The relationship between serum calprotectin levels and disease activity in patients with subacute thyroiditis

H. CENGIZ[1], T. DEMIRCI[1], C. VARIM[2], E. GÖNÜLLÜ[3]

[1]Department of Endocrinology, Sakarya University Medicine Faculty, Sakarya, Turkey
[2]Department of Internal Medicine, Sakarya University Medicine Faculty, Sakarya, Turkey
[3]Department of Rheumatology, Sakarya University Medicine Faculty, Sakarya, Turkey

Abstract. – **OBJECTIVE:** Increased calprotectin (S100A8/A9) levels have been demonstrated in many acute and chronic inflammatory processes. Subacute thyroiditis is an inflammatory disease of the thyroid gland. In our study, we investigated the value of this inflammation marker in the diagnosis and follow-up of subacute thyroiditis.

PATIENTS AND METHODS: Patients with subacute thyroiditis admitted to our clinic between November 2018 and January 2020 were included in the study. In the acute phase of the disease, fT4 (free thyroxin), TSH (Thyroid Stimulant Hormone), CRP (C Reactive Protein), ESR (Erythrocyte Sedimentation Rate), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), Creatinine, WBC (White Blood Cell), Absolute Lymphocyte and Neutrophil Count (ALC, ANC) parameters were detected and recorded. After complete resolution of the disease, the same laboratory parameters and acute phase reactants were again detected. Additionally, Calprotectin determination was performed in the acute phase and recovery period. Persistent hypothyroidism was determined by 6th-month TSH levels.

RESULTS: Thirty-six patients were included in the study. Along with the classical acute phase reactants and ANC, there was a significant increase in the Calprotectin levels in the acute inflammatory phase of the disease compared to the recovery period (96. 92-37.98, $p<0.001$). Neither classical acute phase reactants and nor calprotectin were found to have a significant effect on the development of permanent hypothyroidism. Calprotectin did not correlate with other acute phase reactants, absolute neutrophil count and TSH levels in both the acute phase and resolution period.

CONCLUSIONS: Calprotectin appears to be an important marker in the diagnosis and follow-up of subacute thyroiditis.

Key Words:
 Subacute Thyroiditis, Calprotectin, S100A8/A9.

Introduction

Subacute Granulomatous Thyroiditis (De Quervain Thyroiditis) is an acute painful inflammation of the thyroid gland. It was first described in 1904 by Fritz De Quervain. The etiology of the disease remains unclear. It usually appears after a viral upper respiratory tract infection, especially in spring and autumn. Although it is claimed that there may be factors such as coxsackievirus, adenovirus and influenzavirus in its etiology, these have not been verified yet[1-3].

There are no internationally established consensus criteria for the diagnosis of subacute granulomatous thyroiditis. Diagnosis is made by combining clinical and laboratory findings[1,4]. The disease has a prominent inflammatory presentation led by fever and neck pain. In the laboratory subclinical or overt thyrotoxicosis due to thyroid destruction and accompanying high ESR and CRP levels prevail. ESR levels are usually higher than 50 mm/h, and most often around 100 mm/h. Pathological findings include the destruction of thyroid follicular cells; infiltration of polymorphonuclear leucocytes in the acute period; mononuclear cells in the subacute period; and giant cell granulomatous inflammation. In severe follicular destruction, permanent hypothyroidism develops at a rate of about 5%[4].

Calprotectin is also known as S100A8/A9 and (myeloid related protein) MRP8/14 or Calgranulin A/B. It is found in plasma as S100A8/A9 heterodimeric complex. It is a cytosolic protein in neutrophils, monocytes, and macrophages under normal conditions. In case of an inflammatory stimulus, it migrates to the cell membrane and acts together with the cytoskeleton. Calprotectin is involved in many proinflammatory processes such as che-

motaxis, neutrophil adhesion, inflammatory cytokine release and apoptosis stimulation. It is also released from stimulated neutrophils and myelocytes into the circulation and has direct bactericidal and fungicidal effects. In addition to physiological processes such as wound healing, these proteins have been shown to be associated the activity of many acute and chronic inflammatory processes[5-8].

The inflammatory features of subacute granulomatous thyroiditis are clear in terms of clinical, laboratory and pathological aspects. However, the inflammatory protein calprotectin has not yet been studied in this disease. We think that this protein would be useful in the diagnosis and follow-up of subacute granulomatous thyroiditis and may correlate with the degree of thyroid damage and permanent hypothyroidism. Our aim is to investigate the usability of this marker in this disease.

Patients and Methods

Thirty-six patients with subacute granulomatous thyroiditis admitted to Sakarya University Education and Research Hospital Endocrinology and Metabolism Outpatient Clinic between November 2018 and January 2020 were included the study. Diagnosis of subacute thyroiditis was made by investigating the acute clinical signs and symptoms (high fever, weakness, neck pain, muscle and joint pain and palpitation), measuring high ESR and CRP levels and accompanying suppressed TSH and high or normal fT3 and fT4 levels in the laboratory, and determining common hypoechogenic pseudo nodular areas compatible with subacute thyroiditis via ultrasonography.

All included patients were over 18 years old. Demographic data; fT4 (free thyroxin); TSH (Thyroid Stimulant Hormone); CRP (C Reactive Protein); ESR (Erythrocyte Sedimentation Rate); ALT (Alanine Aminotransferase); AST (Aspartate Aminotransferase); Creatinine; WBC (White Blood Cell), Absolute Neutrophil Count (ANC); and Absolute Lymphocyte Count (ALC) parameters were recorded. After complete resolution of the disease by proper treatment, the same laboratory parameters and acute phase reactants were again measured by the same methods to check correlation with disease activity. Thyroid function tests (fT4, TSH) were used to detect permanent hypothyroidism at the six-month follow-up. In addition, blood samples were collected

and stored for the determination of Calprotectin during the acute and resolution phases of the disease.

Patients with acute or chronic infections; inflammatory and rheumatic diseases that may affect blood parameters and acute phase reactants; those who had used steroids in the last two months; undergone major surgery in the past three months; pregnant women; and those with chronic renal failure, chronic liver disease, congestive heart failure, hematological malignancy or solid organ malignancy, were excluded from the study. Although an association between inflammatory bowel disease and subacute thyroiditis has been demonstrated at the case level in few publications[9], there is no accepted predisposition. Faecal calprotectin was not studied, as accompanying inflammatory bowel disease was among our exclusion criteria.

Informed Consent and Ethics Approval

Informed consent was obtained from all patients participating in the study. Local Ethics Committee approval was obtained from the Sakarya University Local Ethics Committee (Approval Date and No:21.11.2018-16214662/050.01.04/96).

Laboratory Analyses

Blood samples were obtained after eight hours of fasting in the morning during diagnosis and follow-up, sent to the laboratory immediately and centrifuged at 2000 rpm for 15 minutes. Biochemical parameters were investigated using a Beckman Coulter® AU680 (Brea, CA, USA) with Beckman Coulter kits. Hemogram parameters examined via a WIC-LYSE for CELL DYN 3700 Kits on the Abbott Cell-Dyn® 3700, Abbott Park, (IL, USA) device. Erythrocyte Sedimentation Rate was performed with Rapida ESR100® in capillary tubes. C Reactive Protein parameter was studied with SIEMENS BNII® (Berlin, Germany) with Cardio Phase hsCRP WN® kits. TSH and fT4 parameters were studied via an Abbott Architect I 2000 SRIL. USA® device with available commercial kits. Ultrasonographic scans were performed by the same author using a GE Medical Systems Logiq® E9 6–15 MHz linear probe (Milwaukee, WI, USA).

To measure calprotectin levels, each blood sample was collected in dry tube in the morning after 8 hours of fasting and centrifuged at 2000 rpm for 15 minutes and stored in an -80°C freezer until laboratory determination. The blood samples were sent to the laboratory under cold chain

conditions. Calprotectin studied by Microplate Reader RT® 2100 C and Microplate Washer RT® 2600 C devices with the appropriate commercial kits using the Micro ELISA method at 450 nm wavelength light. Intraassay variability was<10%.

Statistical Analysis

In the sample size calculation, power analysis was performed using Cohen's standard effect sizes. When the effect size was taken as 0.5, Type 1 error (α) 0.05 and type 2 error (β) 0.20, the required sample size was calculated as at least 34 patients.

Data analysis was performed by using SPSS-22 for Windows (Statistical Package for Social Science®, SPSS Inc. Chicago IL, USA). The variables were investigated using visual (histograms, probability plot) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they were normally distributed. We performed analyses to describe and summarize the distributions of variables. The continuous variables were expressed in terms of mean and standard deviation or as median and interquartile range, depending on the normality of their distribution. In two different periods of the disease, the Wilcoxon test was preferred to compare non-parametric variables, while a paired Student's t-test was used for variables with normal distribution. Logistic regression was conducted to assess whether the predictor variables, such as certain inflammatory markers, significantly predict permanent hypothyroidism. Since calprotectin variable did not show normal

distribution, the correlation coefficients and their significance were calculated using a Spearman test. The statistically significant two tailed *p*-value was considered as <0.05.

Results

Thirty-six subacute thyroiditis patients were included in the study. Twenty-seven (80.6%) were female and 9 (19.4%) were male. The mean age of the patients was 44.1 (± 8.8) years. TSH, fT4, ESR, CRP, WBC, ANC and ALC values were compared in the acute inflammatory period and recovery period of the disease. TSH, fT4, ESR, CRP, WBC and ANC levels were statistically significantly different between the two periods; however, there was no difference between ALC results. Analysis results for both baseline characteristics and between the two periods are summarized in Table I.

Calprotectin levels were compared between the two periods of the disease. The median levels of calprotectin were 96. 92 ng/ml (IQR: 24. 47-130. 37) in the acute inflammatory period and 37. 98ng/ml (14.02-20. 52) during the recovery period (*p*<0.001) (Table II).

Possible effects of inflammatory parameters in acute phase of disease on the development of permanent hypothyroidism were evaluated via logistic regression analysis. The following parameters were analyzed: Calprotectin (OR = 1.003, CI 95%: 0. 994-1.011, *p* = 0. 544); ESR (OR = 1.018, CI 95%: 0. 967-1.073, *p* = 0. 474); CRP (OR = 0. 991, CI 95%: 0. 951-1.032, *p* = 0. 654); WBC (OR

Table I. Real time PCR primers.

	Baseline*(acute inflammatory phase)	Recovery phase of the disease*	*p*-value
Women/Men (%)	**27/9** (80.6/19.4)		
Age (years)	**44.1** (± 8.8)	-	–
ALT (IU/L)	**19.5** (15.00-32.25)	-	–
AST (IU/L)	**19.5** (16.00-24.00)	-	–
Creatinine (mg/dL)	0.56 (± 0.13)	-	–
ESR (mm/h)	**81.17** (± 23.19)	**19.00** (13.0-26.75)	**< 0.001**
CRP (mg/L)	**31.05** (17.62-46.12)	**3.23** (3.23-4.16)	**< 0.001**
WBC (10^3/mm³)	**8.27** (± 2.44)	**6.72** (± 1.85)	**<0.001**
ANC (10^3/mm³)	**5.31** (± 2.00)	**3.73** (± 1.44)	**<0.001**
ALC (10^3/mm³)	**2.20** (± 0.70)	**2.28** (± 0.59)	0.446
TSH (μU/L)	**0.010** ([< 0.001]-0.037)	**3.51** (± 2.69)	**< 0.001**
fT4 (pmol/L)	**19.13** (15.59-31.37)	**11.33** (10.49-12.4l)	**< 0.001**

ALT; alanine aminotransferase, AST; aspartate aminotransferase, ESR; erythrocyte sedimentation rate, CRP; C-reactive protein, WBC; white blood cells, ANC; absolute neutrophil count, ALC; absolute lymphocyte count, TSH; thyroid stimulating hormone, fT4; free-T4 (thyroxine). *Descriptive results for continuous variables were expressed as mean and standard deviation or as median and interquartile range, depending on the normality of their distribution.

Table II. Calprotectin levels in the acute and recovery period of the disease.

	Acute inflammatory phase of the disease	Recovery phase of the disease	*p*-value
Calprotectin (ng/ml)	96.92	37.98	< 0.001

= 1.071, CI 95%: 0. 321-3. 572, *p* = 0. 911); and ANC (OR = 0. 801, CI 95%: 0. 190-3.380. *p* = 0. 763). None of the variables had a statistically significant effect on the development of permanent hypothyroidism (Table III).

Correlation analysis was performed for inflammatory parameters between both acute and recovery period of disease. No significant correlations were found between Calprotectin levels and other markers such as ESR, CRP, WBC, ANC and ALC and TSH levels in either the acute or recovery period. These findings are summarized in Table IV.

Discussion

Subacute granulomatous thyroiditis is a relatively common and acute inflammatory disease of the thyroid. Its prevalence varies between 3-12/100 000. It constitutes 5% of clinically apparent thyroid diseases. The disease is more common in women, with an F/M ratio of around 4-7/ 1[1,4].

Diagnosis of subacute granulomatous thyroiditis is typically clinical. It generally progresses with acute developing prominent inflammation. High acute phase reactants are typically detected in patients with appropriate symptomatology. Using thyroid scintigraphy, iodine and technetium uptakes are seen to decrease. In ultrasonography, diffuse enlargement of the thyroid gland and hypoechoic pseudo nodular patchy shadows are detected[4].

While the immunological infrastructure of the disease has not yet been fully elucidated, it is thought that cellular immunity plays a major role. Thyroid autoimmunity does not appear to play a primary role in the disorder. Although anti-TPO and Anti-Tg positivity are common, this has been shown to be a result of the destruction of thyroid follicular cells. HLA-B35 positivity is present in 70% of patients. In addition, recent studies have shown an association with the HLA-B * 18: 01 and DRB1 * 01 subgroups[4,10-12]. The definite etiopathogenesis has not yet been elucidated. However, it is now clear that there is a clinical, laboratory and pathologically intense inflammatory burden in this disease.

Calprotectin, also known as S100A/B heterodimer complex or MRP8/14, was first isolated from granulocytes by Fagerhol and Dale in 1980[5]. It is a member of the S100 protein family. The term S100 comes from the solubility of this proteins in 100% ammonium sulphate. Currently, more than 20 proteins have been identified in this group[6-8]. It was originally described as an antibacterial and antifungal molecule, and is found in polymorphonuclear leukocytes, monocytes and macrophages, but not in lymphoid series. It can also be detected in various tissues such as osteoclasts epidermal cells and microvascular endothelium in some special situations and serves as an important mediator of the innate immune system. It forms an average of 40% (30-60%) of the cytosolic molecules in neutrophiles. The heterodimer complex has calcium (Ca^{++}) and zinc (Zn) binding sites. In case of an inflammatory stimulus, intracellular

Table III. The results of logistic regression analysis*.

Independent variables	Odds ratio (OR)	(% 95 confidence interval)	*p*-value
Calprotectin	1.003	0.994-1.011	0.544
ESR	1.018	0.967-1.073	0.474
CRP	0.991	0.951-1.032	0.654
WBC	1.071	0.321-3.572	0.911
ANC	0.801	0.190-3.380	0.763

ESR; erythrocyte sedimentation rate, CRP; C-reactive protein, WBC; white blood cells, ANC; absolute neutrophil count. *The dependent variable is the presence of permanent hypothyroidism. 0: no permanent hypothyroidism, 1: permanent hypothyroidism exists.

Table IV. The correlation of serum calprotectin levels with other parameters.

	Acute inflammatory phase of the disease		Recovery phase of the disease	
	r	*p*	*r*	*p*
ESR	0.017	0.921	-0.089	0.605
CRP	0.036	0.836	-0.084	0.627
WBC	-0.024	0.888	-0.316	0.061
ANC	-0.055	0.751	-0.260	0.126
ALC	-0.070	0.684	-0.159	0.354
TSH	-0.076	0.661	0.098	0.570

ESR; erythrocyte sedimentation rate, CRP; C-reactive protein, WBC; white blood cells, ANC; absolute neutrophil count, ALC; absolute lymphocyte count, TSH; thyroid stimulating hormone.

Ca++level rises and Ca++ binds the Ca binding site and the molecule is activated. After activation, cytosolic proteins are carried into the membrane and act together with the cytoskeleton. They are also released from activated neutrophils[6,13].

Calprotectin has many defined tasks in the inflammation cascade. Neutrophil and monocyte migration, chemotaxis, stimulation of pro-inflammatory cytokines such as IL-1, IL-6, TNFα, proapoptotic effects, and direct antifungal and antibacterial effects are some of these functions. Therefore, its levels also increase in case of infection and wound healing after surgery[6,8,14].

Calprotectin has been studied in many acute and chronic inflammatory diseases and even malignancies, in addition to infectious processes. It has been found to be correlated with disease activity and these correlations were found to be more significant from classical acute phase markers most of rheumatologic diseases such as rheumatoid arthritis, ankylosing spondylitis, Behcet's disease, and vasculitis[7,14]. In addition, it has been found that faecal calprotectin correlated very well with the intestinal activity of inflammatory bowel disease and Behcet's disease; thus, it can be used to determine the response to treatment[15,16].

Calprotectin has also been studied in the context of many types of cancer, including lung cancer, hepatocellular cancer, colorectal carcinomas, breast, and prostate cancers. Increased serum levels of Calprotectin have been found along with S100A8/A9 gene amplifications in cancerous tissues[5,10]. In three studies on thyroid cancer, an increase inS100A8/A9 has been shown to be associated with dedifferentiation and metastatic tendency[17-19]. In another study conducted in Turkey[20], calprotectin serum levels were found to be significantly higher in the preoperative phase than in the postoperative phase and control group

among patients with thyroid papillary carcinoma.

Calprotectin has also been studied in many endocrinological and metabolic entities. There have been numerous studies relating to obesity, insulin resistance, diabetes mellitus, diabetic microvascular complications, and polycystic ovary syndrome. In these investigations, calprotectin has been shown to be associated with disease activity, insulin resistance and complication rates[21-26].

Calprotectin has also been analyzed in Graves ophthalmopathy[27]. Blood Calprotectin levels have been found to be high and S100A8/A9 mRNA expression found to be elevated in orbital fibroblasts. Also, the level of Calprotectin has been found to be correlated with thyroid stimulating immunoglobulin levels and graves ophthalmopathy activity score. In the second study conducted by Korkmaz et al[28], S100A8/A9 levels were found to be significantly higher than the baseline population among Graves and Hashimoto thyroiditis patients and are correlated with other oxidative markers.

In our literature search, we have not encountered calprotectin studies in patients with subacute thyroiditis. In our study, in the acute inflammatory period of the disease, a significant increase was observed in calprotectin level, together with ESR, CRP, WBC and ANC levels, and its levels decreased significantly during the recovery period. In many previous studies calprotectin has been shown to be more correlated with disease activity in inflammatory diseases than classical acute phase reactants. For example, in rheumatoid arthritis, serum and synovial calprotectin levels have been found to be more correlated with disease activity and permanent joint damage than CRP[8]. In addition, fecal Calprotectin level is more valuable than classical acute phase markers in the activation of Beh-

cet enteritis and inflammatory bowel diseases[16]. Based on these data, we attempted to determine the relationship of this marker with disease activity in our subacute thyroiditis patients. Our marker was found to be correlated with disease activity as well as classical acute phase reactants. However, no correlation was found between calprotectin and other inflammatory parameters and thyroid hormone levels during both the acute and resolution periods.

In our previous study, there was a positive correlation between permanent hypothyroidism and acute phase ESR levels[1], while in our current study, neither calprotectin nor ESR levels were found to associated with permanent hypothyroidism. Our sample size was small and the number of patients who develop permanent hypothyroidism is also very low. This may be why we could not demonstrate the predictive effects of calprotectin on permanent hypothyroidism.

Some studies have shown that this tissue destructive effect of calprotectin may be limited by zinc chelation[8,13]. It remains to be studied whether patients may benefit from zinc replacement in addition to conventional treatment, and whether the level of permanent hypothyroidism would decrease.

The main limitations of our study are that it was a single-centered study; the number of patients was low; the follow-up time was limited; and there was no separate control group. However, our study showed that calprotectin can be used as an additional activity marker in subacute thyroiditis, especially in the active inflammatory phase. Additionally, it may be a target molecule in disease treatment. Larger randomized controlled trials are needed to demonstrate its relationship with persistent tissue damage and permanent hypothyroidism, and to determine whether it should be a treatment goal to reduce the inflammatory load.

Conclusions

In our study, a significant increase was observed in the acute inflammatory period of the disease with increased calprotectin levels together with ESR, CRP, WBC and ANC, while these values decreased significantly during the recovery period. We were unable to demonstrate the effects of calprotectin and other inflammatory markers on persistent hypothyroidism. No correlations were found between calprotectin and other inflammatory parameters and thyroid hormone levels during both the acute and resolution periods. While our study has a limited number of patients, we were able to demonstrate the value of calprotectin levels in diagnosing subacute thyroiditis, especially in the acute period.

ORCID
H. Cengiz 0000-0002-5216-3368; T. Demirci 0000-0002-9579-4530; C. Varim 0000-0002-8369-0857; E. Gönüllü 0000-0002-6990-4206.

References

1) Cengiz H, Varim C, Demirci T, Cetin S. hemogram parameters in the patients with subacute thyroiditis. Pak J MedSci 2020; 36: 240-245.

2) Martino E, Buratti L, Bartalena L, MariottiS, Cupini C, Aghini-Lombardi F, Pinchera A. High prevalence of subacute thyroiditis during summer season in Italy. J Endocrinol Invest 1987; 10: 321-323.

3) Desailloud R, Hober D. Viruses and thyroiditis: an update. Virol J 2009; 6: 5.

4) Burman KD, Baker JR. Subacute thyroiditis uptodate clinicalreview. Online available at http://www.uptodate.com Accessed at April 2020.

5) Fagerhol MK, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. Bull Eur Physiopathol Respir 1980; 16: 273-282.

6) Stríz I, Trebichavský I. Calprotectin - a pleiotropic molecule in acute and chronic inflammation. Physiol Res 2004; 53: 245-253.

7) Kopeć-Mędrek M, Widuchowska M, Kucharz EJ. Calprotectin in rheumatic diseases: a review. Reumatologia 2016; 54: 306-309.

8) Ehrchen JM, Sunderkötter C, Foell D, Vogl T, Roth J. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J Leukoc Biol 2009; 86: 557-566.

9) Shizuma T. Concomitant thyroid disorders and inflammatory bowel disease: a literature review. Biomed Res Int 2016; 2016: 5187061.

10) Volpé R, Row VV, Ezrin C. Circulating viral and thyroid antibodies in subacute thyroiditis. J Clin Endocrinol Metab 1967; 27: 1275-1284.

11) Stasiak M, Tymoniuk B, Stasiak B, Lewiński A. The risk of recurrence of subacute thyroiditis is HLA-dependent. Int J Mol Sci 2019; 20: 1089.

12) Stasiak M, Tymoniuk B, Michalak R, Stasiak B, Kowalski ML, Lewiński A. Subacute thyroiditis is associated with HLA-B*18:01, -DRB1*01 and -C*04:01-the significance of the new molecular background. J Clin Med 2020; 9: 534.

13) Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biol Pharm Bull 2003; 26: 753-760.

14) Gebhardt C, Németh J, Angel P, Hess J. S100A8 and S100A9 in inflammation and cancer. Biochem Pharmacol 2006; 72: 1622-1631.

15) Hatemi G, Esatoglu SN, Yazici Y. Biomarkers in vasculitis. Curr Opin Rheumatol 2018; 30: 30-35.

16) Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. Clin Exp Gastroenterol 2016; 9: 21-29.

17) Ito Y, Arai K, Ryushi, Nozawa, Yoshida H, Tomoda C, Uruno T, Miya A, Kobayashi K, Matsuzuka F, Kuma K, Kakudo K, Miyauchi A. S100A9 expression is significantly linked to dedifferentiation of thyroid carcinoma. Pathol Res Pract 2005; 201: 551-556.

18) Ito Y, Arai K, Nozawa R, Yoshida H, Hirokawa M, Fukushima M, Inoue H, Tomoda C, Kihara M, Higashiyama T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Miyauchi A. S100A8 and S100A9 expression is a crucial factor for dedifferentiation in thyroid carcinoma. Anticancer Res 2009; 29: 4157-4161.

19) Reeb AN, Li W, Sewell W, Marlow LA, Tun HW, Smallridge RC, Copland JA, Spradling K, Chernock R, Lin RY. S100A8 is a novel therapeutic target for anaplastic thyroid carcinoma. J Clin Endocrinol Metab 2015; 100: E232-242.

20) Tabur S, Korkmaz H, Özkaya M, Elboğa U, Tarakçıoglu M, Aksoy N, Akarsu E. Serum calprotectin: a new potential biomarker for thyroid papillary carcinoma. Tumour Biol 2015; 36: 7549-7556.

21) Chen S, Jiang M, Ding T, Wang J, Long P. Calprotectin is a potential prognostic marker for polycystic ovary syndrome. Ann Clin Biochem 2017; 54: 253-257.

22) Calcaterra V, De Amici M, Leonard MM, De Silvestri A, Pelizzo G, Buttari N, Michev A, Leggio M, Larizza D, Cena H. Serum calprotectin level in children: marker of obesity and its metabolic complications. Ann Nutr Metab 2018; 73: 177-183.

23) Ortega FJ, Sabater M, Moreno-Navarrete JM, Pueyo N, Botas P, Delgado E, Ricart W, Frühbeck G, Fernández-Real JM. Serum and urinary concentrations of calprotectin as markers of insulin resistance and type 2 diabetes. Eur J Endocrinol 2012; 167: 569-578.

24) Burkhardt K, Schwarz S, Pan C, Stelter F, Kotliar K, Von Eynatten M, Sollinger D, Lanzl I, Heemann U, Baumann M. Myeloid-related protein 8/14 complex describes microcirculatory alterations in patients with type 2 diabetes and nephropathy. Cardiovasc Diabetol 2009; 8: 10.

25) Abu El-Asrar AM, Alam K, Siddiquei MM, Van den Eynde K, Mohammad G, De Hertogh G, Opdenakker G. Myeloid-related protein-14/MRP-14/S100A9/calgranulin B is associated with inflammation in proliferative diabetic retinopathy. Ocul Immunol Inflamm 2018; 26: 615-624.

26) Tabur S, Korkmaz H, Ozkaya M, Aksoy SN, Akarsu E. Is calprotectin a novel biomarker of neuroinflammation in diabetic periferal neuropathy? Diabetol Metab Syndr 2015; 7: 36.

27) Kim JW, Ko J, Kim J, Yoon JS. Proinflammatory effects of calprotectin in Graves' orbitopathy. Ocul Immunol Inflamm 2020; 28: 156-163.

28) Korkmaz H, Tabur S, Savaş E, Özkaya M, Aksoy ŞN, Aksoy N, Akarsu E. Evaluation of serum S100A8/S100A9 levels in patients with autoimmune thyroid diseases. Balkan Med J 2016; 33: 547-551.