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Cathelicidin as a link between sarcoidosis and tuberculosis

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Abstract. *Setting:* Sarcoidosis and tuberculosis share notable clinical, radiological, histological, and immunological similarities. The importance of vitamin D has long been investigated in these two granulomatous lung diseases. Cathelicidin is an antimicrobial peptide of the innate immune system, directly induced by vitD3. *Objective:* To evaluate the role of cathelicidin in sarcoidosis and tuberculosis development. *Design:* The study included 30 consecutive patients with active lung tuberculosis, 30 patients with sarcoidosis, and 20 healthy controls. 25-hydroxyvitamin D [25(OH)D] and cathelicidin levels were measured in blood samples. *Results:* Vitamin D levels were significantly higher (p<0.001) in tuberculosis patients (22.5±9.96 ng/ml) than in sarcoidosis patients (11.75±8.92 ng/ml). Severe vitamin D deficiency was as frequent as 47% in sarcoidosis patients compared to only 3% in tuberculosis patients. Cathelicidin levels were significantly higher in the control group (120.37±41.03 pg/ml) than in sarcoidosis (67.68±38.03 pg/ml) and tuberculosis (68.74 ±39.44 pg/ml) patients (p<0.001). However, no significant difference in cathelicidin levels was observed between tuberculosis and sarcoidosis patients (p=0.966). The optimum cathelicidin cut-off value to distinguish sarcoidosis patients from healthy controls was 107.14 pg/ml (sensitivity 81.5%, specificity 71.2%). *Conclusion:* Cathelicidin appears to play different roles in the development of granulomatous lung disease. *(Sarcoidosis Vasc Diffuse Lung Dis 2015; 32: 222-227)*

Key words: Chathelicidin, 25(OH)D, vitamin D, tubercolosis

Introduction

Mycobacterium tuberculosis is a very common pathogen worldwide (1). It is the cause of tuberculosis and a putative cause of sarcoidosis in a proportion of cases; therefore, this pathogen is believed to be the link between tuberculosis and sarcoidosis. The relationship between these two granulomatous diseases, which share similar clinical, radiological, and histological properties, still remains a paradox (2). However, it is important to discriminate between these two conditions, especially for the selection of different treatment options (3).

Granuloma formation is a consequence of persistent infection with some certain pathogens like such as mycobacteria, or as a or results from of poorly degradable antigens in some individuals with, not able to overcome them due to some defects of in their immune system (3,4).

Antimycobacterial activity is dependent on cell-mediated immune responses involving macrophages

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and T cells. These cells are known to express vitamin D receptors (VDR) (5, 6). Moreover, monocytes and macrophages can produce 1α-hydroxylase, the enzyme that converts 25-hydroxyvitamin D [25(OH)D] into the biologically active $1.25(OH)_2D3$ (7, 8).

Cathelicidin is an antimicrobial peptide belonging to the innate immune system. It is secreted by neutrophils, macrophages, and epithelial cells, and its production and activation is dependent upon vitamin D (8, 9). Cathelicidin regulates the innate immune system through bactericidal, antiviral, anti-endotoxic, and chemoattractant activities; therefore, it is involved in the first line of defense against *M. tuberculosis* (10-12). Researchers have shown that elevated cathelicidin levels are correlated with improved mycobactericidal activity, which partly explains the correlation between the beneficial effects of 25(OH)D and increased cathelicidin production (8).

In recent years, the association between hypovitaminosis D, cathelicidin levels, and infection with *M. tuberculosis* has been reported by several studies and meta-analyses (13-19). However, there have been contrasting results on the association of cathelicidin with sarcoidosis. Clinical studies mostly focused on cathelicidin levels in bronchoalveolar lavage fluids of sarcoidosis patients revealed contradictory results. (20) The association between alveolar macrophage cathelicidin deficiency and clinical disease status in sarcoidosis has been recently reported (21).

Based upon such knowledge, we compared blood cathelicidin and vitamin D levels of tuberculosis and sarcoidosis patients to healthy controls, with the aim of discriminating between these two diseases and elucidating their association.

Methods

Study design and population

This prospective cross-sectional study included 30 consecutive patients with active lung tuberculosis and 30 patients with sarcoidosis, admitted to the Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital in 2013.

The control group (n=20) consisted of healthy volunteers. Table 1 lists the clinical features for all cases.

Sarcoidosis cases (n=30) were recruited from patients undergoing routine clinical evaluation for the initial diagnosis of sarcoidosis (Table 1). All diagnoses were confirmed by pulmonary histology demonstrating non-necrotizing granulomas in the absence of Mycobacterium tuberculosis, other infection agents or further etiologies. We didn't perform bronchoalveolar lavage for sarcoidosis in our study. According to the chest radiographic staging system classification (22), 27 out of 30 sarcoidosis patients were included in Stage I, with bilateral hilar lymphadenopathy, and the remaining three patients were included in Stage II, with bilateral hilas lymphadenopathy and infiltrates.

Tuberculosis patients (n=30) had all for acid-fast bacilli (AFB) positivity in their sputum smears and were all clinically active lung tuberculosis patients. 26 out of 30 patients had cavities visible in chest X-rays.

All blood samples were collected at baseline before the initiation of specific treatments.

Patients with a history of tuberculosis, sarcoidosis, cancer, allergy, or autoimmune disease, and patients who were pregnant during inclusion were not included in the study.

Cathelicidin and Vitamin D measurements

Plasma cathelicidin antimicrobial peptide levels were analyzed using a commercially available ELISA kit (Uscn Life Science Inc. Wuhan); 25(OH)D concentrations were determined by high- performance liquid chromatography (HPLC) (ImmuChrom GmbH, Heppenheim, Germany). Both assays were performed at the Istanbul University Cerrahpasa Medical Faculty Biochemistry Laboratory.

Vitamin D status was defined at different 25(OH)D cut-offs: 62.5-200 nmol/L (25-80 ng/ml), adequate; 25-60 nmol/L (10-24 ng/ml), insufficient; and <25 nmol/L (10 ng/ml), severely deficient, according to the ELISA kit recommendations.

Statistics

For continuous variables, the mean ± standard deviation was reported according to data distribution. Statistically significant differences between the means were determined by the Student-t test or the

Table 1. Baseline characteristics and Vitamin D levels of the study groups and the control group

Characteristics	Sarcoidosis (n=30)	Tuberculosis (n=30)	Control (n=20)
Age	44.9±10.4 (28-67)	35.6±16.2 (17-70)	42.3±14.5 (22-69)
Sex (female / male)	21/9	12/18	6/14
Radiology	Staging Stage 1: 27 (90%) Stage 2: 3 (10%)	Lung cavity 26 (87%)	
Vitamin D levels and status			
25 (OH) D level (ng/ml)	11.75±8.92	22.5±9.96	37.75±18.04
Severely deficient (<10 ng/ml)	14 (47%)	1 (3%)	0
Insufficient (10–25 ng/ml)	13 (43%)	16 (53%)	7 (35%)
Adequate (>25 ng/ml)	3 (10%)	13 (44%)	13 (65%)

Mann-Whitney U test. A *P*- value <0.05 was considered statistically significant. Comparisons on cathelicidin and vitamin D levels between the groups were based on Kruskal-Wallis testing. Bonferroni corrected Mann-Whitney U test was used for Post Hoc analysis. Spearman's correlation coefficient analysis was used to explore the association between vitamin D and cathelicidin levels. The receiver operating characteristic (ROC) curve was constructed to obtain a cut-off point for cathelicidin levels in sarcoidosis.

Statistical analyses were performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013) program.

Ethics

Ethics committee approval was received from the ethics committee of Yedikule Chest Diseases and Chest Surgery Training and Research Hospital. All participants gave their informed consent.

Results

The characteristics of the sarcoidosis, tuberculosis, and control groups are shown in Table 1.

The median 25(OH) D levels for sarcoidosis patients were 11.75±8.92 ng/ml. Severe vitamin D deficiency was observed in 47% of sarcoidosis cases. The median 25(OH)D levels for tuberculosis patients were 22.5±9.96 ng/ml. These were significantly higher than in sarcoidosis patients (p<0.001). Only 3% of tuberculosis patients suffered from severe vitamin D deficiency. In the control group, the mean 25(OH)D levels were 37.75±18.04 ng/ml, which were statistically higher (p<0.001). No severe vitamin D deficiency was observed in the control group (Table 1).

Cathelicidin levels were calculated for each group and gender differences were analyzed.

The mean cathelicidin levels for sarcoidosis patients were 67.68±38.03 (11.53-130.50) pg/ml; for tuberculosis patients they were 68.74 ±39.44 (10.80-182.12) pg/ml; and in the control group they were 120.37±41.03 (36.04-190.67) pg/ml. No differences in cathelicidin levels were observed between male and female patients with sarcoidosis, tuberculosis, or in the control group (p=0.1, p=0.18, and p=0.88), respectively.

Cathelicidin levels were significantly higher in the control group than in sarcoidosis and tuberculosis patients (p<0.001). However, no significant differences in cathelicidin levels were observed between tuberculosis and sarcoidosis patients (p=0.966). Vitamin D and cathelicidin levels were not correlated in the sarcoidosis and tuberculosis groups, respectively (Spearman correlation coefficient 0.012 p-value =0.951/ 0.252 8, p-value=0.196)

Following a quantitative analysis by ELISA of cathelicidin levels in 30 blood samples, ROC curves were used to assess the potential use of blood cathelicidin detection in patients with sarcoidosis. The

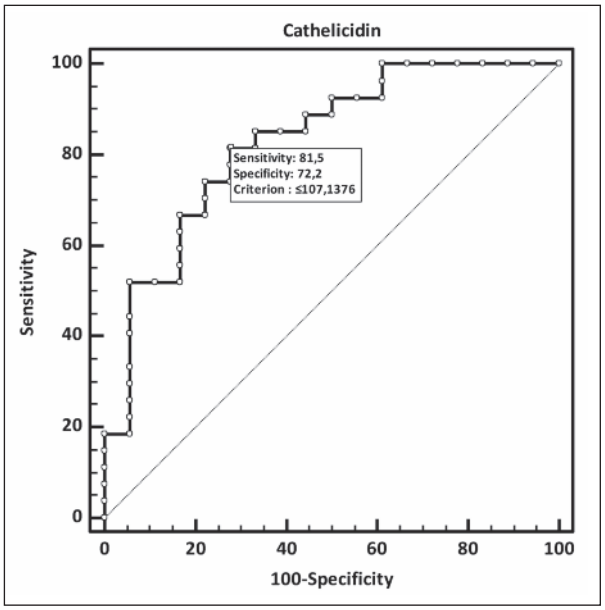

Fig. 1. Evaluation of cathelicidin as a marker for sarcoidosis. The ROC curve of cathelicidin for distinguishing sarcoidosis from healthy controls was based on an optimum cut-off value of 107.14 pg/ml corresponding to 81.5% sensitivity and 71.2% specificity. The area under the ROC curve was 0.734 (95% CI, 81.7–99.9).

area under the ROC curve (AUC), representing cathelicidin levels for the diagnosis of sarcoidosis was 0.734 (95% CI, 81.7-99.9). The analysis rendered an optimum cut-off value of 107.14 pg/ml, corresponding to lower values discriminating sarcoidosis from healthy controls with 81.5% sensitivity, 71.2% specificity, 81.5% positive predictive value, and 72.2% negative predictive value (Figure 1).

Discussion

The main finding of this study was that blood cathelicidin and vitamin D levels are statistically lower in sarcoidosis and tuberculosis patients than in healthy controls. Our findings echo observations from other researchers who also showed a higher prevalence of vitamin D insufficiency in tuberculosis patients (13-19). Another important observation was the lack of a correlation between systemic concentrations of vitamin D and cathelicidin, which was also in agreement with previous tuberculosis studies (23).

Hypovitaminosis D is important for sarcoidosis and seems to be a potential risk factor for disease activity (24). Vitamin D levels were significantly lower in sarcoidosis patients than in tuberculosis patients. This could be useful as an indicator of sarcoidosis versus tuberculosis in countries with a high tuberculosis prevalence. More prospective studies with a larger sample size are needed.

So far, very few studies have evaluated the role of cathelicidin in sarcoidosis. The first study to assess cathelicidin levels in bronchoalveolar lavage fluid (BAL) of sarcoidosis patients aimed to measure cathelicidin as a marker of enhanced activity of the epithelial barrier system in patients with pulmonary sarcoidosis and suggested that elevated BAL cathelicidin levels were responsible for the low frequency of respiratory infections in these patients (20). This study demonstrated that cathelicidin is located in alveolar macrophages, bronchial epithelial cells, and bronchial glands, suggesting it has a defensive role in the airway mucosa. It also showed similar levels of cathelicidin mRNA in the BAL fluid of two Scandinavian patients with sarcoidosis and three control patients. Barna BP et al. reported reduced cathelicidin levels in the BAL of severe sarcoidosis patients but these levels were not reduced when non-severe sarcoidosis patients were compared to controls. They suggested that cathelicidin deficiency might delay the resolution of sarcoidosis inflammatory pathways (21). The difference in these two studies could be explained by the difference in vitamin D levels in these two patient populations, proposing that the role of cathelicidin in the activation of the innate immune system may be most significant in patients with an increased susceptibility to vitamin D deficiency, like our population with 47% severe hypovitaminosis D (3).

The differential diagnosis of sarcoidosis from tuberculosis, lymphoma, and lung cancer is ultimately important for its management, and the pathological evaluation of the tissue sample is the gold standard. Until now, various biomarkers have been used as simple diagnostic marker of sarcoidosis: serum amyloid A (SAA), soluble interleukin 2 receptor (sIL-2R), lysozyme, the mucin-like high-molecular-weight glycoprotein KL-6, and angiotensin-converting enzyme (ACE) (25). Probably, the most well known is ACE, which has an 84% positive predictive value and a 74% negative predictive value, with higher values suggested for tuberculosis (26). Cathelicidin levels of 107.14 pg/ml, with lower levels in-

dicative of disease, have a sensitivity of 81.5%, a specificity of 71.2% and positive and negative predictive values of 81.5% and 72.2%, respectively. In our study, these findings are important for proposing blood cathelicidin levels as potential non-invasive markers for the diagnosis of sarcoidosis.

The main limitation of our study is the sample size. Our data needs to be confirmed in longitudinal studies to verify the association between different blood cathelicidin levels and the severity of sarcoidosis.

To the best of our knowledge, this is the first prospective case-control study reporting lower blood cathelicidin levels and no correlation between vitamin D and blood cathelicidin in sarcoidosis. This outcome emphasizes the effect that the innate immune system has on these two diseases and also provides a link between them. It is also the first study to detect a cut-off value for cathelicidin, with lower values suggestive of sarcoidosis. Furthermore, our study was strengthened by the measurement of serum 25(OH)D levels, which are an objective measure of body vitamin D status, which is significantly lower in sarcoidosis.

In summary, cathelicidin status was lower in granulomatous lung disease such as sarcoidosis and tuberculosis. Further studies are warranted to confirm our results and to further investigate the underlying mechanisms and possible role of cathelicidin as a future treatment option.

Author contributions

Each author has contributed substantially to this paper. E.K. was the principal investigator of the study. M.G.O, E.C. and L.P.O. contributed to the study design. A.B. and E.S.A. were responsible for acquisition of data. H.U. conducted statistical analysis, interpreted results, and drafted the initial manuscript. B.O. and H.U. realized the laboratory part of the study. L.P.O. and M.G.O. participated in the data interpretation and contributed to the final draft of the manuscript with important intellectual content.

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