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ORIGINAL ARTICLE

THE PHAGOCYTIC ACTIVITY OF PERIPHERAL BLOOD MACROPHAGES IN COPD PATIENTS

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Rationale: *Macrophages numbers are elevated in the lungs of smokers and those patients with COPD, where they accumulate in the alveoli, bronchioles and small airways. The slow progression and chronicity of COPD parallels the chronic increase of macrophages that is seen in sites of tissue injury / damage and supports the concept that macrophages are at least in part responsible for the pathological consequences.*

Aim of the Work: *The aim of this work was to study the phagocytic activity of peripheral blood macrophages in COPD patients, either in the stable and during acute exacerbation compared to the normal subjects, which might be reflected on the therapeutic intervention strategies.*

Design: Prospective case – control study.

Subjects and Methods: *Seventy subjects were enrolled in this study, divided into two groups: Group I: 50 patients with COPD and Group II: 20 healthy volunteers serving as a control group.*

All subjects were submitted to ABG analysis, spirometric studies and assessment of phagocytic function, namely phagocytic and lytic indices using opsonised suspension of candida of their peripheral blood macrophages.

Results: *Results showed a highly significant statistical difference as regards both phagocytic and lytic indices among COPD patients (group 1) and control group (group 2) where both indices were significantly lower among the COPD group as compared to the control group indicating obvious phagocytic dysfunction among peripheral blood macrophages in COPD patients, and both indices were significantly lower in patients in exacerbation than patients in stable state.*

Conclusion: *this study is directed to the evaluation of the phagocytic function of peripheral blood macrophages among COPD patients, results revealed that there is actually a phagocytic dysfunction and that it is not always positively correlating to the severity of the disease. This also draws the attention to the peripheral blood cell population in COPD disease which had not taken its share of proper assessment. Hence, it would be recommended to include the peripheral blood cell population and macrophages in particular while searching in the pathophysiology and management of COPD disease.*

Keywords*: peripheral blood macrophage, phagocytic function, COPD.*

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. The airflow limitation is usually progressive, may be accompanied by an abnormal inflammatory responseof the lung to noxious particles or gases.(1) Chronic obstructive pulmonary disease is a major cause of chronic morbidity and mortality throughout the world, and it is the fourth leading cause of death in the world.(2)The characteristic symptoms of COPD are cough, sputum production and dyspnea upon exertion. Chronic cough and sputum production often precede the development of airflow limitation by many years and these symptoms identify individuals at risk of developing COPD.(3)

A diagnosis of COPD should be considered in any patient who has symptoms of cough, sputum production, or dyspnea, and /or a history of exposure to risk factors for the disease .The diagnosis is confirmed by spirometry. The presence of post bronchodilator FEV1<80% of the predicted value in combination with a FEV1/ FVC< 70% confirms the presence of airflow limitation that is not fully reversible.(4) The peripheral airways become the major site of airways obstruction in COPD. The structural changes in the airway wall are the most important cause of the increase in peripheral airways resistance in COPD.(5) Inflammatory changes such as airway edema and mucus hypersecretion also contribute to airway narrowing. The irreversible component of airflow limitation is primarily due to remodeling of the small airway.(6) Alveolar macrophages are the major immunocompetent cell population in the lower human respiratory tract, and are likely to play a role in the initiation, modulation and eventual termination of the inflammatory response that characterizes COPD.(7) There is marked increase (5-10 folds) in the numbers of macrophages in airways, lung parenchyma, BAL fluid and sputum of smokers and patients with COPD, most of which reach the lung via the blood.(8)

Aim of the work: The aim of this present study was to assess the phagocytic function of peripheral blood macrophages of COPD patients as compared to normal population and if it is related to the severity of the disease. Phagocytic index and lytic index using opsonised suspension of candida were used as reflection of phagocytic ability in this study.

METHODS AND SUBJECTS

The present study was conducted upon 70 subjects from those who are regularly attending the chest outpatient clinic in Ain-Shams University Hospital and also those who are admitted to the chest department and chest care unit.

The studied population was divided into 2 groups: ***Group (1):*** 50 patients with COPD diagnosed according to the GOLD guidelines and classifications, and ***Group (2):*** 20 healthy volunteers matched for age and sex serving as a control group. ***Group (1)*** was subdivided into two subgroups according to the disease state: ***Group (A):*** 25 COPD patients in a stable condition either smokers or ex-smokers and ***Group (B):*** 25 COPD patients in an acute exacerbation and most of them are ex-smokers.

The classification of cases is according to the GOLD 2006.

The staging is based on airflow limitation as measured by spirometry by measuring the postbronchodilator forced expiratory volume in one second (FEVI) and comparing it with the forced vital capacity (FVC) of the patient. This classification is essential for diagnosis and provides a useful description of the severity of pathological changes in COPD.

Stage 1: mild COPD; characterized by mild airflow limitation (FEV1/FVC < 70%, but FEV1 is still > 80% predicted).

Stage 2: moderate COPD: characterized by worsening of airflow limitation (50% <FEV1 <80% predicted) and usually the progression of symptoms. Stage 3: severe COPD: characterized by further worsening of airflow limitation

(30% <FEV1<50% predicted), increased shortness of breath, and repeated exacerbations. Stage 4: very severe COPD: characterized by severe airflow limitation (FEV1<30% predicted) or the presence of chronic respiratory failure.[9]

COPD patients are defined as having an exacerbation of the disease if they suffer from increased breathlessness, accompanied by wheezing and chest tightness, increased cough and sputum, change of the colour of sputum and fever.

The most common causes of an exacerbation are infection of the tracheobronchial tree and air pollution.

All subjects were submitted to ABG analysis, spirometric studies and assessment of phagocytic function, namely phagocytic and lytic indices using opsonised suspension of candida of their peripheral blood macrophages.

Steps of assessment of phagocytic function of cells:

Isolation of cells: A thick drop of blood (0.5 cm.) is placed directly on clean dry glass slide with a designed well using a corrector. The slide is then left in humid chamber at 37C for 30 minutes, after which the excess clot is removed and the slide is washed gently with PBS (Phosphate buffer saline).

Preparation of candida: A loopful of candida growth is suspended in PBS and washed two times & then resuspended in pooled fresh normal serum (collected from 7-10 normal fresh specimens). The count of candida is adjusted between 3-5X 100000 /ml poled serum.

The prepared opsonised suspension of candida is placed over the well with the isolated cells and left in humed chamber at 37 C for 45 minutes. After incubation excess candida is washed by PBS and the slide is left to dry then stained using Leishman stain.

$$\text{Phagocytic index} = \frac{\text{No. of phagocytosed candida/100 cells}}{100}$$

$$\text{Lytic index} = \frac{\text{No. of lysed candida / 100 cells}}{100}$$

STATISTICAL ANALYSIS: Statistical analysis of the results was performed according to Duncan Multiple Comparison Test and the results are presented as (mean and SD).

Statistical significance was determined by using unpaired T test analysis of the variance.

P value = level of significance.

P > 0.05 ----- insignificant correlation (NS) >

P < 0.05 ----- significant correlation (S).

P < 0.001 ------ highly significant correlation (HS).

RESULTS

Table 1. The studied population in the present study.

	Cases		Control
Number	50		20
Disease state	Stable	Exacerbated	
	25	25	
Smoking history	Smoker	Smoker	Smoker
	3 (12%)	5 (20%)	4 (20%)
	Ex smoker	Ex smoker	Ex smoker
	22 (88%)	20 (80%)	5 (25%)
	Non smoker	Non smoker	Non smoker
	Non (0%)	Non (0%)	11 (55%)

Table 1 shows the studied population in the present study where the total number of subjects in the present study is 70 subjects subdivided into 2 groups as follows: (group 1) composed of 50 cases of COPD patients. This group is subdivided into two equal groups, one of them contains 25 cases with COPD in remission state (stable) and the other group contains 25 patients in acute exacerbation, then we had 20 healthy volunteers served as a control group. The smoking history was illustrated for each group and they are all males.

Table 2. Comparison between patients and control group as regards phagocytic index and lytic index.

Parameter	Disease State	N	Mean ± SD	F	P	Sig.
Phagocytic index	Control	20	7.5±0.51 [a]	913.13	0.000*	HS
	Stable	25	2.6±0.76 [b]			
	Exacerbation	25	0.16±0.37 [c]			
lytic index	Control	20	7.15±0.37 [a]	1758.79	0.000*	HS
	Stable#	25	0.8±0.65 [b]			
	Exacerbation#	25	0±0 [c]			

Table 2 shows comparison between patients whether in stable state or in acute exacerbation and controls as regards phagocytic, and lytic indices using Duncan Multiple Comparison Test and the results are presented as (mean ± SD). There is a highly significant statistical difference between groups as regards phagocytic and lytic indices. Both indices are significantly lower in patients group compared to control group, and both indices are significantly lower in patients in Exacerbation than patients in stable state.

Table 3. Age, ABG parameters and FEVI in COPD and control subjects.

Age	control	20	49.4 ± 9.12 [a]	15.487	0. 000*
	stable	25	54.24 ± 7.7 [a]		
	Exacerbation	25	63.56 ± 9.43 [b]		
pH	control	20	7.35 ± 0.01 [a]	15.026	0. 000*
	stable	25	7.34 ± 0.01 [a]		
	Exacerbation	25	7.32 ± 0.03 [b]		
pco2	control	20	35.75 ± 1.89 [a]	80.663	0. 000*
	stable	25	48.20 ± 1.26 [b]		
	Exacerbation	25	57.72 ± 4.85 [c]		
po2	control	20	96.3 ± 1.56 [a]	842.188	0.000*
	stable	25	89.88 ± 1.64 [b]		
	Exacerbation	25	60.20 ± 4.93 [c]		
FEV1%	control	20	93.05 ± 3.58 [a]	971.300	0.000*
	stable	25	85.56 ± 2.77 [b]		
	Exacerbation	25	50.04 ± 4.24 [c]		

Table 3 shows comparison between the stable patients and patients in exacerbation and controls as regards age, PH, PCO2, PO2 FEV1% using Duncan Multiple Comparison Test. Results are presented as mean ± SD. There is no statistical difference between the group of stable patients and controls as regards age and pH while there is highly significant statistical difference between them in all other parameters.

Table 4. Correlation between phagocytic and lytic indices and PH, PCO2, PO2, FEV1% among patients in the stable state.

	Phagocytic Index			Lytic Index		
	R	P	Sig.	R	P	Sig.
PH	-0.009	0.965	NS	0.188	0.368	NS
PCO2	-0.087	0.680	NS	0.,051	0.808	NS
PO2	-0.040	0.850	NS	0.212	0.308	NS
FEV1%	-0.106	0.613	NS	-0.005	0.982	NS

Results demonstrated that PH, PCO2, PO2, FEV 1% has no significant statistical correlation to phagocytic and lytic indices.

Table 5. Correlation between phagocytic and lytic indices and PH, PCO, PO2, FEV 1% among patients at exacerbation.

	Phagocytic index			Lytic index		
	R	P	Sig.	R	P	Sig.
PH	0.152	0.469	NS	-	-	-
PCO2	-0.204	0.329	NS	-	-	-
PO2	0.253	0.223	NS	-	-	-
FEV1%	0.232	0.264	NS	-	-	-

Table 5 shows correlation between phagocytic and lytic indices and PH, PCO, PO2, FEV1% among patients at exacerbation using Pearson correlation. And results demonstrated that PH, PCO2, PO2, FEV1% has NO significant statistical correlation to phagocytic and lytic indices.

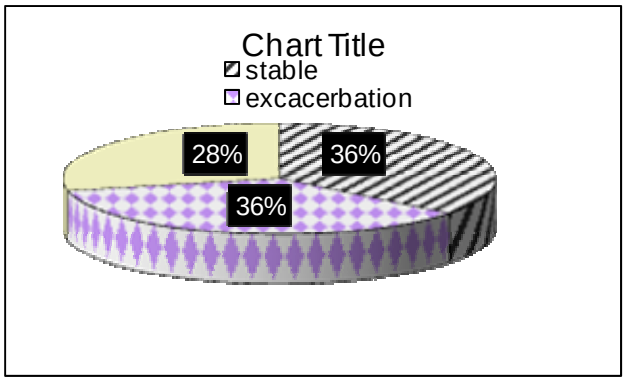

Fig 1. The studied population in the present study.

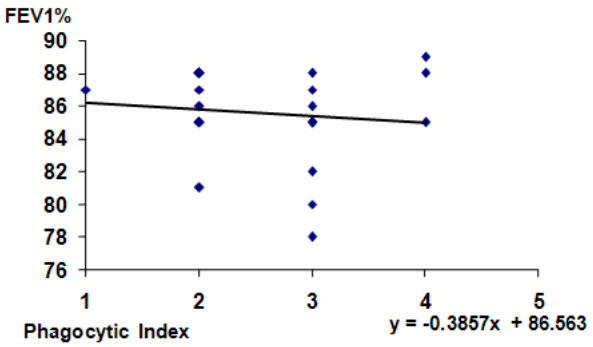

Fig 2. Correlation between phagocytic index and FEVI in COPD patients

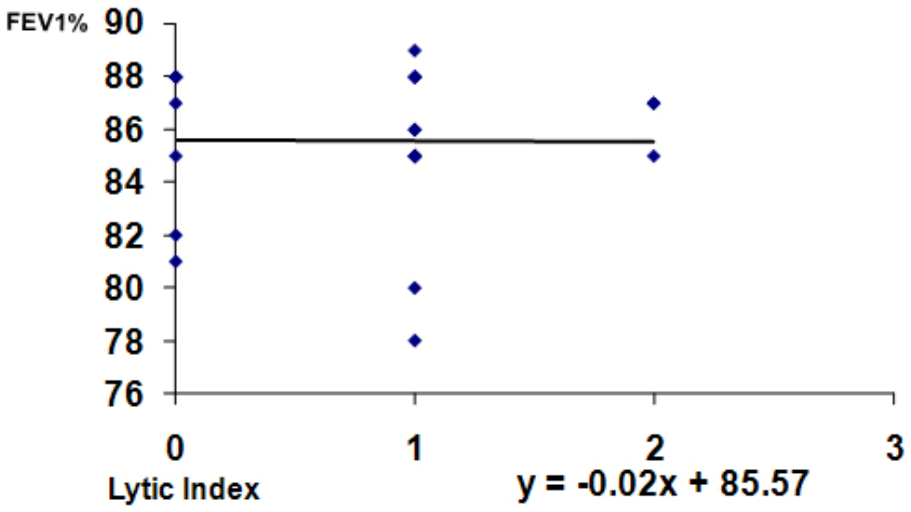

Fig 3. Correlation between lytic index and FEVI in COPD patients.

DISCUSSION

COPD is a disease state characterized by airflow limitation that is not fully reversible. It is usually progressive if exposure to noxious agents is continued.[10] The characteristic symptoms of COPD are cough, sputum production and dyspnea upon exertion. Chronic cough and sputum production often precede the development of airflow limitation by many years and these symptoms identify individuals at risk of developing COPD.[11] COPD can coexist with asthma, however the inflammation characteristics of COPD is distinct from that of asthma. Patients with COPD are heterogeneous in terms of their Clinical presentation, co-morbidities, underlying lung pathology, disease severity, and rate of disease progression.[12]

Thus it is highly unlikely that a single measure can accurately assess the severity of COPD, predict patient prognosis, and evaluate the effectiveness of therapy, thereby measuring all the dimensions of the disease.[13] Yet traditionally, the forced expiratory volume in one second (FEV1) has been used extensively as a global measurement for COPD. While the long term excessive decline of FEVI is the path gnomonic abnormality of COPD and may indeed relate to mortality, FEV1 varies little over short periods of time, even exacerbations, and it relates weakly to other clinical manifestations such as quality of life or exercise tolerance. It is clear, therefore, that other measures (besides FEV1) need to be identified and validated to allow a more complete and clinically relevant assessment of patients with COPD.(14).Exposure to inhaled noxious particles and gases causes inflammation of the lungs that can lead to COPD if the normal protective and / or repair mechanisms are overwhelmed or defective.[15] In addition to inflammation, two other processes thought to be important in the pathogenesis of COPD are: an imbalance of proteinases and antiproteinases in the lung, and oxidative stress. Pathological changes in COPD are found in the central airways, peripheral airways, lung parenchyma and pulmonaryvasculature.[16] Inflammatory changes such as airway edema and mucus hypersecretion also contribute to airway narrowing. The irreversible component of airflow limitation is primarily due to remodeling of the small airways.[17] COPD is characterized by an increase in neutrophils, macrophages and T-lymphocytes in various parts of the lung.[18] Normal PBM (peripheral blood monocytes) migrate to the lungs and mature into alveolar macrophages.[19]Alveolar macrophages are the major immunocompetent cell population in the lower human respiratory tract, and are likely to play a role in the initiation, modulation and eventual termination of the inflammatory response that characterizes COPD.[20] Alveolar macrophages release mediators such as TNF-alpha, interleukin 8, and leukotrien B4, which promote neutrophilic inflammation.[21]

There is marked increase (5-10 folds) in the numbers of macrophages in airways, lung parenchyma , BAL fluid and sputum of smokers and patients with COPD, most of which reach the lung via blood.[22] Seeking for more information about this important effector cell in COPD, studies have been conducted along the years for its functional assessment and thereby its effect on COPD development and severity.[23] The aim of this present study was to assess the phagocytic function of peripheral blood macrophages of COPD patients as compared to normal population and if it is related to the severity of the disease. Phagocytic index and lytic index using opsonised suspension of candida were used as reflection of phagocytic ability in this study.

The results of the present study show a highly significant statistical difference as regards both phagocytic and lytic indices among COPD patients (group 1) and control group (group 2) where both indices were significantly lower among the COPD group (phagocytic index was 2.6 ± 0.76 in pts. with stable COPD and 0.16 ± 0.37 in pts. with COPD in acute exacerbation , while the lytic index was 0.8 ± 0.65 in stable COPD pts. and 0.0 in COPD patients in acute exacerbation) as compared to the control group (7.5 ± 0.51 and 7.15 ± 0.37 respectively) indicating obvious phagocytic dysfunction among

peripheral blood macrophages in COPD patients.

One should question; whether the apparent phagocytic dysfunction observed among peripheral blood macrophages interrelate to the alveolar macrophage function?

There is an established fact that there is a marked increase (5-10 fold) in the numbers of macrophages in airways, lung parenchyma, BAL fluid and sputum of smokers and patients with COPD. A careful morphometric analysis of macrophage numbers in the parenchyma of patients with emphysema showed a 25- fold increase in the numbers of macrophages in the tissue and alveolar space compared with normal smokers approximately matched for cigarette exposure.[24] Furthermore, macrophages are localized to sites of alveolar wall destruction in patients with emphysema and small airway disease.[25] This huge recruitment of macrophages (initially derived from blood monocytes) into the different lung spaces explains the defective macrophage function in peripheral blood samples.

Similarly, we can assume that the phagocytic impairment observed in peripheral blood macrophages of COPD patients impairs the immune system of the body as a whole, needless to say the immunity of the respiratory system is included, therefore increasing the susceptibility of the body to invading micro-organisms with subsequent increase in frequency of exacerbations which aggravate the pathophysiology of the disease.

Moreover, macrophages activated under conditions of oxidative stress can lead to a more enhanced inflammatory response. Coupled with an impairment of the phagocytic response, this can lead to ineffective clearance of apoptotic cells and secondary necrosis, which results in failure to resolve the inflammatory response and establishment of a chronic inflammatory state.[26] One may question; whether the apparent phagocytic dysfunction observed in COPD patients correlates to other parameters that measure the severity of the disease as: FEV1, PO2, PCO2, and PH?

There was no significant correlation between both phagocytic & lytic indices of peripheral blood macrophages and FEV 1% indicating that the degree of phagocytic dysfunction of peripheral blood macrophages of COPD patients does not necessarily correlate with other parameters measuring the severity of the disease.

These findings reflect the nature of COPD disease in which the inflammatory process may be persistent and going on despite the apparent improvement in the clinical condition and pulmonary functions and even after smoking cessation which is a major cause of oxidative stress that leads to rapid deterioration of the pathophysiology of the disease.

In 2005, studying the phagocytic activity of peripheral blood macrophages was conducted on asthmatic patients. This research work was done in Ain - Shams University hospital, and the results showed a highly significant statistical difference as regards both phagocytic and lytic indices among asthmatics were significantly lower than the control group. There was also a significant positive correlation between both phagocytic & lytic indices of peripheral blood macrophages and PO2, i.e: the more impaired the phagocytic function of peripheral blood macrophages of asthmatics, the worse the oxygenation.[27]

These results are different from those obtained from the present study on COPD patients. This reflects the basic difference between bronchial asthma and COPD as two obstructive lung diseases with apparent similar clinical picture and clinical coarse, but with very different underlying pathology and pathophysiology.

In asthma, airflow limitation is largely reversible, either spontaneously or with treatment, and does not progress in most cases. On the other hand, airflow limitation in COPD is usually progressive and poorly reversible. In asthma, the chronic

inflammation causes an associated increase in airway responsiveness to a variety of stimuli, leading to recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and in the early morning. Many cells are involved in the inflammatory response in asthma and, among these, CD4+ Type-2 lymphocytes, mast cells and eosinophils are thought to play a crucial role. In COPD, the poorly reversible airflow limitation is associated with an abnormal inflammatory response of the lungs to noxious particles or gases.[28]

Asthma and chronic obstructive lung disease (COPD) are both associated with structural "remodeling" inappropriate to the maintenance of normal lung function.[29] In conclusion, this study is directed to the evaluation of the phagocytic function of peripheral blood macrophages among COPD patients, results revealed that there is actually a phagocytic dysfunction and that it is not always positively correlating to the severity of the disease. This also draws the attention to the peripheral blood cell population in COPD disease which had not taken its share of proper assessment. Hence, it would be recommended to include the peripheral blood cell population and macrophages in particular while searching in the pathophysiology and management of COPD disease.

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