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Original Article

Serum levels of inflammatory markers in patients with thyroid dysfunction and their association with autoimmunity status

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Abstract: Introduction: Autoimmune inflammation plays an important role in several types of thyroid dysfunctions such as Hashimoto's thyroiditis and Graves' disease. In the present study, the levels of serum inflammation markers and their association with thyroid functions were assessed in patients with thyroid dysfunction. Materials and methods: 86 hypothyroid, 67 hyperthyroid patients and 59 healthy volunteers were included in the study. All patients with thyroid dysfunctions were also divided into two groups as autoimmune and non-autoimmune. Erythrocyte sedimentation rate (ESR) and mean platelet volume (MPV) were measured along with C-reactive protein (CRP) and pro-calcitonin (PCT) levels. Results: While ESR, CRP and PCT were significantly elevated in patients with hypothyroidism and hyperthyroidism, MPV was significantly decreased in both groups. Serum levels of ESR and PCT were significantly elevated in patients with autoimmune and non-autoimmune thyroid disease compared to the control group. Mean platelet volume was significantly decreased in hypothyroidism, hyperthyroidism and non-autoimmune groups compared to the control group. Conclusion: Significant changes in the levels of inflammation markers for both autoimmune and non-autoimmune thyroid disorders observed in current study confirm that inflammation has an important role on pathogenesis of thyroid dysfunctions regardless of their thyroid dysfunction type.

Keywords: Thyroid dysfunction, inflammation, erythrocyte sedimentation rate, C-reactive protein, pro-calcitonin, mean platelet volume

Introduction

Acute-phase reactants (APRs) are known with their involvement as pro-inflammatory molecules in various inflammatory diseases. Most of the APRs generally elevate during various immunologically mediated conditions such as infection, trauma, surgery, burns, tissue infarction, or cancer [1]. They are being used as clinical markers in the diagnosis and management of some diseases, since they reflect the presence and intensity of inflammation. However, according to the recent studies some of the APRs might have also pro-inflammatory properties [2]. C-reactive protein (CRP), ferritin, pro-calcitonin (PCT), fibrinogen and erythrocyte sedimentation rate (ESR) are the well-known APRs. Moreover, the associations between mean platelet volume (MPV) and inflammation have been previously demonstrated [3]. Elevation of fibrinogen causes aggregation of erythrocytes, which results in faster sedimentation of those cells. This phenomenon constructs the basis to the rationale of using ESR for the assessment of systemic inflammatory response to any antigens like lipopolysaccharides [4].

Procalcitonin is the precursor protein of calcitonin that mainly is produced by the C cells of the thyroid gland [5]. It is also produced extra-thyroidal organs such as; lung, liver, pancreas and colon [6, 7]. Procalcitonin behaves like an APR similarly to other positive reactants and its production elevates with inflammatory stimuli and infections as some other APRs [8]. The normal range of PCT is 0.01 ng/ml, however it increases up to 0.5-1 ng/ml during viral infections or inflammatory diseases. Procalcitonin is particu-

larly used as an auxiliary test for diagnosing sepsis and predicting prognosis for the patients treated in the intensive care units [9, 10]. Most commonly used APR is C-reactive protein, which is a globulin type protein, produced by the liver. It rapidly increases in cases of inflammation and tissue damage, and quickly returns back to normal levels as soon as the patient's recovery [11, 12]. Thyroid dysfunction is a common health problem among adults and it can be accurately diagnosed with laboratory tests [13, 14]. Hashimoto's thyroiditis (HT) and Graves' disease are the thyroid dysfunctions, which occur due to the inflammation caused by the autoimmunity and other thyroid diseases are considered to be unrelated with autoimmune processes. It was previously shown that some APRs levels increase with several thyroid diseases [15-19]. In the present study, the levels of serum inflammation markers and their association with thyroid functions were assessed in patients with both autoimmune and non-autoimmune based on thyroid dysfunctions. There are not any comprehensive studies about this subject according to the literature until recently.

Materials and methods

We aimed to determine the association between the levels of serum inflammation markers with thyroid functions in patients with thyroid dysfunction. A total of 86 hypothyroid (82 females, 4 males) and 67 hyperthyroid (63 females, 4 males) patients who were admitted to the outpatient clinic of Gaziantep University School of Medicine, Departments of Internal Medicine and Endocrinology & Metabolism between April 2012 and January 2013 were included into the study. All patients with thyroid dysfunctions were divided into two groups as autoimmune (HT and Graves's disease) and non-autoimmune thyroid dysfunctions in order to elucidate the effect of auto-immunity on APRs. Patients were evaluated with physical examination, laboratory tests and thyroid ultrasonography for thyroid dysfunctions. Control group consisted of 59 (54 females, 5 males) healthy volunteers. The age and BMI of patients and controls were matched.

Blood pressure was measured with sphygmomanometer from the right arm after 5 minutes of resting. The participants completed a questionnaire regarding their sociodemographic characteristics.

Exclusion criteria included; pregnancy, smoking, consuming alcohol, steroid and non-steroid anti-inflammatory drugs and immunomodulators usage, having some diseases such as diabetes mellitus, renal failure, liver insufficiency, malignancy, infectious diseases, and other autoimmune diseases.

Patients who were under anti-hypertensive treatment due to previously diagnosed hypertension and the patients whose blood pressure was over 140/90 mmHg during the screening were also excluded from the study. The blood samples were drained after 12 hours of fasting and complete blood count (CBC), PCT, CRP, ESR, thyroid stimulating hormone (TSH) and free thyroxine (fT4) levels were measured. Blood samples were taken into standard biochemistry tubes with gel for the measurement of TSH and fT4 levels with chemiluminescent microparticle immunoassay (CMIA) method by Abbott Architect c2000i device. Blood samples were collected into tubes with citrate, and Vacuette SRS 100/II device was used for the calculation of ESR with Westergren method. Measurement of PCT levels were completed by electrochemiluminescence immunoassay method on Elecsys 2010 device with standard gel-containing biochemistry tubes. Measurements of CRP levels were completed by immunonephelometry assay on Dade Behring Nephelometer II device with standard gel-containing biochemistry tubes. Blood samples were collected in tubes with ethylenediamine tetraacetic acid, were used for measuring MPV levels with impedance method on Beckman Coulter device.

Statistical analyses

The distribution of the continuous variables was evaluated with Kolmogorov Smirnov test. Normally distributed variables between 2 independent groups were compared with Student t test whereas Mann Whitney U test was used for non-normally distributed variables. ANOVA and Tukey multi-comparison tests were used for comparison of more than 2 normally distributed groups whereas Kruskal Wallis and Dunn multiple comparison methods were used for non-normally distributed variables. Chi-square test was used for the analysis of the association between categorical variables. The results

Table 1. Hypothyroid, hyperthyroid and control groups' levels of CRP, ESR, MPV and PCR

APR	Hyperthyroid (n=86)	Hyperthyroid (n=67)	Control (n=59)	Significance P*
ESR (mm/h)	23.52±14.23[a]	22.13±12.88[b]	7.03±4.61	0.001
CRP (mg/L)	5.62±4.75[a]	5.75±5.27[b]	3.59±0.87	0.007
PCT (ng/mL)	0.03±0.01[a]	0.03±0.01[b]	0.02±0.01	0.001
MPV (f/L)	8.62±0.99[a]	8.61±1.10[b]	9.08±1.25	0.024

*P<0.05 is significant, Kruskall Wallis Test; [a]P<0.05, controls vs hypothyroid patients, Dunn multiple comparison test; [b]P<0.05 controls vs hyperthyroid patients, Dunn multiple comparison test. APR = Acute phase reactant; CRP = C-reactive protein; PCT = pro-calcitonin; ESR = erythrocyte sedimentation rate; MPV = mean platelet volume.

Table 2. Analysis of ESR, CRP, PCT and MPV levels for Autoimmune, Non-Autoimmune and Control groups

APR	Autoimmune (n=76)	Non-Autoimmune (n=77)	Control (n=59)	Significance P*
ESR (mm/h)	22.56±15.25[a]	23.25±11.91[b]	7.03±4.61	0.001
CRP (mg/L)	5.36 ±4.31	5.98±5.56	3.59±0.87	0.087
PCT (ng/mL)	0.03±0.01[a]	0.03±0.01[b]	0.02±0.01	0.001
MPV (f/L)	8.96±1.07	8.28±1.89[b]	9.08±1.25	0.001

*P<0.05 is significant, Kruskall Wallis Test; [a]P<0.05, controls vs autoimmune patients, Dunn multiple comparison test; [b]P<0.05, controls vs non-autoimmune patients, Dunn multiple comparison test; APR = Acute phase reactant; CRP = C-reactive protein; PCT = pro-calcitonin; ESR = erythrocyte sedimentation rate; MPV = mean platelet volume.

of the above mentioned analyses were interpreted according to 95% significance level (P<0.05). Statistical Package for Social Sciences (SPSS) for Windows version 22-package program was used for statistical analyses and statistical significance level was set as P<0.05.

Ethics

The research protocol was approved by the Clinical Researches Ethics Committee of Gaziantep University (approval no: 03.04.2012/147) and conducted according to the Declaration of Helsinki. Informed consents of the patients were obtained.

Results

Erythrocyte sedimentation rate, CRP and PCT levels were significantly higher in the hypothyroid and hyperthyroid groups, compared to the control group (P=0.001, P=0.007 and P=0.001 respectively), on the other hand there was no statistically significant difference for hypothyroid and hyperthyroid patients (P=0.753, P=0.982 and P=0.991 respectively).

Mean platelet volume level of hypothyroid and hyperthyroid groups was significantly lower compared to the controls (P=0.024), however there was no statistically significant difference between MPV of hypothyroid and hyperthyroid patients (P=0.997). **Table 1** summarizes the results for ESR, CRP, PCT and MPV values for hypothyroid, hyperthyroid and control groups.

Table 2 demonstrates the comparisons of ESR, CRP, PCT and MPV levels analysis for autoimmune, non-autoimmune and control groups. Mean ESR and PCT levels were higher in patients with both autoimmune and non-autoimmune thyroid disease, compared to the control group (P=0.001 each). C-reactive protein levels were also higher in patients with both autoimmune and non-autoimmune thyroid dysfunctions compared to the control group, but was not statistically significant (P=0.158 and P=0.162, respectively).

Similarly, MPV was lower in patients with autoimmune thyroid dysfunctions compared to the control group, but was not statistically significant (P=1.00). However, patients with non-autoimmune thyroid dysfunctions had significantly lower mean MPV compared to the controls (P=0.001).

Discussion

In this study while ESR, CRP and PCT levels were significantly higher in the hypothyroid and hyperthyroid groups, compared to the controls, there was no statistically significant difference between hypothyroid and hyperthyroid groups. While ESR and PCT levels were significantly higher in both autoimmune and non-autoimmune compared to the controls, there was no statistically significant difference between autoimmune and non-autoimmune groups. Increased CRP level in thyroid dysfunctions was reported in previous studies. In a cross-section-

al study CRP levels were found to be increased in patients with subclinical hypothyroidism [16]. Yao et al. reported that CRP levels of patients with dermatomyositis whom suffered from subclinical hypothyroidism were higher than who did not [20]. Czarnywojtek et al. reported that the serum CRP concentration was increased in hyperthyroidism and hypothyroidism. Several signs and symptoms of hypothyroid disease led us to think that hypothyroidism may cause inflammation. Increased CRP in hypothyroidism might be due to the result of an interaction of IL-6 on TNF-alpha and IL-1. However the other mechanisms that results in elevated CRP in both hyper- and hypothyroidism is still a question. Additionally the lack of thyroid hormones causes a slower metabolic rate and hence results in with impaired biochemical processes. As a consequence CRP clearance rate may result with elevated serum CRP levels. Similarly, slow CRP uptake in target cells might also add to this phenomenon. On the contrary, hyperthyroidism causes rapid metabolic activity, which may result in adrenergic nervous system hyperactivity, immune system stimulation and significantly increased peripheral blood flow. Thus conditions which might result in an increase of CRP concentration [21]. Tuzcu et al. showed that patients with subclinical hypothyroidism had higher serum hs-CRP than healthy subjects [22]. Lee et al. did not detect any statistically significant difference in hs-CRP levels among hyperthyroid, subclinical hyperthyroid, hypothyroid and subclinical hypothyroid and control groups [23].

In our study the increased ESR in patients with thyroid dysfunction confirms the findings of previously published studies. Erden et al. demonstrated significantly increased ESR, fibrinogen, and serum amyloid A in patients with euthyroid HT patients compared to the controls. This study proves that mild systemic inflammation continues in patients with HT, although they reach euthyroid state [15]. Taddei et al. found that the patients with subclinical hypothyroidism and HT had significantly higher ESR than the control subjects. This may be due to chronic activation of the immune system due to HT [24]. In another study of these researchers, they found higher CRP and IL-6 levels in subclinical hypothyroid patients when compared to the healthy controls, similar to the previous study. They concluded that HT patients had low-grade chronic inflammation [25].

Nylen et al. demonstrated that PCT levels increase in inflammatory diseases and this molecule can be an important marker of systemic inflammation as it is closely related to the mortality [26]. Nijsten et al. reported that PTC behaves as an APR and its production elevates with inflammatory stimuli and infections [8]. Procalcitonin was thought to be produced via TNF-α and IL-6 during the infection and inflammation [7, 10]. According to our study PCT levels increased in hypothyroid, hyperthyroid, autoimmune and non-autoimmune groups compared to the control. With enlighten of all previously mentioned studies, it is possible to conclude that PCT, independent of autoimmunity, increases in thyroid diseases.

We found the Mean MPV of hypothyroid, hyperthyroid and non auto immune patients were lower compared to the controls. Although there are limited publications which analyzed the MPV changes in thyroid dysfunctions, there are many studies in the literature which demonstrates decreased MPV in inflammatory diseases. For example, MPV is found to be decreased among patients with highly inflammatory diseases such as Chron's disease, familial Mediterranean fever and rheumatoid arthritis [27-29]. In a study mean MPV levels were found significantly higher in the euthyroid HT group than in the control group [30]. Contrary to our findings some studies reported the increased MPV level in subclinical hypothyroidism [31-34]. Panzer et al. observed the platelet changes such as lower platelet counts and increased MPV in hyperthyroidism [35]. The most common diagnosis was HT in the hypothyroid group whereas it was Graves' disease in the hyperthyroid group. These findings bring up the idea that the alterations in serum inflammation markers can be explained with autoimmunity. Significant changes in serum inflammatory marker levels in patients with non-autoimmune thyroid dysfunctions strongly suggest that mild systemic inflammation exists in all types of thyroid diseases. Alteration of inflammatory marker levels has been independent from etiology of thyroid disease. Statistically significant difference in serum inflammatory marker levels among different types of thyroid problems also supports this hypothesis.

To our knowledge, this is the first original article that evaluates PCT level with the condition of

thyroid dysfunction. Considering previous studies which demonstrated the alteration of ESR, CRP, PCT, MPV levels in inflammatory diseases, we believe that further studies for elucidating the etiopathogenesis of thyroid dysfunctions must focus on inflammation.

Conclusion

Significant changes in the levels of inflammation markers such as ESR, CRP, PCT, and MPV in autoimmune, non-autoimmune, hypothyroid and hyperthyroid disorders confirm the role of inflammation in the pathogenesis of thyroid dysfunctions regardless of thyroid dysfunction type.

Disclosure of conflict of interest

None.

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