Original Article

COPD

Bronchoalveolar lavage fluid (BALF) differential leucocyte count in Patients with Chronic Obstructive Pulmonary Diseases

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ABSTRACT

Objective: The aim of this study is to determine Bronchoalveolar lavage fluid (BALF) differential leukocyte count (DLC) in patients suffering from chronic obstructive pulmonary disease (COPD) and to identify the predominant cell type in our set of population.

Study Design: It is a cross sectional study.

Place and Duration of Study: This study was conducted at a Tertiary Care Hospital in Karachi, Pakistan from March 2012 till October 2012.

Material and Methods: The study comprises of 140 patients with COPD with no treatment or poor compliance to treatment and no history of any interventional procedures. All patients have undergone pulmonary function tests (PFTs) to differentiate obstructive pattern disease from restrictive. Fiber optic bronchoscopy was also done, after which 10 cc of BALF was collected and run for DLC.

Results: Patients were divided in two groups A and B according to gender, and each group was further divided in to two subgroups on the basis of age i.e. above 40 years and below 40 years. All patients have higher percentage of lymphocytes as compared to polymorphs.

Conclusion: A higher concentration of lymphocytes as compared to polymorphs in COPD patients is considered to be an uncommon in Pakistan as per literature survey is concerned, because very less work is done on BALF analysis. Our study suggests that percentage of lymphocytes in patients suffering from COPD is quite high and cases are not restricted to classical acute pulmonary infections.

Key Words: Bronchoalveolar lavage fluid, Chronic Obstructive Pulmonary Diseases, Pulmonary function tests, Polymorph nuclear cells, Lymphocytes

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) has been the dominant focus of airways research interest, at least until recent times [1]. A multitude of studies have examined its tapestry of airway inflammation and remodeling[2]. Sophisticated theories have resulted, encompassing environmental exposures, epithelial repair, and epithelial-mesenchymal signaling[2]. Therapeutic successes have followed, encouraging more interest and further investment. COPD is generally considered a self-inflicted condition that results in the majority of cases due to chronic smoking and the only effective treatment to date is long term oxygen therapy and bronchodilator drugs as an adjuvant therapy[3]. Neutrophils, macrophages, and CD8+ T lymphocytes are known and predictable responders to a sustained noxious insult[4]. However, in recent years something of a renaissance has occurred. COPD inflammation research has burgeoned, a rise in lymphocytes cellular concentration in majority of cases suffering from COPD has been reported by some recent studies[5].

Animal model studies and in vitro experiments have explored the roles of specific inflammatory cells in COPD, while bronchoscopy, sputum induction and lung resection studies have described airways inflammation in vivo. All have helped to confirm dominant positions in the inflammatory hierarchy for lymphocytes. Other inflammatory cells such as neutrophils, mast cells, eosinophils and natural killer cells have been credited with less importance[5,6].

The pathogenesis of smoke-induced COPD has been evaluated in a number of studies showing the accumulation of inflammatory cells in patients airways, predominantly lymphocytes in samples (sputum, bronchial and bronchoalveolar lavage) representative of intraluminal inflammation and mainly lymphocytes and macrophages in tissue specimens[6]. While the reason(s) for this presentation is still not completely clear; subsequently these studies have opened the field to new questions[6,7].Since the relevance of airway inflammation in COPD has been demonstrated, the next step is the characterization of the different features of air-way inflammation, such as the nature of chemotactic signal(s) and the contribution of the various cell types

causing airway inflammation through the release of specific inflammatory mediators[7].

In recent years, number of studies related to pulmonary disorders has used interventional techniques which were proved to be a successful method in getting the true sample of differential cell count from effected tissue. There are various interventional techniques which are being used e.g. sputum induction, bronchial and bronchoalveolar lavage but the most accredited method is bronchoalveolar lavage[8,9].

Bronchoalveolar lavage (BAL) is commonly and frequently performed as one of the first line procedures for diagnosing pulmonary lesions and infiltrates etc. BAL entails advancing a fiber optic bronchoscope distally into a subsegment bronchus, followed by instillation of saline, which is retrieved along with cellular debris. Pneumothorax, hemothorax, or exacerbations of respiratory failure may occur rarely due to BAL[9].

Extensive Literature Survey revealed that many interventional studies have been conducted to analyze differential leukocyte counts in various pulmonary diseases via BALF in recent years but no such type of interventional study has been done in Pakistani population[10]. In this regard, this study was designed in Karachi, Pakistan to target COPD patient's population of nonatopic current smokers and tobacco users, with the aim of investigating the cellular characteristics of bronchoalveolar lavage fluid in these patients.

MATERIALS AND METHODS

The study was conducted in Karachi, the largest city of Pakistan with a population of approximately 23.5 million [11] belonging to different ethnicities. It is a cross sectional study conducted between March 2012 to October 2012 in which 140 patients were recruited with history of chronic obstructive pulmonary diseases either with no treatment or poor compliance to treatment and with no history of any interventional procedures.

A written informed consent and a detailed medical and surgical history were taken from all patients. Pulmonary function tests (FEV1, FVC, and FEVI/FVC) were performed to diagnose obstructive lung disease.

All patients underwent fiber optic bronchoscopy in which 10 cc of bronchoalveolar lavage fluid was collected. All bronchoscopies were conducted by pulmonologist at tertiary care hospital and BALF differential leukocyte count was performed in Ziauddin University Hospital laboratory.

BAL was performed using fiber optic bronchoscope. The patients were premedicated with atropine, and given local anesthesia (tetracaine 0.5%) to relax the larynx and bronchial tree.BAL was performed by standardized washing of the middle lobe four times with 50-mL aliquots of sterile saline (0.9%NaCl) at 37ºC.Recovered BALF was kept on ice in a siliconized specimen trap, and was separated from cellular components by centrifugation (5 min, at 350 x g). Supernatants were directly stored at -70ºC after an additional centrifugation step (10 min, at 1,000 x g). Cells were washed twice, counted, and suspended in minimal essential medium (MEM; Gib-co, Grand Island, NY) supplemented with 1% bovine serum albumin (BSA; OrganonTeknika, Boxtel, the Nether-lands). Preparations of cell suspensions were made in a cytocentrifuge (Shandon, Runcorn, UK). Cytospin slides of BALF cells were stained with May-Grünwald-Giemsa (MGG; Merck, Darmstadt, Germany) for cell differentiation.

A 3cc sample of blood was taken to estimate erythrocyte sedimentation rate (ESR) by westergren method as per protocol, to identify active inflammatory status.

RESULTS

All subjects (n=140) were divided in two equal groups according to gender. Group A and Group B and each group were sub divided into two equal groups on the basis of age i.e. above 40 years and below 40 years. All patients were belonging to low socio economic status. Males were mostly laborers in leather or hosiery factories with known addictions of smoking and gutka. All gave the history of on and off upper and lower respiratory tract infections that was treated with empirical antibiotics, bronchodilator drugs and steroid therapies without any proper investigations (Table 1). All females were house wives using coal and kerosene stoves with similar past medical history as males. 34 females showed addiction towards huqa, in age group above 40 years. 36 females of age group less than 40 years showed addiction to gutka (Table 1).

All patients BALF were checked for differential leucocyte count as shown in Table 2. Ingroup A, above 40 years age subgroup showed an average white blood cell (WBC) count of 9.9 x 10^9/L, with 35% polymorphonuclear cells and 65% Lymphocytes. Erythrocyte sedimentation rate was 60. Age group below 40 years, showed an average WBC count of 10.7 x 10^9/L, with 22% polymorphonuclear cells and 78% Lymphocytes. Erythrocyte sedimentation rate was 73 in this subgroup. In group B, above 40 years showed an average WBC count of 11.0 x 10^9/L with 30% and 70% polymorphonuclear cells and Lymphocytes respectively. Erythrocyte sedimentation rate was 80. In group below 40 years age, showed an average WBC count of 10.3 x 10^9/L, with 25%. polymorphonuclear cells and 75% Lymphocytes. Erythrocyte sedimentation rate was 77. No gender variation was noted in underlying pulmonary disorders, especially those associated with bronchopulmonary obstruction (viz. Bronchiectasis, emphysema, asthma, tuberculosis, and malignancy).

Table No. 1: Comparison of different female age group less than 40 years showing addiction to gutka

Gender	Age Group	Age	S/E Status	Occupation	Residence	Habits	Treatment History
Group A Males (n=70)	< 40	23	LSE	factory worker	Karachi	Smoker (35)	Symptomatic therapy
	>40	55	LSE	factory worker	Sindh	Smoker (35)	Symptomatic therapy
Group B Females (n=70)	<40	21	LSE	House wife	Karachi	Gutka (36)	Symptomatic therapy
	>40	49	LSE	House wife	Karachi	Huqa (34)	Symptomatic therapy

S/E: socio economic, LSE: low socio economic, A/B: antibiotics

Table No.2: Different gender age groups giving different averages with regard to WBC, PMN, ESR, Lymphocytes

Gender	Age	Average WBC count (x 10^9/L)	Average PMN%	Average ESR mm/hr	Average Lympho-cytes %
Male	Above 40	9.9	35	60	65
	Below 40	10.7	22	73	78
Female	Above 40	11.0	30	80	70
	Below 40	10.3	25	75	68

WBC: white blood cell; PMNs: Polymorphonuclear neutrophils; Lym: lymphocytes; ESR: erythrocyte sedimentation rate

DISCUSSION

Our study suggests that bronchoalveolar lavage is an important tool and valuable interventional technique for accurately evaluating the inflammatory and immune processes of the human lung[9,10]. Although this lavage recovers only those cells and proteins that are present on the epithelial surface of the lower respiratory tract as compared to open lung biopsies that also provides an information of those constituents, which are representative of the inflammatory and immune systems of the alveolar structures and this is also evident by other studies[10,11]. The peculiar feature of BAL technique due to which it becomes superior is that the clinician may obtain sufficient materials from diseased individuals to allow characterization of not only the types of cells and proteins present but also the detailed examination can also be done to see other biomedical markers[11]. Such observations have been useful in defining the inflammatory and immune capabilities of the normal lung and provide a basis for the study of lung disease [12]. From the data already acquired, it is apparent that bronchoalveolar lavage will yield major insights into the pathogenesis, staging, and therapy decisions involved in these disorders as compared to sputum induction and complete blood count.

Studies in adults with COPD have described alterations in the cellular composition of BALF[13, 14], but our study suggests that BALF can also be expected to play a vital role in assessment of disease stage, patient`s therapeutic response, prognosis and immunological status of COPD as also reported by study done in USA by Tuder RM et al in 2012[14]. Defining the BALF differential cell count is an essential prerequisite for the interpretation of changes observed in lung diseases and this study was done as a first step to define the cellularity of BALF in adults suffering with chronic pulmonary diseases in our set of population.

Very little information is available concerning BALF lymphocyte sub count in COPD population. One of the studies showed that total WBC count was not significantly different as compared to control but the number of lymphocytes was increased and their ratio tended to be high but not reaching the level of statistical significance[14]. Our subjects with clinically favorable course of the disease showed a distinct high ratio of BALF lymphocytes, but further phenotypic analysis had not been performed. Biswas SK et al compared the significance of BALF cellular analysis with peripheral complete blood count in 2012 and reported that the number of cells present in peripheral blood picture is 30 % less than the actual cellular count at the site of infection[15].

We observed a high percentage of lymphocytes in the total WBC counts of the BALF samples in our study population. The above finding is in concordance with the marked intra and inter individual variability previously demonstrated in a COPD population [16,17] making the analysis of differential cell counts more suitable for differentiating COPD from other lung diseases[15,16]. The interesting finding of our study is that the total cells as well as lymphocytes in our population tended to be higher in both age groups that is above 40 and below 40 years with no gender variation, although the exact molecular mechanism(s) for this increase in total cell count has not been explored yet, but it might be either related to smoking or some other factor(s).[17,18]

We have assessed the smoking history of the patients by questionnaire, and were able to correlate active as well as passive smoking with absolute cell count of the recovered BALF. However, questionnaires are not considered a reliable tool in determining smoking exposure[19]. While we found a relationship between active and passive smoking with BALF cellularity but more detailed study is recommended to address this question.

A considerable rise was also observed in the relative proportion of lymphocytes in smokers and tobacco chewers both as compared to healthy non smoking adult population, which is also supported by the study done in 2007 by Sun YP et al on non smokers. In the mentioned study mean lymphocyte counts was found between 4–18% from the cells recovered from BALF [20,21]. While most of the studies reporting mean values of less than 10%. The mean fraction of lymphocytes in our study was 65±12% with a median of 12%, which is higher than in most studies done on COPD patients [23-25]. As it has been reported in larger series of COPD subjects, cells were not normally distributed, with individual subjects exceeding lymphocyte counts of 50% as observed in our study. This is well above the range reported in healthy nonsmoking adult volunteers[26]. However there is no reported data on BALF analysis of tobacco chewers but this is study is the first step towards BALF cellular analysis of COPD subjects chewing tobacco and will be available for comparison with future studies.

We are unable to scientifically explain the rise in the differential and total cell counts observed in our study. As has been demonstrated in COPD patients, some individuals may have subclinical alveolitis without clinical, radiological or functional abnormalities[27]. This phenomenon can be expected to occur in COPD population due to any prior or ongoing subclinical respiratory tract infections may have caused changes in the cellularity on the bronchoalveolar surface.

It has become an established fact that acute respiratory viral or bacterial infections occur very frequently in COPD patients[28] that may increase the incidence of non specific infections in this group, could influence the variability of lymphocyte counts. Whether the higher lymphocyte counts that we observed were a transient phenomenon or persisted for longer periods could only be clarified if subjects were followed longitudinally, which was not feasible in our study population.

ESR is performed in all subjects that it is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential)[29]. When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called 'rouleaux', which settle faster[30], in our study it was used as a protocol to identify active inflammatory status, as it is reported by Vimalanatha S et al in 2008 that ESR is a simple, in expensive and sensitive method of identifying acute inflammatory status.

In summary, we have studied cellular constituents of BALF in COPD patients. Significantly more lymphocytes and less granulocytes were present in our set of population. Lymphocytes were the predominant cell type, followed by granulocytes. Absolute lymphocyte counts were higher in both age groups, probably due to improper and poor compliance to treatment, recurrent subclinical infections, smoking, tobacco chewing and poor occupational standards. This study provides a first insight into BALF cellularity in the population of Karachi, and will be the basis for further studies in patients with COPD.

CONCLUSION

All patients with chronic obstructive lung diseases showed border line leucocytosis, significant lymphocytosis, raised ESR and mild to moderate dyspnea on the basis of examination and investigations. Hence our study throws a spot light on cellularity of BALF in a subpopulation of Karachi and provides a new horizon for research in COPD that will help towards better understanding of disease.

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