Original Investigation
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Comparison of the plasma levels of cathepsin-L and granulysin between patients with psoriasis and healthy controls

Psoriazis hastaları ve kontrol gruplarında plazma katepsin-L ve granulizin düzeylerinin karşılaştırılması

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Abstract

Background and Design: Psoriasis is a chronic papulosquamous disease where histologically epidermal hyperproliferation and infiltration involving natural killer cells and cytotoxic T-cells are observed. These cells have been shown to carry cytolytic molecules containing high amount of perforin, granzyme B and granulysin (GNLY). The roles of these molecules in the pathogenesis of psoriasis are still disputed, with serum GNLY and cathepsin-l (CL) levels thought to be associated with cellular immunity. In this study, we investigated the relationship between the severity and duration of psoriasis and the levels of CL and GNLY.
Materials and Methods: Prospective and randomized study of 40 patients (23 males, 17 females) with psoriasis who admitted to hospital between December 2014 and August 2015, and 40 age and sex-matched healthy controls (23 males, 17 females) were investigated. CL and GNLY serum levels were measured by ELISA method.
Results: There was no significant differences in GNLY and CL levels between psoriasis patients and the control group (p=0.243 and p=0.606). There was also no statistically significant difference between psoriasis patients with low Psoriasis Area Severity Index (PASI) (≤10) and those with high PASI (>10) (p=0.86 and p=0.61) score.
Conclusion: There are studies that have shown GNLY and CL in the psoriazis are important markers for disease pathogenesis. However, according to the results of this study, CL and GNLY levels are not sufficient markers to indicate the level of cellular immunity and disease severity in psoriasis. Future studies are needed on this subject with a wider range of patients.
Keywords: Psoriasis, cathepsin, granulysin

Öz

Amaç: Psoriazis, histolojik olarak epidermal hiperproliferasyon ve doğal katil hücreler ile sitotoksik T-hücreleri içeren infiltrasyonun gözlendiği, kronik papüloskuamöz bir hastalıktır. Bu hücrelerin yüksek miktarda perforin, granzim B ve granulizin (GNLY) içeren sitolitik molekülleri taşıdığı gösterilmiştir. Bu moleküllerin psoriazis patogenezindeki rolleri hala tartışmalıdır, serum GNLY ve katepsin-L (CL) seviyelerinin selüler immünite ile ilişkili olabileceği düşünülmektedir. Bu çalışmada psoriaziste hastalık şiddeti ve süresiyle, CL ve GNLY seviyelerinin ilişkisini araştırdık.
Gereç ve Yöntem: Prospektif ve randomize bu çalışmaya, Aralık 2014-Ağustos 2015 tarihleri arasında başvuran 40 psoriazis (23 erkek, 17 kadın) hastası ile yaş, cinsiyet uyumlu 40 gönüllü (23 erkek, 17 kadın) dahil edildi. CL ve GNLY serum seviyeleri ELISA yöntemiyle ölçüldü.
Bulgular: CL ve GNLY seviyelerinde, psoriazis hastaları ve kontrol grubu arasında istatistiksel olarak anlamlı fark yoktu (p=0,243 ve p=0,606). Düşük Psoriazis Alan Şiddet İndeksi (PAŞİ) skoru (≤10) olan psoriazis hastaları ile yüksek PAŞİ skoru (>10) olan hastalar arasında da istatistiksel olarak anlamlı fark yoktu (p=0,86 ve p=0,61).

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Sonuç: Psoriyatik deride bakılan CL ve GNLY'nin, hastalık patogenezi açısından önemli belirteçler olduğuyla ilgili çalışmalar mevcuttur. Fakat bu çalışmanın sonucuna göre, CL ve GNLY seviyelerinin, psoriaziste hücresel immünite düzeyini ve hastalık şiddetini göstermede yeterli belirteçler olmadıkları düşünülmektedir. Bu konuda daha geniş hasta serileri ile ileri çalışmalar yapılmasına ihtiyaç vardır.
Anahtar Kelimeler: Psoriazis, katepsin, granulizin

Introduction

Psoriasis is an immune-mediated, chronic disease which is characterized by sharply-circumscribed, erythematous papules and plaques[1]. Moreover, psoriasis is considered to be a systemic disorder which is not limited to the skin and found to be accompanied with various comorbidities[2]. While the pathogenesis of psoriasis is not exactly known, it is assumed that both the innate and adaptive immune systems have a role on it. The proinflammatory cytokines, such as interleukin-2 (IL-2), IL-6, IL-8, and IL-12, interferon gamma (IFN-γ) and tumor necrosis factor alpha, increase in both serum and lesional skin[3]. T lymphocytes and natural killer (NK) cells are demonstrated involving in cytotoxic immunity and manifest cytotoxic activity through perforin/granzyme-dependent granule exocytosis and this pathway is associated with cathepsin and granulysin (GNLY) which may be related to psoriasis as well[4,5].

Cathepsin-L (CL) is one of the lysosomal cysteine proteases which plays a significant role in regulation of immune response, in ensuring antigen presentation, the adhesion and migration processes and securing the degradation of cytokines and growth factors[4]. Many studies showed that CL may be an indicative of cellular immunity[4,6,7].

GNLY is a cytolytic granule-associated protein that works in a synergistic manner with perforin and induces apoptosis[8]. It is present in activated cytotoxic T lymphocyte (CTL) and NK cells and measurement of serum GNLY level has been found to be beneficial in indicating cytotoxic immunity[9]. Serum CL and GNLY levels which are thought to be indicators of cytotoxicity in psoriasis, were not analyzed before. The aim of this study is to compare these molecules' levels in patients with psoriasis with those of a control group and evaluate the relation between them and the Psoriasis Area Severity Index (PASI).

Materials and Methods

The necessary approval for the study was received from the University of Health Sciences Turkey, Dışkapı Yıldırım Beyazıt Training and Research Hospital Ethics Committee (approval no: 2015/22/20). The patients and the control group members were informed on the procedures to be applied and all participants provided signed consent before the study.

Study sample

This was a randomized, prospective case-control study that involved 40 psoriasis patients (23 men, 17 women) who applied to the dermatology clinic between December 2014 and August 2015, as well as 40 healthy volunteers (23 men, 17 women).

The dermatological examination of all patients participating in the study was carried out by the same physician, and the demographic characteristics, additional systemic diseases and PASI scores of these patients were recorded.

For the measurement of human serum GNLY, the Boster Immunoleader/USA brand 1x96 type GNLY kit with a reference range of 0.1 ng/mL to 200 ng/mL was used (Human GNLY, ELISA kit, Boster Immunoleader/USA) and for human serum CL, the Ebioscience brand 1x96 type CL kit with a reference range of 1.7 ng/mL to 100 ng/mL was used (Human CL, ELISA kit, Ebioscience, BMS257).

Statistical Analysis

During statistical calculations, patients with a PASI value of ≤10 were analyzed as the mild group while those with a PASI value of >10 were analyzed as the moderate to severe group. Mean counts were statistically analyzed by using SPSS (v16; SPSS Inc., Chicago, IL, USA).

Results

The study involved 40 psoriasis patients and control group of 40 people. The group of psoriasis patients consisted of 23 men (57.5%) and 17 women (42.5%). There was no significant difference in regard to gender or age between the patients and control group (47.6±10.2 and 47.5±10). The PASI scores of the psoriasis patients varied between 2.0 and 40.0 while the PASI score average for the control group was 13.9±8.1. The average duration of the disease was identified as 16.82±11.7 (1 to 40 years).

The average CL level of psoriasis patients was 9.78 ng/mL (1.70 to 28.73 ng/mL) and of the control group was 11.82 ng/mL (1.78 to 59.96 ng/mL) (Table 1). No statistically significant difference was found (p=0.243) (Figure 1). The comparison of CL levels according to PASI scores did not yield any correlation (Figure 1); neither did the comparison of CL levels according to disease duration in psoriasis patients (Figure 2).

The average GNLY level of psoriasis patients was 3.83 ng/mL (0.96 to 12.29 ng/mL) and of the control group was 3.62 ng/mL (0.98 to 9.13 ng/mL) (Table 1). No statistically significant difference was found (p=0.606) (Figure 3). The comparison of GNLY levels according to PASI scores did not yield any correlation (Figure 3); and neither did the comparison of GNLY levels according to disease duration in psoriasis patients (Figure 2).

Table 1. The mean and minimum-maximum values of cathepsin-L and granuylsin in patients with psoriasis and healthy controls

	Cathepsin-L		
	Mean	**Minimum-Maximum**	**p***
Psoriasis (n %)	9.78	1.70-28.73	0.243
Control (n %)	11.82	1.78-59.96	
	Granulysin		
	Mean	**Minimum-Maximum**	**p***
Psoriasis (n %)	3.83	0.96-12.29	0.606
Control (n %)	3.62	0.98-9.13	
*			

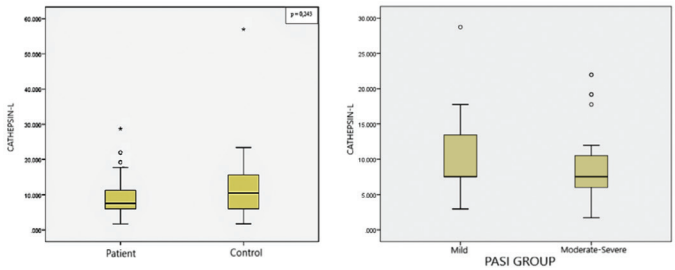

Figure 1. Comparison of plasma cathepsin-L levels (a) between patients with psoriasis and healthy controls, (b) among the patients according to Psoriasis Area Severity Index scores
PASI: Psoriasis Area Severity Index

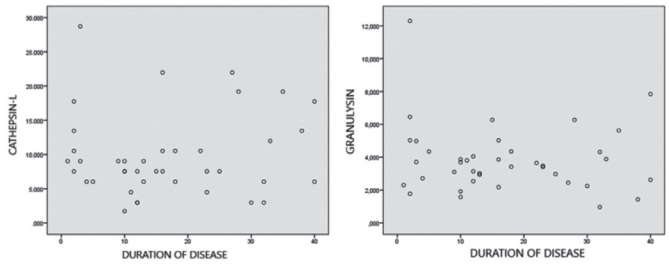

Figure 2. Scatter diagram of (a) plasma cathepsin-L levels according to the duration of the disease, (b) plasma granulysin levels according to the duration of the disease

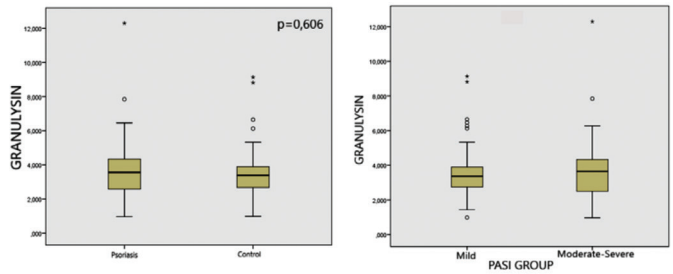

Figure 3. (a) Comparison of plasma granulysin levels between patients with psoriasis and healthy controls (b) Comparison of plasma granulysin levels among patients according to Psoriasis Area Severity Index
PASI: Psoriasis Area Severity Index

Discussion

Psoriasis is one of the most studied inflammatory skin diseases in the world[10]. In spite of all the studies, the etiopathogenesis is not clear yet. It has been demonstrated that dendritic cells, macrophages, mast cells, neutrophils and keratinocytes are also involved in the pathogenesis beneath T cell immunity[11,12]. Both Th1 and Th17 cells stimulate the release of cytokines such as tumour necrosis factor alpha, IFN-γ, IL-12, IL-17A, IL-22 and IL-23 and initiate the inflammatory process[12]. Until now, many indicators that may bring more clarity to the progress of the disease have been studied but none have been found to be directly related to it.

Cathepsins are proteases which have important roles in various physiological processes[13]. A study showed that after CL injection there was a decrease in serine protease inhibitor levels which may stated that CL may have a role in the inflammatory process[14]. In another study involving patients with rheumatoid arthritis, psoriatic arthritis, gout arthritis and unidentified arthritis revealed that matrix metalloproteinase-1, CL and cathepsin B were expressed in the synovial membrane aspirates of patients with early inflammatory arthritis, while no expression was observed in normal synovium. It could be contributing to joint destruction in the early period and has a major role in initiating and maintaining both inflammation and angiogenesis[15].

In another study, CL and hurpin (CL inhibitor) expressions and localizations were analyzed immunohistochemically in the skins of people with various inflammatory and neoplastic diseases; and intensive CL expression was detected in skin subject to diseases such as psoriasis, atopic dermatitis and squamous cell carcinoma[16]. Similarly, CL, B, H and D levels[17,18] and transglutaminase 3[19] were found to be high in psoriatic epidermis cases.

However, despite the fact that CL is shown to be elevated in the psoriatic skin, there has been no known study to our knowledge that examines the level of CL in the serums of psoriasis patients. In this study, the results of our analysis yielded lower but statistically insignificant CL levels in the patient group when compared to the control group. Upon comparison of patients according to PASI scores, the CL levels in the moderate-severe psoriasis group were once again found to be lower but statistically insignificant. This may be related to the fact that the cytotoxic T and NK cells, which release CL in peripheral blood, decrease in peripheral blood due to the migration to the area of inflammation. It can also be explained by the lack of any role cytotoxicity may have had in systemic inflammation.

In a study, serum GNLY levels and immunohistochemical GNLY expression were analyzed in patients with alopecia areata (AA), and as a result, the serum GNLY levels are stated to be a cytotoxicity indicator that can demonstrate the disease activity and prognosis in acute AA patients[20]. A study including patients with psoriasis revealed that GNLY expressions in patients were found to be significantly higher in comparison to healthy control group and also, the higher their GNLY levels were related to the duration of disease. According to these results, it was believed that GNLY could have an important role in the pathogenesis of psoriasis[21].

On the other hand, in a study involving psoriatic arthritis (PsA) patients. T levels of CTL and NK cells that contain GNLY in active-phase PsA were found to be always higher than those of others in the study, although the difference was not significant[22]. None of the current studies on psoriasis involves analysis of both GNLY and CL serum levels at the same time. Our study was unique as both indicators were analyzed at the same time.

Study Limitation

Due to the limited number of patients in our study, studies that are more extensive in this regard are needed to arrive at a definitive conclusion.

Conclusion

There are studies that claim, cytotoxic immunity may have a role in the pathogenesis of psoriasis. While CL and GNLY levels were demonstrated to be increased in the lesional skin of psoriasis patients; however, the serum levels of these molecules were never analyzed before for both molecules at the same time. According to the results we have obtained, serum GNLY and CL levels are not sufficient indicators in determining

the cell immunity level and the disease prognosis through the ELISA method in psoriasis. However, further research is required in this topic.

Ethics

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Dışkapı Yıldırım Beyazıt Training and Research Hospital Ethics Committee (approval no: 2015/22/20).

Informed Consent: All participants provided signed consent before the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.H.A., Design: H.H.A., Data Collection or Processing: H.H.A., S.B., Ş.Ö., A.Ö., Analysis or Interpretation: H.H.A., M.G., Literature Search: H.H.A., S.B., M.G., A.Ö., Writing: H.H.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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