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Inflammatory Infiltrate in the Respiratory Tract and Their Relationship to Respiratory Function Among Asthmatics in Accra, Ghana

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Abstract: ***Although many studies have been conducted into the cause, pathogenesis and lung changes in asthmatic patients, not much has been done in Ghana. This study sought to find out the inflammatory infiltrate in the airways tract and their relationship with respiratory function among asthmatics in Korle-Bu Teaching Hospital, Accra, Ghana. One hundred and sixty-five subjects (77 controls, 88 asthmatics age range: 12-75 years, median age 43.5) participated in the study. Subjects completed a risk factor questionaire and provided sputum for cell count and spirometer was used for the lung function test. However, in patients with acute attack, the spirometry was done 4 weeks after the attack. Of the 88 asthmatics, thirty-three (40.74%) had FEV_1 predicted values less than 50% compared with only 15 (9.48%) of controls. Forty-one (51.25%) and 59 (76.62%) of asthmatics and controls had FVC predicted value greater than or equal to 70% while 11 (13.75%) and 3 (8.90%) of asthmatics and controls respectively had FVC predicted value less than 50%. Twenty-six percent of the sputum smears from asthmatics and 1.2% of controls contained eosinophils (p-value- 0.014). Macrophages were present in 86% of smears from control and 46% smears from asthmatics, while neutrophils were found in 94% of smears from both asthmatics and controls (p-value-0.002). This study added to the fact that sputum cytology can be used to assess airway inflammation in asthmatics and concluded that, the effect of asthma on lung function is influenced by the predominant inflammatory cell type in the airways.***

Keywords: Asthma, airways, sputum cytology, spirometry

1. Introduction

Asthma is a serious, sometimes life threatening disease of the airways which is complex and characterised by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness and an underlying inflammation. The interaction of these features of asthma determines the clinical manifestations, severity of asthma and response to treatment. There are a number of risk factors that increase the chances of developing asthma. Some of the risk factors are avoidable such as exposure to smoke and eating certain foods while others like family history are not avoidable or modifiable.

Asthma causes changes in the airways which include epithelial cell damage, hyperplasia of goblet cells, activation and metaplasia of goblet cells, infiltration of the respiratory tract with inflammatory cells and production of copious amount of sputum [1]; some of these changes can occur early in the disease process [2].

Repeated inflammatory process in persistent asthmatic attack leads to airway remodeling and eventually to irreversible airway obstruction and difficulty in breathing [3, 4]. A large cohort study [5] show that asthma patients develop a progressive decline in pulmonary function correlated with age, sex, duration of asthma and asthma severity. Early diagnosis and intervention is necessary to reduce impact of asthma on lung function. Airway remodelling in adult asthma also provides an explanation for the accelerated decline in lung function observed over time [6].

In asthma, the bronchial epithelium is stressed and damaged, with shedding of the columnar cells into the airway lumen. This damage and ensuing repair responses orchestrate airway inflammation and remodeling through activation of myofibroblasts in the underlying lamina reticularis [7] resulting in reduction in lung function. However, the rate of FEV_1 decline in younger subjects increase only when the baseline function is poor [5].

Quantitative cell counts from sputum processed by standardized methods are reliable in the study of the airways and assessment of the severity of inflammatory process in the airways of asthmatics [8].

Though the prevalence of asthma is known in some African countries (Ethiopia 9.1%, Kenya 15.8%, Nigeria 13.0%, South Africa 20.3%, Algeria 8.7%, Morocco 10.4%, and Tunisia 11.9%) [9]. However, the prevalence asthma among children aged between 9-16 in an urban centre in Ghana was 4.8% based on the study criteria and doctor-diagnosis and 7.4% were classified asthmatic by study criteria but not doctor-diagnosed (missed diagnosis) [10]. There are no national statistics on asthma including its morbidity and mortality rates in Ghana [11]. In Korle-Bu Teaching hospital it was revealed that out of 2,752 admissions to the Emergency Room, 252 (9.1%) were due to bronchial asthma [12]. Currently there has been an increase in asthma cases among adults in Ghana.

It was therefore necessary to determine the the cellular changes and lung function of Ghanaian asthmatics and acertain the state of the respiratory tract in asthmatics and improve their management in Ghana. This study suggest that the pathogenic pathway in asthma may vary from the

existing knowledge and has provided a baseline for further studies into the pathogenesis of asthma in Ghanaians, with particular reference to the significantly high neutrophil and macrophage counts in Ghanaian asthmatics.

2. Material and Methods

2.1 Study Site and Population

The study was carried out between January-June 2010 at the Departments of Pathology and Anaesthesia, Asthma clinic at Korle-Bu Teaching hospital (KBTH) and Korle-Bu polyclinic. KBTH is the largest tertiary medical institution in Ghana with about 1600 beds. It is situated in the nation's capital, Accra in the Ablekuma district and also serves as a teaching hospital for the University of Ghana Medical School, School of Allied Health Sciences and other Health institutions. It serves as a referral hospital for hospitals and clinics in and out of the country. Korle-Bu polyclinic is adjacent to KBTH, it serves as a primary care medical centre. About 400 patients attend the clinic in a day. It takes care of newly diagnosed cases of asthma and their follow-up.

The following selection criteria were employed in the recruitment of the study subjects: clinically diagnosed asthmatics between 12 to 75years. and controls: non-asthmatics, between 12-75 years, have no known respiratory disease and non- smokers. The study protocol was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School, Accra, Ghana.

Following inform concent, spirometer (Puritan-Bennett Renaissance®II spirometer) was used to determine the Forced vital capacity (FVC), slow vital capacity (SVC), forced expiratory volume in 1 second [FEV_1], FEV_1% and peak expiratory flow rate (PEFR) for each of the study participant.

2.2 Specimen Collection

2ml of **s**putum was collected from each of the study participant into a clean dry containers labelled with the subjects identification number. Sputum sample was collected twice from each study participant with acute asthma. The first sample was collected spontaneously during the asthmatic attack and second sample collected 4 weeks after the acute attack.

2.3 Sputum Induction and Collection

Sputum was induced by nebulization with 5% hypertonic saline in all the study participant except the study participants with acute asthmatic attack whose first sample was collected spontaneously during the athmatic attack. The participants were given clean water to rinse their mouth before the induction. Subjects were asked to spit out saliva into a container that was provided and sputum into the specimen containers provided.

2.4 Smear preparation

The sputum specimens were liquefied using 0.1% dithiothreitol phosphate-buffered solution of pH 7.2 in a ratio of 1:4. After a period of between 1 – 3h (The time range depended on the consistency of the sputum), the liquefied sputum samples were cenfrifuged at 2000 rpm for 5 min. The supernatants were discarded and the deposit resuspended by gentle taps to the bottom of the test tubes. Two drops of the suspended deposits were placed on clean grease free frosted-end glass slides labelled with the subjects identification numbers. Using the squash method, four smears were prepared for each specimen.

2.5 Fixation

Three of the smears were wet fixed in 95% ethanol and one air dried (at room temperature) in absolute methanol.

2.6 Staining and Mounting

After fifteen minutes of fixation, the smears were stained as follows: wet fixed smears: Papaniculaou stain, Congo red stain and Periodic Acid-Schiff stain and dried fixed smears stained with Leishman stain. Papaniculaou stain was the routine stain used. The rest were confimatory stains. The wet fixed smears stained with Papaniculaou stain were for identification of the epithelial cells and inflammatory cells. Eosinophils were confirmed with Congo red stain and goblet cells with Periodic Acid-Shiff stain. Leishman stain was used to confirm the various types of inflammatory cells. The stained smears were dehydrated in ascending grades of ethanol; from 50% through to 100% . They were then cleared in xylene and mounted with DPX using 24x50mm coverslip.

2.7 Microscopy

The mounted slides were allowed to set or dry at room temperature. Olympus (CX41RF) light microscope was used for the assessment . x10 objective was used to scan through the smear and x40 and x100 objectives were used for detailed evaluation.

The epithelial cells, goblet cells and the various inflammatory cells in the smears were counted. The presence of ciliated columnar cells were recorded and the squamous cells were described and counted in categories as normal, metaplastic, degenerated, anucleate and the presence of cytoplasmic and nuclear vacuolation. The presence of perinuclear halo was also recorded.

2.7 Cell Count

The cells were counted using x100 objective. The Papaniculaou stained slides were used to count the epithelial cells (squamous and columnar), goblet cells and inflammatory cells. Twenty-five high power fields were randomly selected for the cell count and description according to their morphological features. The cells were categorized into the various cell types such as squamous cells, columnar cells, neutrophils, eosinophils, lymphocytes, alveolar macrophages, basophils, mast cells and plasma

cells. The cell count and descriptions were entered on a specially design result form.

2.8 Statistical Analysis

Data was entered into SSPS version 16 for statistical analysis. Percentages, proportions, ranges, means and standard deviations were used to summarize the data collected. Chi-square analysis was used to check for association between demographic variables and outcome variables. Paired t-test was used to compare variables within the groups while unpaired t-test was used to compare variables between controls and cases at 95% significant level.One-way analysis of variance (ANOVA) was used to compare mean values among groups of subjects. Parsons' correlation was used to compare variables and Pillai's trace (0.0 – 1.0) was used to determined the effect of the inflammatory cells on lun function.

3. Results

Between January to June 2010, 165 participated (88 asthmatics; 13 males age range 19-75, females age range 12-70 and 77 controls; 16 males age range 19-75, 61 females age range 12-70).

There was statistically significant difference in the values of FEV_1, PEFR and FEV_1/FCV (p-value-0.001, 0.040 and 0.012 respectively) between the controls and asthmatics. Thirty-three (40.74%) of the asthmatics and 15 (9.48%) of controls had FEV_1 predicted values less than 50%. Three (3.75%) and 20 (25.97%) asthmatics and controls respectively had FEV_1 predicted values greater than or equal to 80%. Forty-one (51.25%) asthmatics and 59 (76.62%) controls had FVC predicted value greater than or equal to 70%. Of the asthmatics, 11 (13.75%) and 3 (8.90%) of the controls had FVC predicted value less than 50%. A high proportion of both controls (83.12%) and asthmatics (58.75%) had FEV_1% value greater than or equal to 75%. There a significant difference between the mean values of FEV_1 for controls and asthmatics (p-value-0.001) and FEV_1/FVC (p-value-0.003).

A significant negative relationship was found between the duration of asthma and the various lung function variables, thus the longer the duration of asthma, the worse the lung function variables. There was a positive correlation between FEV% and FEV_1 (r=0.394; p= 0.001) between controls and asthmatics.

Twenty-six percent and 1.2% of the smears from asthmatics and controls respectively contained eosinophils (p-value-0.014) while macrophages were present in 86% of smears from control and 46% smears from asthmatics. Neutrophils were found in 94% of all smears (both control and asthmatics), having mean numbers of 4.20 (controls) and 37.70 (asthmatics); p-value-0.002.There was a significance difference between the mean number of lymphocytes (38.81 in asthmatics as compared to 7.77 in controls); p-value-0.001. Only one smear from the asthmatics contained Goblet cells. In ten (11.36%) of the smears from asthmatics were found "spindle" cells. Chi square analysis showed a significant association between participants with asthmatic status and the presence or absence of metaplastic cells (p=0.001). Metaplastic cells were found in 88% of smears from controls and 64% of smears from asthmatics.

The inflammatory changes in the smears include: perinuclear halo in 12.5% of smears from asthmatic, degenerated squamous cells were found in 36% of smears from asthmatics and 2.5% of smears from non-asthmatics. Deciliated columnar cells, damaged columnar cells, cytoplasmic vacuoles and intranuclear vacuoles were present in smears from asthmatic patients only.
The mean numbers of lymphocytes, neutrophils, eosinophils, macrophages and total squamous cells between controls and asthmatics (Table 1). The mean numbers of all the cellular components after 4 weeks of acute attack were statistically significant (Table 2).

Multivariate analysis; Pillai's trace (0.0-1.0) was used to check the effect of the various inflammatory cells on lung function of the asthma subjects. The study showed that lymphocytes have the greatest influence on the lung function variables (Pillai's trace 0. 212 and p-value of 0.015).

Table 1: Comparisons of mean cellular changes variables among controls and asthmatics

Cellular Infiltrate	*Code*	*Mean*	*S.D.*	*P-Value*
Neutrophils	Controls	4.2	6.07	
	Asthmatics	37.7	83.78	0.002
Lymphocytes	Controls	7.77	12.74	
	Asthmatics	38.81	39.75	0.001
Eosinophils	Controls	0.24	1.4	
	Asthmatics	9.32	28.62	0.014
Basophils	Controls	0	0	
	Asthmatics	0	0	
Macrophages	Controls	4.78	7.86	
	Asthmatics	16.44	24.77	0.001
Goblet Cells	Controls	0	0	
	Asthmatics	0.03	0.25	0.321
Columnar Cells	Controls	2.38	3.93	
	Asthmatics	1.44	2.23	0.08
Total Squamous Cells	Controls	97.18	43.52	
	Asthmatics	73.95	43.5	0.002
Normal S.C	Controls	98.16	43.52	
	Asthmatics	64.67	37.93	0
Degenerated S.C	Controls	2.29	4.37	
	Asthmatics	6.2	8.94	0.003
Vacuolated S.C.	Controls	0.14	0.44	
	Asthmatics	0.02	0.13	0.045
Anucleate S.C.	Controls	3.09	3.05	
	Asthmatics	3	4.14	0.895

Table 2: Comparisons of mean cellular changes variables before and after Treatment

	Code	Mean	S.D.	P-Value
Neutrophils	Before	39.5	6.27	
	After	32.53	8.39	0.004
Lymphocytes	Before	101.25	5.96	
	After	19.6	7.55	0.001
Eosinophils	Before	20.88	7.4	
	After	0.73	2.11	0.001
Basophils	Before	0	0	
	After	0	0	
Macrophages	Before	7.13	3.86	
	After	17.87	3.31	0.001
Goblet Cells	Before	0	0	

	After	0	0	
Columnar Cells	Before	2.69	1.93	
	After	6	2.66	0.005
Total Squamous Cells	Before	51.57	13.52	
	After	50.44	12.81	0.782
Normal	Before	43.71	14.93	
	After	31.13	18.28	0.019
Degenerated S.C	Before	6.71	2.37	
	After	5	3.32	0.062
Vacuolated S.C.	Before	0.29	0.14	
	After	0.06	0.01	0.001
Anucleate S.C.	Before	0.71	0.06	
	After	2	1.16	0.001

4. Discussion

Eosinophils according to research are said to be a predominant feature of asthma [13, 14, 15, 16], on the contrary Nair *et al.* [17] and Haldar *et al.* [18] found out that not all asthma is eosinophilic, but the rate of asthma exacerbations is associated with eosinophils.

The results of this study agrees with that of Nair *et al.* [17] and Haldar *et al.* [18] since about 76.5% smears from the asthmatics did not contain any eosinophils. 54% (15) of these were from asthmatics with acute attack. This confirms the findings that, asthma exacerbation is as a result of allergic reaction or a viral infection [16, 19, 20].

Although it is well accepted that asthma is characterized by eosinophilic infiltration, there has been accumulating evidence that prominent neutrophilia occurs in situations associated with severe asthma [21, 22, 23]. Significant sputum neutrophilia in asthmatics in this present study supports this observation, with increase neutrophilia in smears of asthmatics with acute asthmatics and those who are stable.

The predominant inflammatory cell type found in the smears of asthmatics in this study was lymphocytes. These findings may be due to the role lymphocytes play in asthma pathogenesis peculiar to asthmatics in this study. T-cell products might also have direct effects on the airways through the recruitment of inflammatory cells, particularly eosinophils. Several pathologic changes occur in the airway epithelium in asthma, but the relationship between these changes; initiation and progression of asthma remains poorly understood. Airway eosinophilia as a characteristic feature of asthma, and it has been speculated that desquamation of epithelial cells may be due to the action of eosinophil granule proteins [24]. This study observed these changes in asthmatic except the presence of degenerated cells which were found in both study groups but of higher quantities in asthmatics than non-asthmatics (6.20±8.94 and 2.29±4.37, p-value-0.003).

Higher numbers of normal squamous cells were found in asthmatics than non-asthmatics in this study, contrary to what Wardlaw *et al.* [25] reported. They recorded increased numbers of epithelial cells in bronchoalveolar lavage from subjects with asthmatics than non-asthmatics. Similar to this study but using biopsies from healthy subjects and asthmatics, Lozewicz *et al.* [26] and Boulet *et al.* [27] have documented epithelial desquamation in healthy subjects that is similar to that found in this study.

Using both actual and predictive values of the parameters based on ATS guidelines, we found a positive correlation between FEV_1 and FEV_1% and a decline in the mean values of FEV_1(p-value-0.001) and FEV_1/FVC (p-value-0.003) in asthmatics compared to non-asthmatics, while similar to recent studies that showing that adult asthma patients have an accelerated decline in FEV_1 [28, 29, 30].

The study observed a significant negative relationship between age and FEV1%, FEV1 and FCV (p-values-0.035, 0.043 and 0.027 respectively) and a positve relationship between the predicted values of SCV and PEFR (p-value of 0.042 and 0.011 respectively) between acute asthmatics and non- acute asthmatics as stated in a large cohort study that asthma patients develop a progressive decline in pulmonary function with age [31]. FEV_1 predictive values seemed lower in younger and elderly subjects (<50%) than middle aged as in the study by Cibella *et al*., [29] who found out that the rate of FEV_1 decline increase in younger subjects. It has also been found that, the airway of adult or elderly individuals who have had chronic asthma for years are the likely candidates for airway remodeling which leads to decline in their lung function [32]. Though majority of the control subjects are exposed to dusty environment that introduces particulate matter into their airways, which may induce an inflammatory process, they have a “healthier” lungs compared to asthmatics, there was a significant difference in the predictive values of FEV1%, FEV1 (p-value-0.01) and FCV (p-value-0.05) between controls and cases.

Although this study showed lymphocytes have the greatest effect on the lung function variables (Pillai’s trace 0. 212 and p-value of 0.015), some studies found eosinophils as having effect on the lung function [33, 34].

5. Conclusion

The result of this study suggest that the pathogenic pathway in asthma may vary from the existing knowledge and has provided a baseline for further studies into the pathogenesis of asthma in Ghanaians, with particular reference to the significantly high neutrophil and macrophage counts in Ghanaian asthmatics. We recommend further study of abnomalities, such as the presence of spindle cells, lack of metaplastic cells, lymphocytes and anucleate cells in smears of asthmatics which may suggest more severe disease requiring improved clinical care to prevent death. The cellular abnomalities in the sputum smears of asthma patients suggest significant abnormalities and epithelial damage that could be prevented. We also recommend early diagnosis and good asthma management with controller (anti-inflammatory) medications to improve lung function, minimize epithelial cell damage and achieve good control.

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