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Investigation Of Levels Of Serum Pentraxin-3 And CRP In Sickle Cell Anemia

Serum Pentraksın-3 Ve CRP Düzeylerının Orak Hücre Anemısında Araştırılması

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ABSTRACT

Sickle cell anemia (SCA) is one of the hemoglobinopathies with a high prevalence both in the world and in our country, so its importance is increasing. Infections are among the most common causes of mortality in SCA patients. Based on this, in this study, it was aimed to investigate the levels of Pentraxin-3 and C-reactive protein, which are acute phase reactants that increase in inflammatory and ischemia conditions, in patients with SCA. A total of 89 individuals, including 44 patients (23-40 years old) and 45 healthy controls (21-48 years old) diagnosed with SAD, were included in the study. CRP levels were studied by immunoturbidimetric method in Cobas Integra 800 device, and PTX3 levels were measured by ELISA method. CRP and PTX3 levels were found to be increased in the patient group with SCA compared to the healthy group ($p < 0.001$). However, when evaluated in terms of PTX3 and CRP levels, it was found that there was no significant correlation between the patient with SCA and healthy control groups ($r = -0.188$, $p = 0.22$; $r = -0.117$, $p = 0.445$; respectively). As a result of the data obtained, we think that PTX3 and CRP with increased levels in many inflammatory diseases can be useful in the follow-up of SCA.

Keywords: Pentraxin-3, CRP, sickle cell anemia, acute phase protein

ÖZET

Orak hücre anemisi (OHA), Dünyada ve de ülkemizde görülme sıklığı yüksek olan hemoglobinopatilerden biridir, bu nedenle önemi artmaktadır. OHA hastalarında görülen en sık mortalite nedenleri arasında enfeksiyonlar yer almaktadır. Bundan yola çıkarak, bu çalışmada, inflamatuvar, iskemi gibi durumlarda artış gösteren akut faz reaktanları olan Pentraksin-3 ve C-reaktif proteinin, OHA'lı hastalarda düzeylerinin araştırılması amaçlandı. Çalışmaya OHA tanısı almış 44 hasta (23-40 yaş arası) ve 45 sağlıklı kontrol (21-48 yaş arası) olmak üzere toplam 89 birey dahil edildi. CRP düzeyleri Cobas İntegra 800 cihazında immünotürbidimetrik yöntemle, PTX3 düzeyleri ise ELISA yöntemiyle çalışıldı. CRP ve PTX3 düzeylerinin, OHA'lı hasta grubunda, sağlıklı gruba kıyasla artmış olduğu bulundu ($p<0.001$). Bununla birlikte, PTX3 ile CRP düzeyleri bakımından değerlendirildiğinde, OHA'lı hasta ve sağlıklı kontrol grupları arasında anlamlı bir korelasyon olmadığı tespit edildi (sırasıyla; $r=-0.188$, $p=0.22$; $r=-0.117$, $p=0.445$). Birçok inflamatuvar hastalıkta düzeyleri artan PTX3 ve CRP düzeylerinin OHA takibinde kullanılabileceğini düşünmekteyiz.

Anahtar Kelimeler: Pentraksin-3, C-reaktif protein, orak hücre anemisi, akut faz proteinleri

INTRODUCTION

Sickle cell anemia (SCA) is one of the most common hemoglobinopathies in the world. SCA is caused by inherited mutations of the globin gene, and abnormal hemoglobin (Hb) S is formed when glutamic acid is replaced by valine at the 6th position of the β chain (Simpson, 2019). In SCA, which is a multisystem disease characterized by the deterioration, stiffness and adhesion of red blood cells, reperfusion damage leading to recurrent microvascular ischemia and chronic

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organ damage and intravascular hemolysis develop. Infections are among the most common causes of morbidity and mortality in patients with SCA. Loss of function ability of the spleen, disruption of IgG and IgM responses to infections, defects in alternative complement pathway and defects in opsonization and phagocytosis abilities of macrophages constitute the causes of increased infection in patients with SCA (Schnog et al., 1998; Serjeant, 2001; Kato et al., 2006; Ünal et al., 2011).

Pentraxins (PTX), which are natural pattern recognition molecules produced in response to infection and tissue damage, have multiple functions. Their best characterized function is the activation of the classical complement pathway in some microorganisms and necrotic cells and thus they contribute to the removal of cellular debris. Pentraxins are divided into two as long and short pentraxins (Günaştı et al., 2017; Meri-Haapasalo, 2019).

Prototypical long pentraxin-3 (PTX3), which is one of the acute phase proteins and an important component of innate immunity, rise in diseases such as acute infections, myocardial infarction and autoimmune inflammatory diseases. PTX3 is released locally at the site of infection by various cell types such as mononuclear phagocytes, fibroblasts, and endothelial cells. PTX3 expression can be induced by proinflammatory cytokines (TNF-α, IL-1ß) as well as in diseases of microbial origin. C-reactive protein (CRP), which is among the short pentraxins, is mainly produced and released in the liver in response to proinflammatory stimuli. (Luchetti et al., 2000; Peri et al., 2000; Fazzini et al., 2001; Muller et al., 2001; Latini et al., 2004; Mantovani, 2008).

The aim of this study was to determine the acute phase reactants PTX3 and CRP levels in patients with SCA and to investigate their roles in the disease.

MATERIAL AND METHOD

Study Group

A total of 89 individuals was included in the study. 44 of them are patients (between 23-40 years old) who applied to the Hematology outpatient clinic and diagnosed with SCA, and 45 of them are healthy controls (aged 21-48 years) who had no history of disease (Table 1).

Table 1. Demographic data of the study group

	Patient		Control	
	Female	**Male**	**Female**	**Male**
n (%)	19 (43,20)	25 (56,80)	26 (57,80)	19 (42,20)
Age	31,9±4,56	30,0±5,78	33,2±8,77	32,5±5,12

Results were given as means (¯x) ± standard deviation (SD); n: number of sample

Patients with any disease other than sickle cell anemia, a known inflammatory disease or infectious disease, a systemic disease such as diabetes, hypertension, cancer, or liver or kidney failure were excluded from the study. This study was approved by ME.U Clinical Research Ethics Committee with the Board Decision dated 23.03.2017 and numbered 2017/71.

PTX-3 and CRP Measurements

Venous blood samples taken into biochemistry tubes from individuals belonging to the patient and control groups were centrifuged at 4000 rpm for 10 minutes, 15 minutes after reaching the laboratory. The serums obtained were divided into aliquots. CRP levels were studied on the same day, within the following 1 hour, by immunoturbidimetric method on Cobas Integra 800 device (Roche Diagnostics Mannheim, GmbH). arithmetic

Serum samples were stored in a -80 °C freezer for PTX-3 measurements. On the working day, PTX-3 levels of serum samples expected to come to room temperature were studied in accordance with the kit content (Pentraxin-Human / SUNREDBIO) with ELISA method by using DSXTM Four-Plate Automated ELISA Processing System microELISA device.

Statistical Method

For age, which is a continuous variable, mean and standard deviation were used from descriptive statistics, while median, minimum and maximum values were used for CRP and PTX3. For gender and patient group, which are categorical variables, number and percentage values were used. Normal Distribution control was done with Shapiro-Wilk Test. Mann Whitney U test was used in patient-control and gender groups for CRP and PTX3 that did not show normal distribution. Student's t test was used to compare the difference between the averages of ages of the patient-control and gender groups. Correlation analysis was performed to investigate the linear relationship between CRP and PTX3. Statistical significance level was taken as $p < 0.05$ for all comparisons.

RESULTS

89 individuals were included in the patient group with sickle cell anemia and in the control group. 44 of them were male (25 patients, 19 controls) and 45 of them were female (19 patients, 26 controls). While the age range of men is between 23 and 41, the age range of women is between 21 and 48. Of the 44 patients, 25 were male (56.8%), 19 were female (43.2%). Of the 45 individuals that make up the control group, 19 were male (42.2%), 26 were female (57.8%). The difference between the averages of ages of the patient and control groups is not statistically significant (p> 0.05) (Tables 2 and 3).

Table 2. Averages of ages in the male and female group

		Age			
Gender	**n**	**Median**	**Min**	**Max**	**p**
Male	**44**	30,50	23,00	41,00	0,248
Female	**45**	33,00	21,00	48,00	

n: number of individuals; p: p values

Table 3. Averages of ages in patient and control groups

		Age						
	Gender	**n**	**Median**	**Min**	**Max**	**Q1**	**Q3**	p
Patient Group	**Male**	25	29,00	23,00	40,00	25,00	34,00	0,246
	Female	19	33,00	25,00	40,00	26,0000	35,00	
Control Group	**Male**	19	34,00	23,00	41,00	30,00	36,00	0,739
	Female	26	32,00	21,00	48,00	24,75	41,25	

n: number of individuals; p: p values

When CRP levels between the patient and control groups were compared, CRP levels in the patient group with SCA were found to be increased compared to the control group (p <0.001). Similarly, PTX3 levels were statistically significantly increased in the SCA patient group compared to the healthy control group (Table 4).

Table 4. Median values of PTX-3 and CRP levels in the groups

	PTX3 (min-max) pg/mL		**CRP (min-max) mg/L**	
Patient	3,43	1,10-28,21	10,66	1,14-149,00
Control	1,91	0,49-10,88	1,78	0,21-77,00
p	<0,001		<0,001	

n: number of sample; p: p value

When PTX-3 and CRP levels were compared between males and females in the patient and control groups separately, the difference between the PTX3 and CRP medians in the female and male groups of the patient group was not found to be statistically significant (p> 0.05). Similarly, the difference between PTX-3 and CRP medians in the female and male groups of the control group was not to be statistically significant (p> 0.05) (Table 5).

Table 5. Median values of PTX-3 and CRP levels in the patient and control groups

		Gender	n	Median	Minimum	Maximum	Q1	Q3	p
Patient Group	**PTX3 (pg/mL)**	Male	25	3,14	1,10	16,40	2,08	5,28	0,139
		Female	19	4,17	1,51	28,21	2,24	12,46	
	CRP (mg/L)	Male	25	14,00	1,50	149,00	6,71	28,85	0,078
		Female	19	7,00	1,14	99,73	4,35	14,90	
Control Group	**PTX3 (pg/mL)**	Male	19	1,94	1,31	10,88	1,63	2,00	0,483
		Female	26	1,90	0,49	9,04	1,72	4,96	
	CRP (mg/L)	Male	19	2,49	0,21	39,37	0,73	6,73	0,662
		Female	26	1,67	0,24	77,00	1,305	3,63	

n: number of sample; p: values of significance with difference of each group; Q:quartile

According to the result of correlation analysis between PTX-3 and CRP levels, which were found to be significantly different between the groups, no significant relationship was found between PTX-3 (r=-0.188; p=0,221) and CRP (r = -0.117; p = 0.445) in the patient group and in the control group.

DISCUSSION

Chronic intravascular hemolysis and recurrent inflammation are frequently encountered in sickle cell patients. In addition, recurrent clinically symptomatic or asymptomatic vascular occlusion with ischemia-reperfusion injury resulting in endothelial activation is frequently observed in patients (Frenette, 2002). Fever and infections are the main reasons for hospital admission in patients with SCA. Therefore, patients with SCA should be evaluated in terms of infection in detail and treated until the presence of infection is completely excluded. Tissue ischemia and infarction developing due to ongoing inflammation increases PTX3 formation and triggers its release into the circulation. In an animal study, PTX3 has been shown to be effective in ischemia-reperfusion injury and has an enhancing role (Nur et al., 2011).

When the data of the studies are evaluated, PTX3 can be considered as an early biomarker of inflammation (Peri et al., 2000; Latini et al., 2004). In many studies, it has been shown that plasma PTX-3 values increase in diseases such as myocardial infarction (Peri et al., 2000), rheumatoid arthritis (Luchetti et al., 2000) and sepsis (Muller et al., 2001). In a study conducted with patients in the intensive care unit, a strong correlation was shown between PTX3 and the severity of infection (Muller et al., 2001).

Nur et al. (2011), in their study on patients with SCA, reported that the levels of PTX3 levels significantly changed both at the beginning and due course of the painful crisis in admission to the hospital with a painful crisis, and that they showed a significant correlation with the duration of hospital stay afterwards. As a result, they reported that PTX3 could be an important indicator of painful crisis severity. In this study, it was found that serum PTX3 levels of sickle cell patients increased significantly compared to the healthy control group.

There are studies arguing that there is a relationship between pentraxine-3 and hemolysis and coagulation (Singer-Ataga, 2008; van Beers et al., 2009). There has even been reported evidence that PTX3 induces endothelial and monocyte TF expression. However, although PTX3 is thought to play an important role in endothelial and coagulation activation, there are data indicating that there is no relationship between PTX3 levels and coagulation markers. In addition, there are disagreements on its effect on hemolysis due to the arising oxidative stress and downstream effects on endothelial and coagulation activation (Napoleone et al., 2002; Napoleone et al., 2004).

According to the data of this study, it was found that CRP levels expected to increase in SCA patients compared to the healthy control group increased as similarity with other studies (Makis et al., 2006). Innate immunity is the first line of host defense. Compared to CRP produced only by the liver, PTX3 is excreted by different cell types corresponding to infection, proinflammatory stimulation and endotoxemia (Muller et al., 2001; Han et al., 2005; He et al., 2010). It has been reported that PTX3 is produced in different tissues and cells including vascular endothelial cells and macrophages and binds to the endothelium rapidly since it is produced in the inflammation site (Mantovani et al., 2006). Unlike CRP, due to its extrahepatic synthesis, PTX-3 levels are thought to be the true independent indicator of disease activity (Fazzini et al., 2001). In addition, studies have shown that proinflammatory cytokines such as TNFα and IL1β are excessively activated due to the induction of sepsis by severe infections that stimulate PTX3 expression (Fornai et al., 2016). It has been reported that PTX3 regulates the development of atherosclerosis by increasing in vitro IL10 production, however, it has also been reported that PTX3 stimulation does not cause the production of IL6 and TNFα, which are pro-inflammatory cytokines (Slusher et al., 2016).

In this study, when the findings were examined in terms of the correlation between PTX3 and CRP levels, no significant relationship was found in the SCA patient and healthy control groups. In a study involving a group of patients with myocardial infarction, it was reported that CRP

levels reached their peak value approximately 2 days after the onset of symptoms. PTX3 levels have been reported to peak on the day of onset of symptoms, even within hours, and return to baseline a few days later. Thus, PTX3 is thought to be valuable as a more potent marker of ischemia-induced inflammation (Peri et al., 2000). However, the PTX3 level peaks much earlier. Therefore, it is thought that PTX3 may decrease as CRP increases and this may eliminate the possibility of a linear relationship (Sprong et al., 2009).

The C-reactive protein reflects chronic inflammation in SAD patients, and the CRP level is significantly increased in patients with asymptomatic sickle cells. This is because CRP is only systemically produced by the liver. PTX3, on the other hand, is produced locally, making this protein more specific. Differences in production of PTX3 and CRP may explain the different time processes observed in SCA or other pathological conditions (Mauri et al., 2008). In further studies, the diagnostic power of determining the peak levels of both acute phase proteins will be increased by specifying the patient group as painful crisis and clinically asymptomatic sickle cell patients.

In conclusion, based on the data we obtained, considering the difference in production sites of PTX3 and CRP and the duration of production, we think that PTX3 is a more useful marker in the early period in inflammation and can be used in the diagnosis of SCA and especially in the follow-up.

Declarations

The authors declare no conflict of interest.

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