18.2	0.557	< 0.001	0.002
ESR (mm/h)	14.55 ± 9.9	19.35 ± 16.4	34.06 ± 13.3	0.373	< 0.001	0.002
SAA (µg/ml)	2002.25 ± 1897.3	2056.44 ± 2052.8	2409.27 ± 1714.1	0.964	0.250	0.563
YKL-40 (pg/ml)	794.24 ± 742.4	817.42 ± 1059.1	964.38 ± 1405.3	0.598	0.813	0.742
PTX-3 (pg/ml)	222.95 ± 81.7	222.45 ± 56.2	252.98 ± 100.6	0.940	0.209	0.394

^aMean ± standard deviation; P*, group 1 vs. group 2; P**, group 1 vs. group 3; P***, group 2 vs. group 3; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; SAA, Serum Amyloid A; PTX-3, Pentraxin-3. The values in bold are statistically significant at P < 0.05.

TABLE 3. Correlation Results

Parameter	CRP (mg/l)	ESR (mm/h)	SAA (µg/ml)	YKL-40 (pg/ml)	PTX-3 (pg/ml)
CRP (mg/l)	–	$r = 0.725^a$, P < 0.001**	$r = 0.508^a$, P < 0.001**	$r = 0.577^a$, P < 0.001**	$r = 0.340^a$, P = 0.002**
ESR (mm/h)	$r = 0.725^a$, P < 0.001**	–	$r = 0.453^a$, P < 0.001**	$r = 0.445^a$, P < 0.001**	$r = 0.315^a$, P = 0.003**
SAA (µg/ml)	$r = 0.508^a$, P < 0.001**	$r = 0.453^a$, P < 0.001**	–	$r = 0.225^a$, P = 0.036**	$r = 0.135^a$, P = 0.219*
YKL-40 (pg/ml)	$r = 0.577^a$, P < 0.001**	$r = 0.445^a$, P < 0.001**	$r = 0.225^a$, P = 0.036**	–	$r = 0.525^a$, P < 0.001**
PTX-3 (pg/ml)	$r = 0.340^a$, P = 0.002**	$r = 0.315^a$, P = 0.003**	$r = 0.135^a$, P = 0.219*	$r = 0.525^a$, P = 0.001**	–

^ar, correlation coefficient; *P > 0.05, statistically not-significant; **The values in bold are statistically significant at P < 0.05, CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; SAA, Serum Amyloid A; PTX-3, Pentraxin-3.

improvement of amyloidosis (26). When SAA levels were examined in our study, SAA levels were found to be the highest in group 3; however, the comparison of groups showed no statistical significance. The elevation in SAA levels in accordance with the severity of the disease gave rise to a thought that this might be used for determination of the disease severity during follow-up. According to SAA results, it may also be used in monitoring the severity of FMF patients.

CRP, SAA, and ESR are significantly elevated during attacks. But also between attacks, a substantial degree of subclinical inflammation can be detected (e.g., increased CRP 34% and increased ESR in 52% of asymptomatic FMF patients (18)). In about 30% of patients with FMF, the laboratory markers of inflammation, including SAA, do not return to normal levels (26). During the selection process, we excluded the patients who could lead to an increase in case of acute-phase reactants (e.g., infectious diseases, autoimmune diseases, chronic metabolic diseases, cancer, drugs, smoking, and alcohols).

YKL-40 has been suggested to be a potential biomarker of inflammation and endothelial dysfunction. Increased circulating YKL-40 levels are found in patients with diseases characterized by inflammation and ongoing tissue remodeling such as inflammatory bowel disease, rheumatoid arthritis, asthma, cancer, liver fibrosis, chronic obstructive lung disease, diabetes,

and cardiovascular diseases (8). It was reported that monitoring multiple diseases with an inflammatory process can be done using YKL-40 levels (9). The relationship between the concentration of YKL-40 with the conventional parameters, proinflammatory cytokines, and sonographic examinations indicates that YKL-40 is associated with progression of inflammatory processes (27). The results of clinical studies in community-acquired pneumonia, active rheumatoid arthritis, obstructive sleep apnea syndrome, breast cancer, and human glioma have disclosed that YKL-40 serum levels are correlated with the severity of diseases (28–32).

This study is the first research which investigated YKL-40 levels in FMF patients in the literature. Comparison of YKL-40 levels between the patient group and the healthy control group revealed a statistically significant elevation. According to the results of the AUC, YKL-40 was identified as a better test for SAA and the PTX-3. In this study, we found that YKL-40 would be statistically deterministic in the diagnosis of FMF disease. We detected that the levels related to the diagnostic performance with respect to the best cut-off values were higher than SAA. In other words, when YKL-40 levels in a patient are higher than the present cut-off value, FMF should be considered and YKL-40 levels might be used as a more useful test than SAA in the diagnosis of FMF disease. As there was a positive correlation with CRP, sedimentation rate, PTX-3, and

Fig. 1. SAA, YKL-40, and PTX-3 ROC Curves. *AUC*, Area under the curve; *SAA*, Serum Amyloid A; *PTX-3*, Pentraxin-3.

SAA, we consider that it might support the other test results reflecting the inflammatory process in FMF disease and it might be used securely in reflecting the sub-clinical inflammation. On the other hand, we predicted that YKL-40 levels might be used in the follow-up as it increases with the severity of the disease. We believe that our study will lead to further research using large samples and will be initiative in enlightening the importance of YKL-40 in FMF disease.

PTX3, being synthesized by several tissues, is a non-specific acute-phase reactant increasing rapidly in case of inflammatory stimulation (33). The blood levels of PTX3 peak after 7–8 h following inflammatory stimulation and are recovered to normal in 3–5 days (34). Yuksel et al. reported that in patients with pediatric FMF, serum PTX-3 levels increased during the attacks of FMF and decreased during the attack-free period, whereas mean levels remain higher than healthy subjects (35). With respect to our results, there was no significant difference between the patients and the healthy control groups. When we evaluated our results, it was seen that PTX-3 levels do not differ significantly for determination of FMF disease diagnosis. PTX-3 increases hours after the acute stimulation and is recovered in days. We suggested that PTX-3 levels might only be used during the acute attacks in FMF patients. Further studies with larger samples comprising the patients in attack period are required.

CONCLUSIONS

Elevated serum YKL-40 concentrations in Turkish patients with FMF were shown in this report. The

correlation of YKL-40 and FMF severity and inflammation is proposed. A possible close relationship between YKL-40 and clinical outcomes of FMF may exist. As a result, in this study YKL-40 levels were investigated for the first time in FMF disease. It was determined that YKL-40 has a potential use in the diagnosis, treatment of FMF disease, and in the follow-up to determine severity; on the contrary, PTX-3 does not have this potential. Further studies are necessary with a larger sample of patients in the attack period.

ACKNOWLEDGMENT

Financial support for this study was provided by Turgut Ozal University Research Coordinator (sequence no: 1, project no: 015-04-2014).

REFERENCES

1. Lidar M, Livneh A. Familial Mediterranean fever: Clinical, molecular and management advancements. *Neth J Med* 2007;65:318–324.
2. Bhat A, Naguwa SM, Gershwin ME. Genetics and new treatment modalities for familial Mediterranean fever. *Ann N Y Acad Sci* 2007;1110:201–208.
3. Chae JJ, Aksentijevich I, Kastner DL. Advances in the understanding of familial Mediterranean fever and possibilities for targeted therapy. *Br J Haematol* 2009;146:467–478.
4. Bakkaloglu A. Familial Mediterranean fever. *Pediatr Nephrol* 2003;18:853–859 Epub 2003 Jun 27.
5. Westermark GT, Westermark P. Serum amyloid A and protein AA: Molecular mechanisms of a transmissible amyloidosis. *FEBS Lett* 2009;583:2685–2690 Epub 2009.
6. Bilginer Y, Bakkaloglu A. Ailevi Akdeniz Atesi ve Amiloidoz. *Turkiye Klinikleri J Int Med Sci* 2006;2:33–39.
7. Vos K, Steenbakkens P, Miltenburg AM, et al. Raised human cartilage glycoprotein-39 plasma levels in patients with rheumatoid arthritis and other inflammatory conditions. *Ann Rheum Dis* 2000;59:544–548.
8. Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? *Cancer Epidemiol Biomarkers Prev* 2006;15:194–202.
9. Rathcke CN, Vestergaard H. YKL-40—an emerging biomarker in cardiovascular disease and diabetes. *Cardiovasc Diabetol* 2009;8:61.
10. Volck B, Ostergaard K, Johansen JS, Garbarsch C, Price PA. The distribution of YKL-40 in osteoarthritic and normal human articular cartilage. *Scand J Rheumatol* 1999;28:171–179.
11. Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. *Dan Med Bull* 2006;53:172–209.
12. Ablij H, Meinders A. C-reactive protein: History and revival. *Eur J Intern Med* 2002;13:412.
13. Cieřlik P, Hrycek A. Pentraxin 3 as a biomarker of local inflammatory response to vascular injury in systemic lupus erythematosus. *Autoimmunity* 2015;48:242–250.
14. Liu S, Qu X, Liu F, Wang C. Pentraxin 3 as a prognostic biomarker in patients with systemic inflammation or infection. *Mediators Inflamm* 2014;2014:421429 Epub 2014.

15. Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum* 1997;40:1879–1885.
16. Pras E, Livneh A, Balow JE, et al. Clinical differences between North African and Iraqi Jews with familial Mediterranean fever. *Am J Med Genet* 1998;75:216–219.
17. Erer B, Demirkaya E, Ozen S, Kallinich T. What is the best acute phase reactant for familial Mediterranean fever follow-up and its role in the prediction of complications? A systematic review *Rheumatol Int* 2016;36:483–487.
18. Korkmaz C, Ozdogan H, Kasapcopur O, Yazici H. Acute phase response in familial mediterranean fever. *Ann Rheum Dis* 2002;61:79–81.
19. Ben-Zvi I, Livneh A. Chronic inflammation in FMF: Markers, risk factors, outcomes and therapy. *Nat Rev Rheumatol* 2011;7:105–112.
20. Levay PF, Retief JH. Causes of high erythrocyte sedimentation rates in an inpatient population. *S Afr Med J* 2005;95:45–46.
21. Örün E, Yalcinkaya F. Familial Mediterranean Fever and amiloidosis in Turkish Medicine. *Journal of the Turkish Society of Nephrology* 2003;12:1–7.
22. Düzoa A, Ozen S. Ailevi Akdeniz Atesinin Kliniği ve Tanısı. *Türkiye Klinikleri J Int Med Sci* 2006;2:12–20.
23. Erdoğan Ö, Öner A. Ailevi Akdeniz Atesi. *T Klin Pediatrı* 2002;11:160–170.
24. Odabas AR. Amiloidozis ve Böbrek Tutulumu. *Türkiye Klinikleri J Nephrol* 2004;2:52–56.
25. Patel H, Bramall J, Waters H, De Beer MC, Woo P. Expression of recombinant human serum amyloid A in mammalian cells and demonstration of the region necessary for high-density lipoprotein binding and amyloid fibril formation by site-directed mutagenesis. *Biochem J* 1996;318(Pt 3):1041–1049.
26. Berkun Y, Padeh S, Reichman B, et al. A single testing of serum amyloid a levels as a tool for diagnosis and treatment dilemmas in familial Mediterranean fever. *Semin Arthritis Rheum* 2007;37:182–188 Epub 2007.
27. Kazakova M, Sarafian V. YKL-40 in health and disease: A challenge for joint inflammation. *Biomedical Reviews* 2013;24:49–56.
28. Wang HL, Hsiao PC, Tsai HT, Yeh CB, Yang SF. Usefulness of plasma YKL-40 in management of community-acquired pneumonia severity in patients. *Int J Mol Sci* 2013;14:22817–22825.
29. Turkyilmaz AK, Devrimel G, Kirbas A, et al. Relationship between pulse wave velocity and serum YKL-40 level in patients with early rheumatoid arthritis. *Rheumatol Int* 2013;33:2751–2756.
30. Wang X, Xing GH. Serum YKL-40 concentrations are elevated and correlated with disease severity in patients with obstructive sleep apnea syndrome. *Scand J Clin Lab Invest* 2014;74:74–88.
31. Wang D, Zhai B, Hu F, Liu C, Zhao J, Xu J. High YKL-40 serum concentration is correlated with prognosis of Chinese patients with breast cancer. *PLoS ONE* 2012;7:e51127.
32. Iwamoto FM, Hottinger AF, Karimi S, et al. Serum YKL-40 is a marker of prognosis and disease status in high-grade gliomas. *Neuro Oncol* 2011;13:1244–1251.
33. Mantovani A, Garlanda C, Doni A, Bottazzi B. Pentraxins in innate immunity: From C-reactive protein to the long pentraxin PTX3. *J Clin Immunol* 2008;28:1–13 Epub 2007.
34. Mauri T, Coppadoro A, Bellani G, et al. Pentraxin 3 in acute respiratory distress syndrome: An early marker of severity. *Crit Care Med* 2008;36:2302–2308.
35. Yuksel S, Karadagli E, Evrengul H, Senol H. Could pentraxin-3 be a new marker for subclinical inflammation in familial Mediterranean fever? *Pediatric Rheumatology* 2015;13 (Suppl 1):P98.