Peripheral Blood Lymphocyte Cell Subsets and Their Association with Lung Functions in Patients with Chronic Obstructive Pulmonary Disease

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Abstract: Chronic obstructive pulmonary disease (COPD) is a treatable and preventable disease state, characterized by progressive airflow limitation that is not fully reversible. In the present study; 101 COPD patients and 52 age-matched healthy nonsmokers (HNS) control subjects were recruited and their pulmonary functions were assessed. The frequencies of $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, B, NK, NKT-like cells and $CD16^+$ were determined using flowcytometry. The potential association of lymphocyte cell subsets with disease severity was further analyzed. Statistically highly significant reduction in the frequencies of $CD4^+$ T cells, and the $CD4^+/CD8^+$ ratio, but increased frequencies of B cells, $CD8^+$ T cells, CD16, $CD3^-CD56^+$ NK and $CD3^+CD56^+$ NKT-like cells were observed in COPD patients compared to controls. There were significant positive correlations between the frequencies of $CD4^+$ T cells and $CD8^+$ T cells and pulmonary functions. A significant positive correlation between $CD4^+/CD8^+$ ratio and FEV_1/FVC was observed. While significant negative correlations between the frequency of B cells and pulmonary functions; and between the frequency of CD16 and FVC% were denoted. There were no significant correlations between the frequencies of all other cell types tested and pulmonary functions. Our data indicated that COPD patients have immune dysfunction which may play key roles in the development and progression of COPD. These findings suggest that modulating immune cells activation may be a new target for the treatment of COPD.
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1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most prevalent chronic adult diseases, affecting more than 200 million people worldwide, and has become the fourth leading cause of death ***(Lopez et al.,2006)***. Current therapies have very limited impact on disease progression, making greater understanding of pathogenesis crucial. Tobacco smoking is established as the main etiological factor for COPD ***(Mannino and Buist, 2007)*** and it is now accepted that COPD is an inflammatory disorder ***(Urbanowicz et al., 2010)***. The persistence of inflammation will produce other modifications such as bronchial fibrosis as well as the hyperplasia of the airway smooth muscle, either directly as an effect of the inflammation, or indirectly as a result of the contraction of airway smooth muscle. All of these modifications lead to the thickening of the bronchial wall which will generate a narrowing of the bronchial lumen and an airflow limitation ***(Petrescu et al., 2013).***Immune dysregulation arises in the peripheral blood of patients with COPD, and might contribute to the pathogenesis of the extra pulmonary effects of the disorder due to the overspill of inflammation in the lung into the circulation ***(Sinden and Stockley, 2010)***. The number of $CD4^+$T lymphocytes and the $CD4^+/CD8^+$ ratio have been shown to be less in COPD patients compared with healthy volunteers ***(Gupta et al., 2007)***, while the numbers of CD8+ T cells and B cells increase as COPD progresses ***(O'Shaughnessy et al.,1997).*** The role of natural killer (NK) and natural killer T (NKT)-like cells is attracting increased attention ***(Culley, 2009)***. These cells represent a small but important proportion of effector lymphocytes. NKT-like cells differ from conventional NK cells in that they are auto reactive and can mediate rapid and sustained production of both Th1 and Th2 cytokines. $CD8^+$T-lymphocytes, NKT-like cells and NK cells are the three main types of killer cells in the immune system and have been implicated in the pathogenesis of COPD ***(Paats et al., 2012)***. There are several studies reported the presence of an increased number of natural killer lymphocytes in the submucosa of the large airways of smokers with COPD ***(Barnes and Cosio, 2004)***. So; in order to verify the immune dysfunction in COPD; we detected the numbers of $CD3^-CD56^+$ NK cells , $CD3^+CD56^+$ NKT-like cells , $CD16^+$, $CD4^+$ T cell, $CD8^+$ T cell, and B cells in the peripheral blood of COPD patients and compared their frequencies with those in healthy nonsmokers (HNS) who acted as controls. Also; we tried to find out the association between the frequencies of these cells and the severity of airflow limitation in the patients, indicated by the forced expiratory volume in one second (FEV_1) %

prediction, forced vital capacity (FVC) and the ratio of FEV_1 to forced vital capacity (FVC).

2. Patients and Methods

Study design and patients:

*This study was carried out in the Department of Medical Microbiology & Immunology and Department of Chest diseases and Tuberculosis; Faculty of Medicine, Sohag University during the period from January 2014 to January 2015. The study included a total of 101 COPD patients (Group I) clinically diagnosed and classified as COPD according to the medical history, current symptoms, chest x-ray and available pulmonary function tests following Global Initiative for Chronic Obstructive Lung Disease guidelines ***(Global Initiative for Chronic Obstructive Lung Disease, 2013)***.

* Patients' mean age was 62.71±7.73 years, including 72 (71.3%) males and 29 (28.7%) females. ***Inclusion criteria:*** Patients were diagnosed as having COPD, if their FEV_1/FVC ratio was less than 70% and FEV_1 was less than 80% after inhaling a bronchodilator, plus manifestations of hyperinflation and enhanced bronchovascular markings in chest x-ray. ***Exclusion criteria:*** COPD subjects were also excluded if they had an exacerbation within the previous 6 weeks, other pulmonary diseases, or severe functional defects of the heart, liver, or kidney.

* A total of 52 apparently healthy nonsmokers age-and gender-matched with normal pulmonary function and without evidence of chronic inflammatory disease; 33 (73.5%) males and 19 (36.5%) females served as the control group (Group II).

* All participants filled out a questionnaire reviewed by an examiner at attendance, had spirometry, chest x-ray performed and blood samples withdrawn.

*An informed oral consent was taken from patients and controls included in the study and the institutional ethical committee approval was obtained.

I. Spirometry:

Lung function data were collected using ***the Master Screen PFT Erich JAEGER*** Spirometer ***(GmbH, Wuerzburg/Germany)***. Lung functions were measured for patients and controls before and 15 min after administration of 200 µg of albuterol/salbutamol. Spirometry measures reported here included the FEV_1% predicted, and FVC, as well as the FEV_1/FVC ratio. The FEV_1% prediction and FEV_1/FVC ratio should be more than 80% and 70% of the prediction, respectively, in normal pulmonary function. FEV_1 % predicted, although not reported separately, was used to stage COPD. Participants were grouped according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) grades 1 through 4 for airflow limitation and the recent GOLD grades A through D for assessing both symptoms and risk ***(Global Initiative for Chronic Obstructive Lung Disease, 2013)***. Correlations between pulmonary function tests and the frequencies of immune cells were analyzed.

II. Flowcytometry analysis of Lymphocyte Subsets.:

EDTA anticoagulated venous blood samples were collected from COPD patients and subjects of the control group. Peripheral blood samples were stained with monoclonal antibodies (mAbs) to determine the frequency of lymphocyte subsets in each individual using flowcytometry. The following mAbs were used in the study: fluorescein-isothiocyanate- (FITC-) conjugated anti- CD4 mAb, phycoerythrin- (PE-) conjugated anti-CD8 mAb, energy coupled dye- (ECD-) conjugated anti-CD16 mAb, phycoerythrin- (PE-) conjugated anti- CD56 mAb, phycoerythrin-cyanin 5 (PE– Cy™5)- (PC5-) conjugated anti- CD3 mAb, phycoerythrin- (PE-) conjugated anti- CD19 mAb purchased from ***Beckman Coulter, France*** for flowcytometric analysis of the frequencies of $CD3^+CD4^+$ (helper T cells), $CD3^+CD8^+$ (cytotoxic T cells), $CD19^+$ (B cells), $CD3^-$ CD56+ (NK cells), $CD3^+$ CD56+ (NKT like cells) and $CD16^+$ among the total lymphocytes. Briefly, 100 µL of blood was used for each staining experiment and the samples were placed into polystyrene tubes ***(Beckman Coulter, France)*** and were subjected to four-color staining with 10µL/test of fluorochrome conjugated monoclonal antibodies (MoAbs):

- anti $CD3^+$PC5/ anti $CD4^+$FITC/ anti $CD8^+$PE (T helper $CD4^+$ cell frequency, T cytotoxic $CD8^+$ cell frequency)
- anti $CD16^+$ ECD ($CD16^+$ frequency)
- anti $CD56^+$PE/ anti $CD3^+$PC5 ($CD3^+$/$CD56^+$ NKT like cells frequency, $CD3^-$/$CD56^+$ NK cells frequency)
- anti $CD19^+$ PE (B cell frequency)

After 20 min incubation at room temperature in the dark, erythrocytes were lysed using 1.0 mL of lysing reagent ***(Versalyse™, Beckman Coulter, France)*** and lysis was allowed for 10 min at room temperature in the dark. Cells were washed twice with phosphate-buffered saline prior to analysis. Cells were analyzed and 10000 events per sample were analyzed by ***Beckman Coulter Epics-XL flowcytometer*** using System II software version 3.0 ***(Coulter, USA)***. Membrane relative mean fluorescence intensity (MFI) of CD3, CD4, CD8, CD19, CD16 and CD56 which is proportional to the number of CD3, CD4, CD8, CD19, CD16 and CD56 epitopes expressed on the cell membrane of peripheral blood cells was estimated in the gated subpopulations by two parameter histograms. **(Figures 1 & 2)**

Statistical Analysis

Data were analyzed using IBM-SPSS version 22 ***(Chicago, USA, 2013)***. Qualitative data were expressed

as frequency and percentage, and quantitative data were expressed as mean±SD. Chi square test was used to compare frequencies of qualitative data and Student's t test was used to compare means in quantitative data. Pearson correlation test (***r***) was used to study the linear relationship between immune cells and respiratory functions. Logistic regression analysis was used for the potential role of different immune cells in the development of COPD. ***P*** *values* < 0.05 were considered significant. ***P*** *values* < 0.01 and ***P*** *values* < 0.001 were considered highly significant.

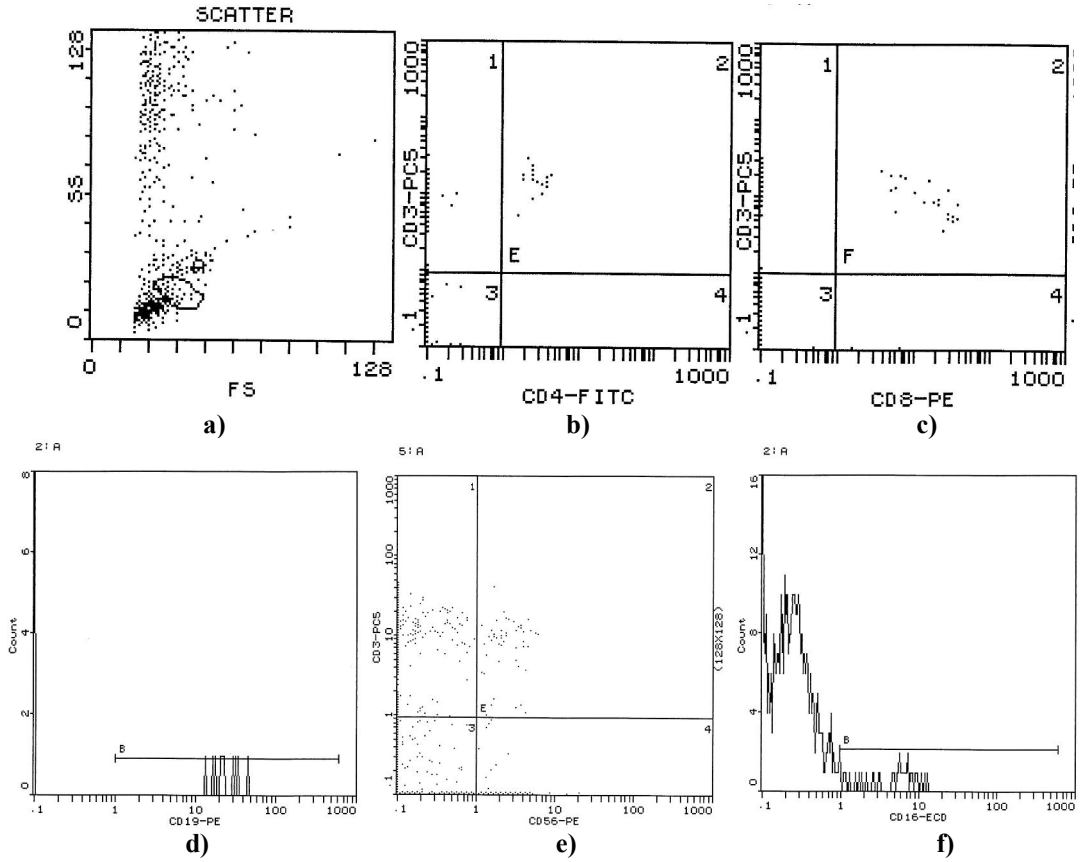
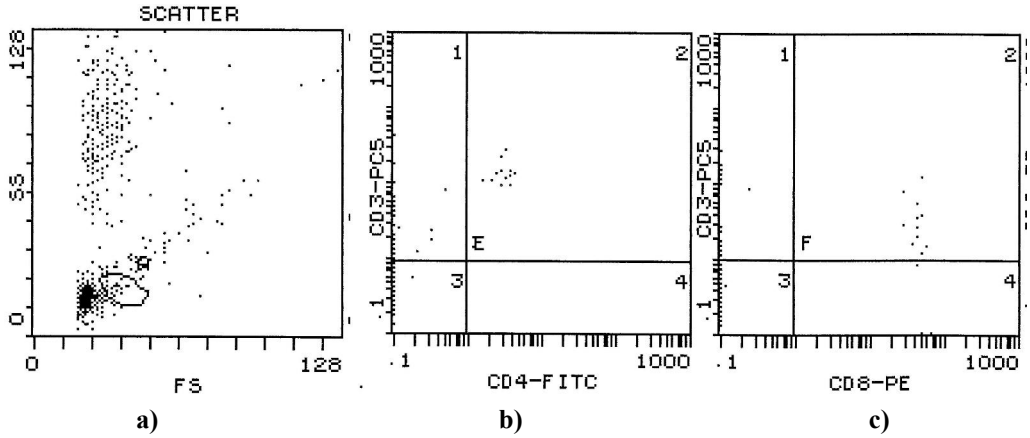
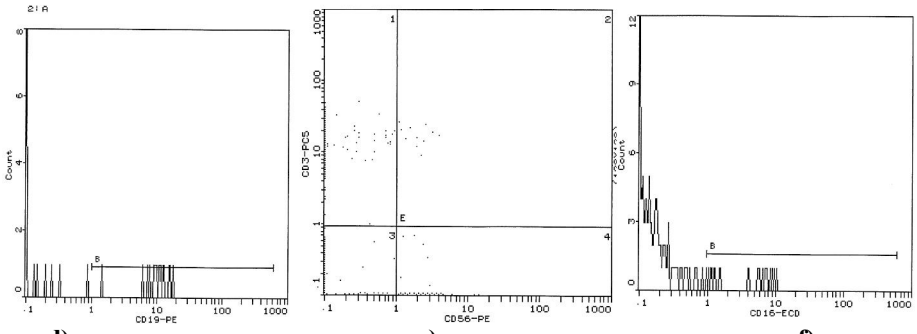


Figure 1: Flow cytometry dot plots; representative histograms demonstrating Lymphocyte subsets in a COPD patient. a) Forward Scatter (FS) vs Side Scatter (SS) plot for identification of lymphocytes. b) Identification of the frequency of $CD4^+$ lymphocytes ($CD3^+CD4^+$) (21.8%). c) Identification of the frequency of $CD8^+$ T lymphocytes ($CD3^+CD8^+$) (26.2%). d) Identification of the frequency of B lymphocytes ($CD19^+$) (25.7%). e) Identification of the frequency of NK cells ($CD3^-CD56^+$) (11.5%) and Identification of the frequency of NKT-like cells($CD3^+CD56^+$) (3.1%).
f) Identification of the frequency of $CD16^+$ on NK cells (9.8%).





d) **e)** **f)**

Figure 2: Flow cytometry dot plots; representative histograms demonstrating Lymphocyte subsets in a control subject. a) Forward Scatter (FS) vs Side Scatter (SS) plot for identification of lymphocytes. b) Identification of the frequencies of $CD4^+$ T lymphocytes ($CD3^+CD4^+$) (35.4%). c) Identification of the frequencies of $CD8^+$ T lymphocytes ($CD3^+CD8^+$) (22.8%). d) Identification of the frequency of B lymphocytes ($CD19^+$) (12.9%). e) Identification of the frequency of NK cells ($CD3^-CD56^+$) (5.6%) and Identification of the frequency of NKT-like cells ($CD3^+CD56^+$) (1.03%). f) Identification of the frequency of $CD16^+$ on NK cells (3.9%).

3. Results

One hundred and one COPD patients (Group I) were included in our study; in addition to 52 apparently healthy individuals as the control group (Group II). The baseline characteristics of the study participants were shown in **table 1**.

Table 1: Demographic data of the study groups

Variable		**COPD Cases**	**Controls**	**Total**
Sex	Male	72 (71.3%)	33 (73.5%)	105 (68.6%)
	Female	29 (28.7%)	19 (36.5%)	48 (31.4%)
Age (Mean±SD)		62.71±7.73	60.58±5.8	61.99±7.19
Smoking	Smoker	48 (47.5%)	-	48 (31.4%)
	X-smoker	29 (28.7%)	-	29 (19%)
	Non Smoker	24 (23.8%)	52 (100%)	76 (49.7%)
Total		101	52	153

Pulmonary functions of the study groups

As expected, pulmonary function tests; namely FEV_1% prediction, FVC% and FEV_1/FVC ratio were statistically highly significant worse in COPD patients compared with controls ($p < 0.001$). **(Table 2)**

Table 2: Mean pulmonary functions of the study groups

Variable	COPD Cases	Controls	*P* value
FEV_1 %	54.55±7.15%	82.35±4.86%	<0.001 (HS)
FVC %	69.81±6.6%	89.65±3.88%	<0.001 (HS)
FEV_1/ FVC ratio	55.67±8.08	79.9±4.08	<0.001 (HS)

*HS: statistically highly significant, FEV_1: forced expiratory volume in one second, FVC: forced vital capacity.

- **Immune Function in COPD Patients:**

Our study revealed that the frequencies of different immune cells in COPD patients were as follows; total lymphocytes (30.43±6.12), B cells (28.96±4.58), CD16 (10±5.76), $CD3^-CD56^+$ NK cells (11.67±3.22) and $CD3^+CD56^+$ NKT-like cells (3.23±2.56) which were greater in COPD patients than those of the healthy controls (22.9±4.18, 15.38±2.74, 4.63±2.5, 6.36±1.87, 1.05±0.78, respectively) with statistically highly significant difference ($p < 0.001$). The frequency of $CD8^+$ T cells (30.77±7.87) was greater in COPD patients compared with healthy controls (26.56±3.4) but the difference was not statistically significant ($p = 0.055$). However; the frequency of $CD4^+$ T cells (25.36±7.23), and the $CD4^+/CD8^+$ ratio (0.82±0.15) were less in COPD patients compared with healthy controls (49.69±10.28, 1.87±0.45 respectively) with statistically highly significant difference ($p < 0.001$). **(Table 3)**

Table 3: Immune cell mean frequencies in COPD cases and controls

Variable	COPD Cases	Controls	*P* value
Total lymphocytes	30.43±6.12	22.9±4.18	<0.001 (HS)
CD4	25.36±7.23	49.69±10.28	<0.001 (HS)
CD8	30.77±7.87	26.56±3.4	0.055 (NS)
CD4/CD8 ratio	0.82±0.15	1.87±0.45	<0.001 (HS)
B cells	28.96±4.58	15.38±2.74	<0.001 (HS)
NK cells	11.67±3.22	6.36±1.87	<0.001 (HS)
NKT cells	3.23±2.56	1.05±0.78	<0.001 (HS)
CD16	10±5.76	4.63±2.5	<0.001 (HS)

*HS: Highly Significant; NS: Non Significant.

- **Correlation between pulmonary functions and immune cell frequencies among COPD cases:**

There was an imbalance of immune function observed in COPD patients as compared with the healthy controls. To demonstrate whether this dysfunction correlated with the severity of COPD, we assessed the correlation between the frequency of lymphocyte subsets and pulmonary functions. Pulmonary functions, especially FEV_1% prediction and the FEV_1/FVC ratio, are often used to evaluate the severity of COPD.

- We found that; there was no significant correlation between pulmonary functions and the frequencies of total lymphocytes, NK cells, and NKT-like cells in COPD patients.
- However; there was a positive correlation between all pulmonary functions tested and the frequencies of $CD4^+$ T cells; namely with FEV1% prediction (r = 0.257, ***P*** = 0.009) with statistically significant ***P*** value; FVC% (r = 0.242, ***P*** = 0.015) with statistically significant ***P*** value and FEV_1/FVC ratio (r = 0.395, ***P*** = <0.001) with statistically highly significant ***P*** value.
- Also, there was a positive correlation between all pulmonary functions tested and the frequencies of $CD8^+$ T cells; namely with FEV1% prediction (r = 0.362, ***P*** = <0.001) with statistically highly significant correlation; FVC% (r = 0.454, ***P*** = <0.001) with statistically highly significant correlation and FEV_1/FVC ratio (r = 0.199, ***P*** = 0.046) with statistically significant correlation.
- There was a positive correlation between the frequencies of $CD4^+/CD8^+$ ratio and FEV_1/FVC ratio (r = 0.286, ***P*** = 0.004) with statistically significant correlation.

Table 4: Correlation between pulmonary functions and immune cell frequencies among COPD cases

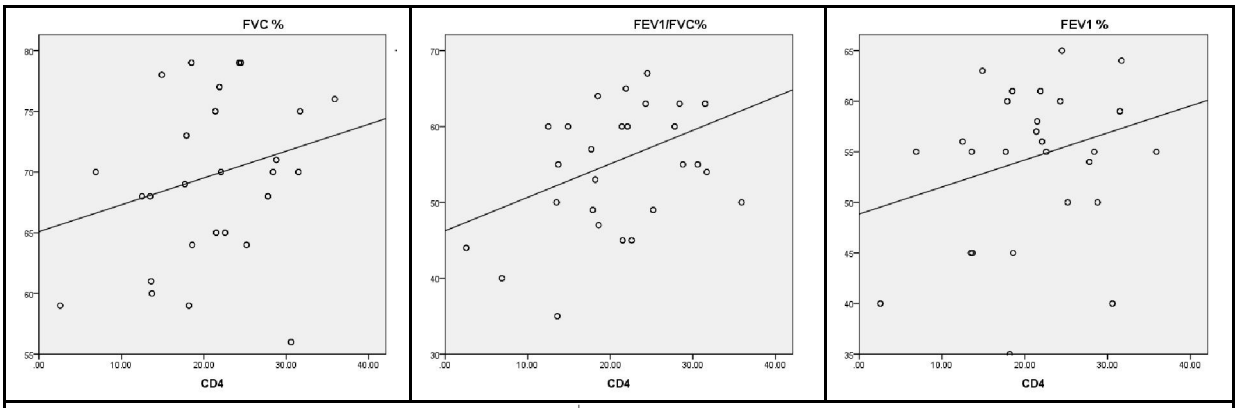
		FEV_1 %	FVC %	FEV_1/FVC
Total lymphocytes	Pearson Correlation	-0.036	0.092	0.164
	P value	0.719	0.361	0.102
CD4	Pearson Correlation	0.257	0.242	0.395
	P value	0.009*	0.015*	<0.001**
CD8	Pearson Correlation	0.362	0.454	0.199
	P value	<0.001**	<0.001**	0.046*
CD4/CD8 ratio	Pearson Correlation	0.039	-0.023	0.286
	P value	0.700	0.820	0.004*
B Cells	Pearson Correlation	-0.306	-0.245	-0.301
	P value	0.002*	0.013*	0.002*
NK cells	Pearson Correlation	-0.031	-0.035	0.161
	P value	0.755	0.729	0.108
NKT cells	Pearson Correlation	0.093	-0.061	0.012
	P value	0.358	0.542	0.908
CD16	Pearson Correlation	-0.009	-0.199	-0.121
	P value	0.929	0.046*	0.230

* ***P*** value is significant, ** ***P*** value is statistically highly significant

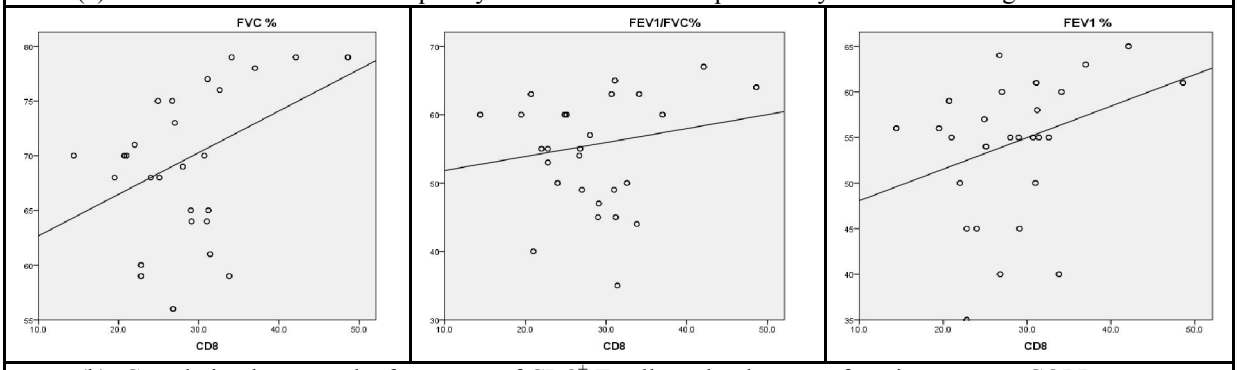
- However; there was an inverse correlation between all pulmonary functions tested and the frequency of B cells; FEV1% prediction (r = -0.306, ***P*** = 0.002) with statistically significant correlation; FVC% (r = -0.245, ***P*** = 0.013) with statistically significant correlation and FEV_1/FVC ratio (r = -0.301, ***P*** = 0.002) with statistically significant correlation.
- Also, we found that the frequency of CD16 was inversely correlated with FVC% (r = −0.199, ***P*** = 0.046) with statistically significant correlation. But there was no significant correlation between other pulmonary functions; FEV_1% prediction and the FEV_1/FVC ratio and the frequency of CD16. **(Table 4, Figure 3).**

Binary univariate logistic regression analysis of all immune cell types as potential risk factors for the development of COPD demonstrated that; all cell types had statistically significant to highly significant relation to COPD. The only exception is $CD8^+$T cells which seem to have no significant relation to COPD development. All these cells would be applied in a multivariate regression analysis model to test if any of them is an independent risk factor for COPD development. **(Table 5)**

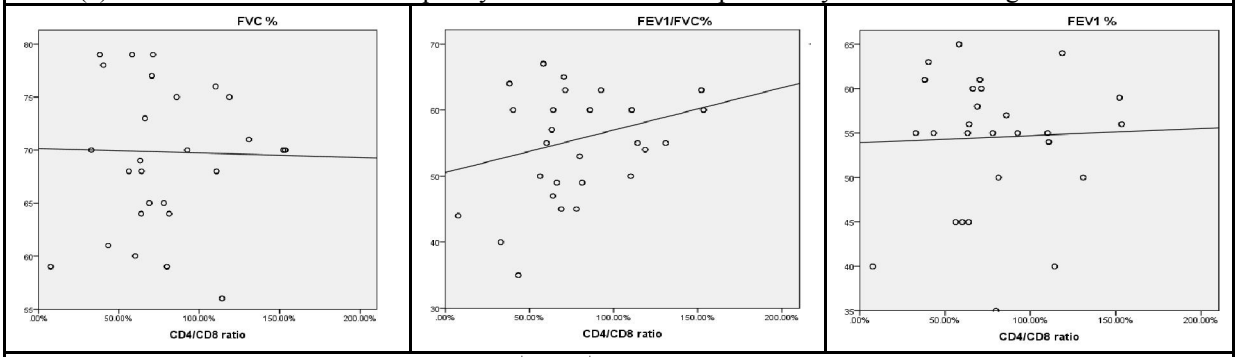
Binary multivariate logistic regression analysis demonstrated that; none of the immune cell types was alone an independent risk factor for COPD development. **(Table 6).**



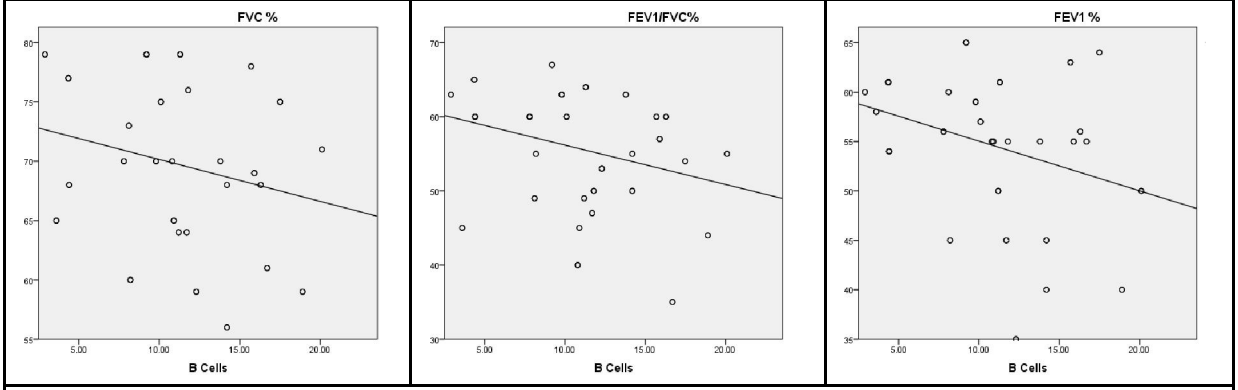
(a). Correlation between the frequency of $CD4^+$ T cells and pulmonary functions among COPD cases.



(b). Correlation between the frequency of $CD8^+$ T cells and pulmonary functions among COPD cases



(c). Correlation between the frequency of $CD4^+/CD8^+$ ratio and pulmonary functions among COPD cases



(d). Correlation between the frequency of B cells and pulmonary functions among COPD cases

Figure 3 (a,b,c,d): Correlation between lymphocyte subsets frequencies and pulmonary functions among COPD cases.

Table 5: Binary univariate logistic regression analysis of the potential role of different immune cells in the development of COPD

	B	S.E.	Wald	*P* value	Odd's ratio	95% C.I. for Odd's	
						Lower	Upper
Total lymphocytes	0.272	0.047	33.299	<0.001(HS)	1.312	1.196	1.439
CD4	-0.749	0.214	12.283	<0.001(HS)	0.473	0.311	0.719
CD8	0.054	0.029	3.556	0.059 (NS)	1.055	0.998	1.116
CD4/CD8 ratio	-0.072	0.014	26.302	<0.001(HS)	0.931	0.906	0.957
B cells	0.163	0.047	11.999	0.001 (S)	1.177	1.073	1.291
NK	0.539	0.092	34.427	<0.001(HS)	1.715	1.432	2.053
NKT	0.950	0.229	17.213	<0.001(HS)	2.586	1.651	4.052
CD16	0.251	0.050	25.233	<0.001(HS)	1.285	1.165	1.417

Table 6: Binary multivariate logistic regression analysis of the potential role of different immune cells in the development of COPD

	B	S.E.	Wald	*P* value
Total lymphocytes	1.632	688.778	0.000	0.998 (NS)
CD4	-17.989	1545.149	0.000	0.991 (NS)
CD4/CD8 ratio	3.529	445.860	0.000	0.994 (NS)
B cells	1.143	913.237	0.000	0.999 (NS)
NK	-4.679	1953.171	0.000	0.998 (NS)
NKT	5.306	1959.239	0.000	0.998 (NS)
CD16	0.884	481.984	0.000	0.999 (NS)

4. Discussion

There is approved remarkable heterogeneity of COPD-associated clinical and biologic phenotypes and that is why therapeutic strategies should be designed in a personalized manner, i.e. based on the knowledge of particular cellular and molecular alterations in the immune system in each individual patient ***(Decramer et al., 2012).*** Besides the local inflammation, it is now clear that also systemic inflammation is of great importance in the pathogenesis of COPD ***(Agusti; 2007)***. However, in contrast with the pulmonary inflammatory component, only little is known about the characteristics and functions of circulating lymphocytes in COPD, and reported findings are often conflicting. Natural killer (NK) cells are innate immune cells secrete an array of cytokines, including IFN-γ, TNF-α, and IL-12 necessary for activation of other immune cells, helping to destroy virus infected and transformed cells via secretion of perforin and other cytotoxic factors ***(Fairclough et al., 2008)***. Smokers have marked depression of NK cell activity and there is recent evidence that exposure of human NK cells to cigarette smoke inhibits IFN-γ and TNF-α production, perforin expression and cytotoxic activity of these cells ***(Mian et al.,2008)***. The numbers and function of NK cells in the lung of healthy and COPD smokers are controversial ***(Prieto et al., 2001)***. In the present study; the frequencies of $CD3^{-}CD56^{+}$ NK cells and $CD3^{+}CD56^{+}$ NKT-like cells were significantly greater in COPD patients compared to HNS, indicating that NK cells and NKT-like cells may be involved in the pathogenesis of COPD. As the pathological hallmarks of COPD are destruction of the lung parenchyma (pulmonary emphysema), inflammation of the central airways (chronic bronchitis) and inflammation of the peripheral airways (respiratory bronchiolitis). The observed damage to the pulmonary tissue could be caused by direct cytotoxic effect against the lung epithelium mediated by the activities of perforin and granzymes ***(Urbanowicz et al., 2010)***. Our results agreed with those of ***Tang et al., 2013.*** However, these results are not consistent with previous findings reported by ***Urbanowicz et al. (2009)*** and ***Chi et al. (2012)*** who reported that; in peripheral blood, the cytotoxic effector function of NK and NKT cells appears to be impaired and their relative number to be reduced in COPD subjects. In the present study of much concern was the finding that; the frequency of CD16 which is an activating receptor of NK cells and NKT cells was greater in COPD patients compared with healthy controls with statistically highly significant difference ($p < 0.001$). Also, we found that the frequency of CD16 was inversely correlated with pulmonary functions tested but with no statistically significant ***P*** value except with FVC% as CD16 was inversely correlated with FVC% with statistically significant ***P*** value ($r = -0.199$, ***P*** = 0.046). CD16 has been identified as Fc receptors FcγRIIIa (CD16a) and FcγRIIIb (CD16b). These receptors bind to the Fc portion of IgG antibodies which then activates the NK cell for Antibody-Dependent Cell-mediated

Cytotoxicity (ADCC) ***(Janeway, 2001)***. With direct cytotoxic effect against the lung epithelium mediated by the activities of perforin and granzymes released from NK cells which driving the pathogenesis of COPD. So; in our study we found increased numbers of NK cells and NKT-like cells expressing activating receptors. These killer cells play key roles in inflammatory responses and activation of these cells can cause the production of inflammatory cytokines and chemokines, that can induce pathological features of COPD and lung tissue damage ***(Wang et al., 2013***).Therefore, we emphasized on the role of these killer cells in the pathogenesis of COPD and it is important to target therapeutic measures to decrease their frequencies and their production of pro-inflammatory mediators in COPD patients. $CD8^+$ T cells play a destructive role in COPD and can release proteolytic enzymes such as granzyme, which causes the death of structural cells ***(Urbanowicz et al., 2009)***. Several studies have shown an increase in $CD8^+$T-lymphocytes within both the peripheral airways ***(Saetta et al., 1998)*** and lower respiratory tract in patients with COPD, but this effect is less conclusive in peripheral blood, with some investigators reporting a decrease ***(Kim et al., 2002)*** and others no change ***(Barcelo et al., 2006)***. In our study; the frequencies of $CD8^+$ T cells were greater in COPD patients compared with healthy controls but the difference was not statistically significant (p =0.055). Also, there was positive correlation between all pulmonary functions tested and the frequencies of $CD8^+$ T cells; FEV1% prediction (r = 0.362, ***P*** = <0.001) with statistically highly significant ***P*** value; FVC% (r = 0.454, ***P*** = <0.001) with statistically highly significant ***P*** value and FEV_1/FVC ratio (r = 0.199, ***P*** = 0.046) with statistically significant ***P*** value. Our findings and the findings of ***Kim et al. (2002)*** suggested that $CD8^+$ T-lymphocyte abnormalities might be not involved in the pathogenesis of airflow limitation in all cases of COPD but only in a subgroup of patients and not in all patients with COPD. Of much concern our findings that; binary univariate logistic regression analysis of all immune cell types as potential risk factors for the development of COPD showed that all cell types showed significant to statistically highly significant relation to COPD. The only exception is $CD8^+$ T cells which seemed to have no significant relation to COPD development. This can be explained by the fact that $CD8^+$ T cells are dependent on $CD4^+$ T cells which act as regulatory elements in the activation as well as the deletion of $CD8^+$ T cells ***(Gupta et al., 2007)***. An important point in our study was the finding that; binary multivariate logistic regression analysis demonstrated that; none of the immune cell types was alone an independent risk factor for COPD development. The explanation of this can be attributed to the inter-relationship between all immune cells tested. In our study; the frequencies of $CD4^+$ T cells were less in COPD patients compared with healthy controls with statistically highly significant difference (**p** < 0.001). These findings are similar to those reported by ***Tang et al. (2013) and Ginns et al. (1982).*** However; there was positive correlation between all pulmonary functions tested and the frequencies of $CD4^+$ T cells; namely with FEV1% prediction (r = 0.257, ***P*** = 0.009) with statistically significant ***P*** value; FVC% (r = 0.242, ***P*** = 0.015) with statistically significant ***P*** value and FEV_1/FVC ratio (r = 0.395, ***P*** = <0.001) with statistically highly significant ***P*** value. These results were not in consistent with those reported by ***Zhu et al. (2009)*** that COPD severity is significantly and inversely associated with the frequency of circulating CD4+ T cells. This difference may be due to the patients' respiratory conditions and matching the condition to pulmonary function determination. It was approved that; a decreased CD4+/CD8+ ratio is a characteristic feature of the pulmonary inflammatory response in COPD ***(Cosio and Guerassimov,1999)***, which is consistent with our results; as the $CD4^+/CD8^+$ ratio were less in COPD patients compared with healthy controls with statistically highly significant difference (p < 0.001). There was positive correlation between the frequencies of $CD4^+/CD8^+$ ratio and FEV_1/FVC ratio (r = 0.286, ***P*** = 0.004) with statistically significant ***P*** value. It has been demonstrated that B-lymphocyte numbers and lymphoid follicles in the small airways of COPD patients are increased ***(Hogg et al., 2004)***, and associated with disease severity. While previous studies observed similar B cell counts in large airways of COPD patients compared to controls ***(O'Shaughnessy et al., 1997)***. In our study; we measured the frequency of B cells in peripheral blood; and it was greater in COPD patients compared with healthy controls with statistically highly significant difference (p < 0.001). And there was negative correlation between all pulmonary functions tested and the frequencies of B cells with statistically significant ***P*** value. Our results were in consistent with other studies stated that; the numbers of B cells increase in peripheral blood as COPD progresses ***(Stefano et al., 1996 & Bosken et al., 1992).*** While our results were not in consistent with ***Tang et al. (2013)*** who reported that; the number of B cells was not significantly different between COPD patients and HNS. Moreover, they found no correlation between the frequency of B cells and pulmonary function. These verify the fact of the remarkable heterogeneity of COPD-associated biologic phenotypes.

Conclusion

The present study described some peripheral blood cell types which may play a role in the pathogenesis of COPD. It is evident that there are changes in balances of lymphocyte cell subsets which can be responsible for immune dysfunction and the development and progression of COPD. Effective prevention of COPD development should include therapeutic strategies aimed at normalization of the disordered components of the immune system to reduce systemic inflammatory mediators in COPD and improve patient morbidity.

Conflict of Interests

The authors state that they have no conflict of interests.

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