Research Article (Araştırma makalesi)

Evaluation of Sputum Eosinophil Level and Presence of Charcot-Leyden Crystals in Patients with Airway Hyperreactivity

Havayolu Hiperreaktivitesi Olan Hastalarda Balgamda Eozinofil Düzeyi ve Charcot-Leyden Kristal Varlığının Değerlendirilmesi

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Özet

Amaç: Bu araştırmanın amacı, eozinofilinin bronş hiperreaktivitesine neden olan bağımsız bir faktör olup olmadığını veya balgamdaki varlığının bu durumda tamamlayıcı bir rol oynayıp oynamadığını özellikle astım vakalarında araştırmaktır.

Gereç ve Yöntemler: Veriler, Kahramanmaraş Sütçü İmam Üniversitesi Tıp Fakültesi Hastanesi Göğüs Hastalıkları servisine yatan 100 kişiden oluşan bir örneklemden elde edildi ve bronş hiperreaktivitesi ile karakterize edilen vakalara odaklanıldı. 30 astımlı, 34 astım dışı solunum hastalığı grubu ve 36 kontrol grubu hastasından oluşan üç grup oluşturuldu. Hastaların ilk servise yatırıldığı anda balgam örnekleri toplandı. Bu nedenle önceden var olan hastalıkların bulgusu olarak da eozinofili ortaya çıkabileceği gözönünde bulunduruldu.

Bulgular: Açıklamak gerekirse; eozinofili oranını üç grupta tanımlandı ve ayrıca smear incelemesinde Charcoat- Leyden kristallerinin varlığını araştırıldı. Çalışmanın sonuçları, astımlılarda eozinofili oranının daha belirgin (%16,6) olduğunu ve geçmiş sonuçları tekrarladığını göstermekteydi. Astımlı olmayan hasta grubundan elde edilen sonuçlar, eozinofili hasta yüzdesinin astımlılardan daha az olduğunu doğrulamaktaydı (%13,3). Hipotezlerle tutarlı olarak kontrol grubu en az yüzdeye (%8,8) sahipti.

Sonuç: Çalışmada eozinofili ile Charcoat Leyden kristallerinin varlığı arasındaki ilişki incelendi. Tüm çabalarımıza rağmen incelenen smearlerin hiçbirinde kristal yapı tespit edilmediğinden herhangi bir korrelasyon görülemedi.

Anahtar kelimeler: Astım, Bronşiyal Hiperreaktivite, Eosinofili.

Abstract

Objective: The focus of this research is to clarify whether eosinophilia is an independent factor in causing bronchial hyperreactivity or if its presence in sputum plays a supplementary role in this condition; especially in asthma.

Material and Methods: Data was obtained from a sample of 100 individuals in the respiratory care unit, concentrating on cases characterized by bronchial hyperreactivity. We made three groups with 30 asthmatics, 34 non-asthmatic respiratory disease group and 36 control group patients. We decided to get the patients' sputum when they were internalized first. Eosinophilia can therefore occur in addition to pre-existing illnesses.

Results: To explain; we defined the ratio of eosinophilia in three groups and also searched for the presence of Charcot-Leyden crystals out of smear examination. The results of the study demonstrate eosinophilia ratio among asthmatics was more prominent (16.6%), replicating past findings. Results from the non-asthmatic patient group confirm that percentage of patients with eosinophilia was lower than that of asthmatics (13.3%). Consistent with hypotheses, control group had the least percentage (8.8%).

Conclusion: The study examined the relation between eosinophilia and the presence of Charcot-Leyden crystals. Despite our efforts, we were unable to observe any signs of coexistence, as no crystal structures were detected in any of the smears examined.

Keywords: Asthma, Bronchial Hyperreactivity, Eosinophilia.

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INTRODUCTION

Inflammation of the airways is an important cause of respiratory diseases such as asthma, chronic obstructive pulmonary disease or chronic cough. Therefore, the collection of airway samples is an essential step for both research and clinical purposes. As a result, sputum sampling is popular as a safe, non-invasive alternative to bronchoscopy, which carries the risk of asthma exacerbation (1,2). Sputum induction is a technique that involves the induction and subsequent processing of expectoration primarily for the analysis of inflammatory cells in the airways in order to understand the underlying mechanism of various inflammatory diseases as asthma. Asthma is a chronic respiratory disease characterized by a persistent inflammatory process in which eosinophils play an important role (3).

Approximately when Osler released his initial textbook, The Principles and Practice of Medicine, highlighting the role of inflammation in asthma, Gollasch discovered eosinophils in the sputum of asthma patients. Afterwards, eosinophils were included of the characteristic process of asthma (4). The percentage of eosinophils in sputum in airway inflammation is a good marker for assessing prognosis in asthma patients. Validation and characterization of biomarkers are crucial for their successful use and should also confirm sensitivity, specificity, and productivity. We are searching if there is a statistically significant correlation between the disease and the eosinophil count in sputum (5). Treatments aimed at normalizing the eosinophil count in sputum may help prevent asthma attacks, and the eosinophil count in the blood is also related to the frequency of attacks. Therefore, the stratification of severe asthmatics as a basis for treatment decisions is complex. Therefore, reliable and sensitive biomarkers such as sputum samples are a valuable tool for physicians (5).

The Charcot-Leyden crystal (CLC) protein was first observed in 1853 by Jean-Martin Charcot in a leukemia patient and later detected by Ernst Leyden in 1872 in the sputum of asthma patients. The Charcot-Leyden crystals, named after him, are rhombic lysophospholipase crystals (hexagonal, bipyramidal) of up to 50 µm in length, which are produced by eosinophils (6). With the disruption of epithelial integrity, the eosinophils in the bronchial mucosa migrate into the bronchial lumen. The lysophospholipase enzyme released by the eosinophils crystallizes in vitro and in vivo. These crystals, which are found in the sputum of patients with airway hyperreactivity, are called "Charcot-Leyden crystals"(6). A protein called galectin-10, which is distributed in the inner and outer regions of the cell membrane, accounts for 10% of the eosinophil protein concentration. When the local Gal-10 concentration exceeds the threshold concentration resulting from the accumulation of these proteins released by eosinophil degranulation, crystallization begins and Charcot-Leyden crystals form (6). If the CLCs were easy to see; the crystals can be examined directly by light and phase contrast microscopy. Because Gal-10 in CLCs contains aromatic residues, it can also be examined directly by fluorescence microscopy (6). May-Grünwald and Papanicolaou staining of the CLCs show colour blue and orange, respectively. CLCs can also occur in eosinophilic diseases such as asthma, allergic reactions, fungal and helminthic infections and rarely in hematologic and neoplastic diseases. These crystals can also be detected in stool examined for parasites, in respiratory secretions or, more rarely, in tissue biopsies (6). We are trying to answer the question here is to decide whether eosinophilia is an independent factor for bronchial hyperreactivity disorders. Eosinophilia causing symptoms manifesting in addition to pre-existing conditions, masked as a worsening (7).

MATERIALS AND METHODS

Study design

In this prospective study, patients with bronchial hyperreactivity and cough were examined between June 2024 to September 2024. The clinical history, diagnosis as well as accompanying diseases were recorded. In the present study, the inclusion criteria were as follows: (1) between 16 and 80 years of age; (2) bronchial hyperreactivity as the symptom of internalization to service; (3) patients had sputum examination. Corticosteroids and specific biological agents have demonstrated positive results in eosinophilia, so we exclude the patients receiving corticosteroids. We collected the data from routine examined sputum samples received from the respiratory diseases ward.

In our study, the examination of sputum samples coming to the Medical Microbiology Laboratory, sputum smear samples were examined using May Grünwald and Gram staining methods. Gram stain revealed the quality of sputum and presence of PMNL in smears (more than 25 PMNL, less than 10 epithelial cells). Presence of Charcot-Leyden Crystals and percentage of eosinophils in Giemsa-stained smears were investigated. The dependent variable of the study was eosinophil percentage and the presence of crystals in the participants. While the presence of airway hyperreactivity in the participants would be investigated with the diagnosis they held in hospital, and demographic data would constitute the independent variable.

For each sputum sample, the number of PMNL per field of view (10 × objective) were recorded. Sputum samples were classified as good quality by three different criteria: (i) <10 epithelial cell (ii) <10 SEC or >25 PMNL. A magnification of 10 to 10 x 10 was used as a measure of the validity and quality of the sputum samples taken. Attention is paid to the presence of fewer epithelial cells and more than 25 polymorphonuclear leukocytes. In addition, the participants in the research group are asthma patients, and the control groups are patients with respiratory pathology but not asthma, and other pathologies referred to the hospital. taken to ensure that they have similar demographic characteristics, and overlapping clusters were used. After ethics committee approval, since the sputum samples coming to the laboratory for routine examination were examined, sputum samples were not taken; therefore, patients would not be asked to provide an informed consent form. Financial support to be provided by the researchers and used for the expenses of the dyes.

Statistical analysis

Frequency (%) was given for categorical variables, mean ± standard deviation, and median (minimum-maximum) for continuous variables. When examining whether there was a statistically significant difference between the eosinophil percentage and the asthma and non-asthmatic groups, the Mann-Whitney U test was used since the assumption of normal distribution was not met. Statistical significance level was accepted as $p<0.05$. Evaluation of the data was done using the SPSS 11.5 for Windows program.

The study followed the international principles of Helsinki and was approved by the Local Ethics Committee (date 04.11.2024/protocol no: 259).

RESULTS

30 patients were all asthmatics. 34 of the patients in our study were not asthmatic but had respiratory pathology; 36 were non-asthmatic and had non-respiratory pathology. Out of non-asthmatic respiratory tract pathologies, upper respiratory tract infections, chronic cough, bronchiectasis, chronic obstructive pulmonary disease, and pleural effusion were present. Nonrespiratory pathologies were coronary heart disease, hypertension, diabetes mellitus, and rheumatoid arthritis.

No correlation was found between age and sputum eosinophilia; the mean age among 34 asthmatic patients was 54, and patients having other respiratory pathologies had a mean age of 58. The third group had a mean age of 54. In studies that took gender into account, the value was found to be higher in women than in men, but in our study, among all groups, all eosinophilia diagnosis in sputum was found in men. Demographic variables such as gender and ethnicity are thought to be responsible for the different values found in various studies on sputum eosinophilia. In our study, all members' ethnicity was the same, and gender couldn't be accounted for because all patients were men, other for 5 women.

What about the difference among groups? The eosinophil counts are used to detect eosinophilic airway inflammation in our study. Eosinophilia in patients with asthma was found in %1 in 5 asthmatic patients (%16.6), %3 in 4 of patients with other respiratory pathologies (%13.3); including chronic obstructive pulmonary disease exacerbation, pulmonary carcinomas, and pneumonia. Moreover, the control group containing non-asthmatic and non-respiratory pathology patients had % 1 of counted eosinophils in 3 of the patients (%8.8).

DISCUSSION

Every day, many patients with lung diseases and numerous complaints are treated both in the outpatient clinic and on the ward. They are usually admitted to the hospital with complaints related to the diagnosis of pneumonia. They may be chronic obstructive pulmonary disease or mainly asthmatic patients. Sputum examination could be used to monitor asthma and could be performed with limited resources. It is a simple, non-invasive and inexpensive method (1). The sputum cell counting by 400 non-tumour cells; the percentage of eosinophilic cells (Eos%) about 2.5% was defined as eosinophilia in the sputum (8). We had the data by smear examination, stained with May Grünwald, after we decided the sputum was a sample taken properly and was qualified. In the sputum of asthmatics, we found 16.6 % having eosinophilia, and in other respiratory pathologies, found to be 13.3%; in the control group, patients with eosinophilic sputum were 8.8%. We found higher percentages in asthmatics, supporting older studies sharing the idea of reduced eosinophil apoptosis and sputum eosinophil load were correlated with the severity of asthma (9).

Patients with eosinophilia with other respiratory pathologies were pneumonias; we thought that all cell types are increasing in number in infections, so the eosinophils. Some diseases exhibit eosinophilia and are referred to as eosinophilic lung diseases of known or

unknown origin. The percentage of eosinophils in peripheral blood can also be determined; bronchoalveolar lavage fluid and sputum are important samples of the assessment (3). We attempt to answer the question of whether eosinophilia is an independent causative factor or the presence of eosinophilia in sputum is an additional causative factor to bronchial hyperresponsiveness disorders that may have existed prior to the finding of eosinophilia. So we decided to get the patients' sputum when they were internalized first. The symptoms caused by eosinophilia can therefore occur in addition to pre-existing illnesses and be masked as an exacerbation. In a study held in Turkey among the general population, blood eosinophil count was measured in 18-79-year-old people; the mean eosinophil count value was 140 eos/μL, and percentiles were not reported. Eosinophilia at 2.59% is an average rate (10).

In our control group, the least extent of eosinophilia was observed; usually, expectorated sputum is examined for specialization of cell types and to measure the presence of inflammation in the airways. We used smear examination for this process(1). Also, laboratory processing of sputum was significantly important to get results right. According to recent studies, the eosinophil counts of our population are also compared with other populations. It is also unclear whether there is a correlation between the disease and the eosinophil count; also, there may be diurnal variation in sputum as in blood. In eosinophilic asthma, a subtype of asthma characterized by an increased concentration of eosinophils in the airways, peripheral blood and sputum, a complex interplay of factors plays a role in the progression of the disease. Although a standard definition of eosinophilic asthma remains not clear, eosinophilic asthma is often indicated by peripheral blood eosinophil counts that surpass specific benchmarks, such as >150 cells/μL, >300 cells/μL, or >400 cells/μL in peripheral blood (1) and eosinophil counts in sputum of greater than 2 to 3% describes eosinophilic asthma in clinical studies (11).

In a study conducted among the Korean population, the cut-off for sputum eosinophilia was 3.5% (1). In another wide study, blood eosinophil count has moderate accuracy in predicting eosinophils in the sputum of 3% or more in asthma patients. Patients with stable COPD Show that blood eosinophil count at a threshold of 0.3/L could help determine the sputum eosinophilia(12). Balazs et al. found that blood eosinophil count is a good surrogate for identifying sputum eosinophilia (>3%) in stable COPD. Nevertheless, the role of peripheral eosinophil count in predicting sputum eosinophilia in patients with chronic cough is still unclear (13).

The limitations of our study indicate that better methods are needed to assess the involvement of eosinophils in the disease. The cell count in induced sputum is the most reliable method for detecting eosinophilic airway inflammation. However, the induced sputum test is not universally applicable due to its time-consuming and labour-intensive procedures. In addition, some patients with chronic cough have a dry cough, making it difficult to obtain sufficient sputum for cell differential analysis. Therefore, it is difficult to find a simple and reliable biomarker for the prediction of eosinophilic airway inflammation by sputum examination that has clinical significance for chronic cough (1).

Ethics: The study followed the international principles of Helsinki and was approved by the Local Ethics Committee (date 04.11.2024/protocol no: 259).

Author Contribution and Conflicts of Interest: The authors contributed equally to the study and have no conflicts of interest to declare.

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