THE PREVALENCE OF ASTHMA PHENOTYPES IN PATIENTS ATTENDING THE CHEST CLINIC AT KENYATTA NATIONAL HOSPITAL

DR REBECCA NJERI KARANJA
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DECLARATION

This dissertation is my original work being presented as part fulfillment for the award of a degree in master of internal medicine, and that to the best of my knowledge it has not been presented at any other university or institution of higher learning.

Signed……..

Date……..

APPROVAL BY SUPERVISORS

This dissertation has been submitted with the approval of my supervisors:

Dr Jared O. Mecha

M.B.Ch.B, MMed MSc-RM (Lond)

Lecturer & Consultant Physician / Pulmonologist

Department of Clinical Medicine and Therapeutics, UON

Signed .....Date.....

Dr George Nyale

M.B.Ch.B, MMed FELL Pulmonology (wits)

Respiratory and Infectious Diseases Unit

Consultant Physician / Pulmonologist

Kenyatta National Hospital

Signed.....Date.....

Prof Kirana M. Bhatt

M.B.Ch.B, MMed, DTM&H, MSc, FRCP

Professor of Medicine & Consultant Physician

Department of Clinical Medicine and Therapeutics, UoN

Signed .....Date.....

Prof Lucy Muchiri

M.B.Ch.B, MMed (Path) PhD

Associate Professor of Pathology and Consultant Pathologist

Department of Human Pathology, UON

Signed.....Date.....

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CHAPTER 1

LIST OF ABBREVIATIONS

ATS	American Thoracic Society
BTS	British Thoracic Society
DALYS	Disability Adjusted Life Years
ENFUMOSA	European Network For Understanding Mechanisms of Severe Asthma
ETS	European Thoracic Society
FEV1	Forced Expiratory Volume in one second
FENO	Fractional Exhaled Nitric Oxide
FVC	Forced Vital Capacity
GERD	Gastro-esophageal Reflux Disease
GINA	Global Initiative for Asthma
ICS	Inhaled Corticosteroid
IL	Interleukin
ISAAC	International Studies of Asthma and Allergy in Children
KNH	Kenyatta National Hospital
LABA	Long Acting Beta Agonists
MMP	Matrix Metalloproteinase
NAEPP	National Asthma Education and Prevention Program
PEFR	Peak Expiratory Flow Rate
PI	Principle Investigator

SABA	Short Acting Beta Agonists
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
TNF	Tumor Necrosis Factor
UON	University of Nairobi
WHO	World Health Organization

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ABSTRACT:

Background: Asthma is a heterogeneous disease characterized by distinct inflammatory and clinical phenotypes. Knowledge of these phenotypes enables individual patient targeted treatment resulting in better control leading to a reduction in treatment costs.

Objective: To determine the prevalence of asthma phenotypes among adult asthmatics attending the chest out-patient clinic at Kenyatta National Hospital.

Methodology: The study was a cross-sectional, descriptive study carried out on asthma patients attending chest clinic at KNH. The inclusion criteria was asthmatic patients, aged 13 years and above and had provided an informed consent/assent. The inflammatory phenotypes were determined using inflammatory cells in sputum. The clinical phenotypes were associated comorbidities which were determined from the case notes.

Data Management: Data was analyzed using SPSS 21.0 software. Continuous data was analyzed into means and medians. Categorical data which included the asthma phenotypes and spirometry findings was presented as percentages. The prevalence of asthma phenotypes was analyzed as proportions using 95% CI. The results from spirometry were graded as mild, moderate, severe and very severe obstruction based on the spirometry findings of FEV1 and were presented as percentages.

Results: Eighty one (81) asthma patients with an average age of 50.1 years and female predominance of 76.5% were studied. The prevalence of inflammatory phenotypes was paucigranulocytic (84%), mixed granulocytic (8.6%), neutrophilic (7.4%) and eosinophilic (0%). The prevalence of clinical phenotypes was allergic rhinitis (76.5%), atopy (67.9%) and GERD (54.3%).

Conclusion: This study shows majorly paucigranulocytic phenotype which commonly has no target therapy, making routine use of inflammatory phenotyping in our patients not beneficial. Among the clinical phenotypes allergic rhinitis was the predominant clinical phenotype.

CHAPTER 1

1.0 INTRODUCTION

Asthma is a heterogeneous disease that is characterized by chronic airway inflammation with different underlying disease processes (1). Eosinophils, neutrophils and cellular elements are involved in the pathogenesis and inflammation process of asthma (2). Asthma phenotypes are as a result of interaction between genetic, epigenetic and environmental factors. The asthma phenotypes are divided into the clinical and inflammatory phenotypes (3). Symptoms and lung function tests have been used to assess the control of asthma but have not been sensitive in reflecting the underlying disease process. There is now increased evidence that suggests that phenotyping asthma according to airway inflammation may enable the identification of subgroups that are most likely to respond to targeted therapy (4).

The eosinophilic phenotype responds to inhaled corticosteroids resulting in better asthma control resulting in reduced exacerbations and hospital admissions. Thus treatment aimed at normalizing eosinophils in the airway results in better outcomes. Neutrophilic asthma has been associated with poor short term response to inhaled corticosteroids (5).

There are approximately 250,000 asthma deaths every year and this is mostly in the low and middle income countries (6). It is estimated that there are 300 million asthma cases worldwide affecting all age groups and this figure is postulated to increase to 100 million in 2025 (7). There have been few studies in Africa on asthma and this has been mainly because of diagnostic challenges. The international Study of Asthma and Allergies (ISAAC) reported that the prevalence of asthma in Africa was increasing and this was especially in children. It has been estimated that the prevalence of asthma in Kenya is 10% with 4 million having asthma (8).

Asthma is an expensive disease to manage and control in terms of drugs, hospital visits and days lost from work or school (9). Asthmatic patients have a poor quality of life with increased personal resource utilization to treat the disease at the expense of other life needs. It has been estimated that about 15 million DALYS is lost due to asthma and this accounts for 1% of all DALYS lost worldwide (10).

CHAPTER 2

2. LITERATURE REVIEW

2.1 HISTORY OF ASTHMA CLASSIFICATION AND PHENOTYPING

In 1918 Francis M. Rackemaan classified asthma as extrinsic with evidence of allergic sensitization and intrinsic with no evidence of allergic sensitization (11). This classification has been replaced by phenotyping of asthma which can translate to a more targeted and individualized treatment of asthma.

Asthma has also been classified based on lung function tests, which mainly measures the severity of airway obstruction. This is also being replaced by the degree of asthma control. These changes have been prompted by the realization of the heterogeneous nature of asthma. Currently there is no consensus on asthma phenotypes but a proposed classification using clinical and inflammatory phenotypes is gathering steam (12). Use of sputum inflammometry studies to determine asthma phenotypes is important as it enables prediction of response to treatment, hence enabling disease pathogenesis to be determined and also to predict risk in the future (13).

Until recently asthma was thought to be a single disease. It has now been established that asthma is a heterogeneous disease consisting of many phenotypes (3). Numerous studies have depicted that asthma is not a uniform disease but consists of several subsets of groupings which differ in disease characteristics and response to treatment. These subsets can be at genetic/epigenetic level genotypes, or at pathophysiologic level endotypes or at outward/manifestation level phenotype. Due to the difficulty of differentiating the pathophysiologic and manifestation levels in asthma the latter two have been lumped together and are referred generally as phenotypes.

The Oxford English dictionary defines a phenotype as the visible or identifiable properties of an organism that are produced as a result of interaction with genetic and environmental factors (12). Visible properties have been used to describe various subtypes of asthma such as clinical, allergic, morphologic and pathophysiologic.

It is hoped that the classification of asthma by phenotypes will improve our understanding of its pathogenesis and therapeutics thus allowing for a personalized targeted treatment of our asthma patients which will eventually lead to better treatment outcomes (13).

At present asthma treatment is based upon the step up approach which relies on symptoms control to up regulate or down regulate medication doses. This approach does not take into account probability of patient response to different treatments. Studies carried out on treatment of asthma have shown that different phenotypes require tailored treatment resulting in better treatment outcomes (3). Asthma treatment based on phenotypes will be most beneficial in those who have failed standard therapy (14).

About 40% of asthmatic patients remain symptomatic despite being on several medications and 5% have difficult to control asthma. It was suggested that high doses of corticosteroids should be used together with long acting beta -2 agonists. The patients should be appropriately investigated to exclude other causes that may impair treatment (15).

Inflammatory phenotypes are established by analysis of induced sputum and include eosinophilic, neutrophilic, mixed granulocytic or paucigranulocytic phenotypes (16). This classification is based on the number of eosinophils and neutrophils cells present in the sputum.

STUDIES ON MANAGEMENT OF ASTHMA USING SPUTUM

Numerous studies carried out have supported normalization of the sputum eosinophils count in the treatment of asthma as this decreases exacerbations without need to increase antiinflammatory treatment.

Green et al in 2002 carried out a randomized control study of asthma exacerbations and sputum eosinophil count of 74 asthma patients who were either managed according to the British Thoracic Society guidelines (standard of care) or treatment guided by normalization of the induced sputum eosinophil count. This study showed that the group treated based on sputum

eosinophils had fewer asthma exacerbations and fewer admissions compared to the BTS group. The sputum eosinophils count was also lower by 63 % in the intervention group (17).

Jatakanon et al in 2000 studied noninvasive markers of airway inflammation of exacerbations that were induced by reducing the dose of inhaled corticosteroids in 15 patients. They reduced the dose of budesonide from 800 mcg to 200 mcg and followed patients for 8 weeks. Nitric oxide and methacholine airway hyperresponsiveness were measured and spirometry as well as sputum induction performed. Seven patients developed mild exacerbations with increased sputum eosinophil count. This suggested that changes in sputum eosinophil count can predict loss of asthma control that is affected by airway inflammation (9).

Jayaram et al (2006) carried out a multicentre randomized study of 117 patients who were divided into two treatment groups depending on clinical strategy and sputum strategy and followed up for 2 years. Clinical strategy was based on clinical symptoms and spirometry while sputum strategy was based on sputum cell counts to guide treatment with corticosteroids with the aim of maintaining low sputum eosinophils counts less than 2%. There were 126 acute exacerbations, 79 in the clinical strategy group and 47 in the sputum strategy group. The time of first exacerbation was longer in the sputum strategy group by 213 days and they were milder. The exacerbations of asthma that required treatment with prednisone was also reduced. The main difference was observed in the eosinophilic exacerbations. There was no effect on non-eosinophilic exacerbation (18).

Simpson et al in 2006 carried out a case control study of 135 patients. They investigated the detection of non eosinophilic asthma using induced sputum. They studied 93 asthmatic patients and 42 normal patients. They found out that 19 patients had neutrophilic asthma, 38 had eosinophilic asthma, 7 had paucigranulocytic and 29 had mixed granulocytic asthma. Noneosinophilic asthma was found to be a stable phenotype both short term (4 weeks) and long term (5 years). Neutrophils were associated with severe asthma that may require intubation and also sudden onset of fatal asthma. This indicates there is a role of neutrophils in severe asthma (19).

TREATMENT

Asthma treatment has traditionally been based on clinical symptoms and assessment of lung function to determine the drugs to use. Knowledge of the various phenotypes has enabled treatment with greater specificity and effectiveness (20).

Eosinophilic asthma responds to corticosteroids while neutrophilic asthma does not responds well to corticosteroids (21). Patients who have persistent eosinophils counts in sputum in severe asthma and are on high doses of inhaled or oral corticosteroids have a favorable response to treatment (22).

According to National asthma Education and Prevention Program (NAEPP) new drugs are being developed that will enable treatment of asthma with reduced dosing of corticosteroids. These drugs will interfere with and block the inflammatory cascade in the airways at various levels (23).

2.2 PREVALENCE OF ASTHMA PHENOTYPES

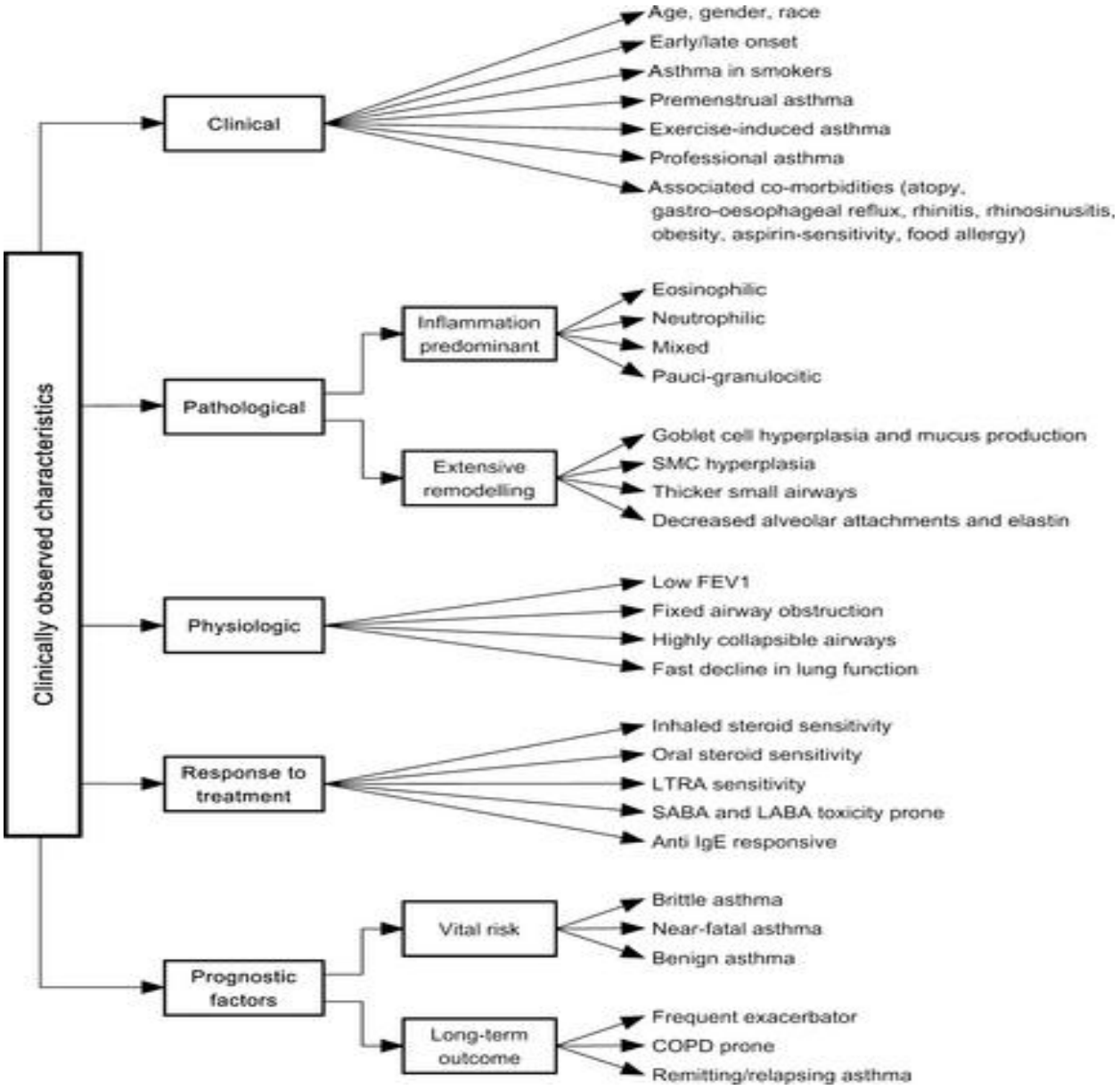
The prevalence of asthma phenotypes has been noted in different countries. Unfortunately there is no consensus yet on the true prevalence of asthma phenotypes. Several studies have been carried out and have given different values. Mohamed et al in 2013, in a study carried out in Egypt of 80 patients found the prevalence of eosinophilic asthma to be 63.8%, neutrophilic 10%, mixed granulocytic 7.5% and Paucigranulocytic 6.2% (24). Simpson et al in 2006 studied 135 patients in Australia. They found the prevalence of eosinophilic asthma to be 41%, neutrophilic 20%, mixed granulocytic 31% and paucigranulocytic was 8% (19).

TABLE 1: SUMMARY OF PREVALENCE STUDIES ON ASTHMA PHENOTYPES

Author	Year	Country	Sample	Prevalence	Design
Romagnoli et al(25)	2002	France	43	Eosinophil-2.5% Neutrophil-5.7% Macrophage-66% Epithelial-0%	Case control
Rosi et al(26)	1999	Italy	99	Eosinophil-12.4%	Cross-sectional study
Schleich et al(4)	2013	Belgium	508	Eosinophil-41% Neutrophil-16% Mixed-3% Pauci-40%	Retrospective study
Pin et al(27)	1992	Canada	34	Eosinophil-18.5% Neutrophil- 23.5% Macrophage- 56% Epithelial- 2.9% Lymphocyte-0.5%	Case control
Simpson et al(19)	2006	Australia	135	Eosinophil-41% Neutrophil- 20% Mixed-31% Pauci-8%	Case control
Mohamed et al(24)	2013	Egypt	80	Eosinophil-63.8% Neutrophil-10% Mixed- 7.5% Pauci-6.2% Undefined-10	Cross-sectional study

The figure below illustrates the proposed features that are used to characterize asthma phenotypes.

FIGURE 1: CLINICALLY OBSERVED CHARACTERISTICS USED TO DESCRIBE ASTHMA PHENOTYPES.



Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes.Allergy. 2012;67(7):835–46.

Inflammatory phenotypes can be classified using invasive methods such as bronchoalveolar lavage and bronchial biopsy. Noninvasive methods include induced sputum, peripheral blood, exhaled gases (FENO) and volatile organic components. Induced sputum is a technique that has

been shown to be reproducible and validated for assessment of airway inflammation and enables us to analyze cells (12).

INFLAMMATORY CELLS

a) Neutrophils

Neutrophils are the first cells that reach the site of inflammation. They have been linked to severe chronic asthma and sudden attacks. They cause phagocytosis and release enzymes and cytotoxic agents and mediators that have an effect on the airways of asthma patients (2). Neutrophils decrease after treatment and resolution of allergy. Platelet activating factor (PAF) induces chemotactic activity of neutrophils and is increased in asthma patients (28). The number of neutrophils in induced sputum was found to be similar in patients with mild to moderate asthma (29). However neutrophils are increased in patients with persistent or acute asthma. This tends to be seen in patients who do not respond to inhaled corticosteroids and have low eosinophils counts (30).

Shaw et al 2007 carried out a study involving 1,197 patients. They aimed to find out the association between neutrophilic airway inflammation and airflow limitation in asthma. They concluded that neutrophilic airway inflammation had a role in persistent airflow obstruction in asthma (31). The level of neutrophils was also increased in biopsy specimens of asthmatic patients. Sur et al in 1993 carried out a study in 7 patients to determine the histological differences in the airways of patients who died from slow and sudden onset asthma. They found that the slow onset group had elevated eosinophils counts while the sudden onset fatal asthma had elevated neutrophils counts in the airways. This suggested that the mechanisms of airway inflammation and narrowing were different in the two groups (32). Elevated neutrophils counts have also been found in submucosal glands of patients with asthma as compared to normal patients (33).

b) Eosinophils

Eosinophils are found in patients with allergic asthma. TH2 results in allergic airway inflammation and is a response when an allergen exposure occurs (34). The allergens are taken

by dendritic and basophils and are then presented to T cells. After induction of T cells by dendritic cells and basophils they develop into TH2 cells. These cells secrete cytokines e.g. IL-3, IL-4, IL-5. A humoral response is then elicited that has elevated IgE antibodies. TH17 produces IL-17 which also plays a role in allergic response. These cells are secreted when there is an allergen exposure to the airways resulting in cytokine secretion. These act on the airways causing pathology and symptoms of inflammation. Due to their central role eosinophils have been targeted in asthma therapy (35). Treatment targeted at eosinophils and its products have been developed. Anti IL-5 drugs such as mepolizumab when used in some asthmatic patients showed significant improvement in the number of exacerbations of patients with prednisone dependent asthma and refractory eosinophilic asthma. It was noted there was no change in airway hyperresponsiveness (36).

2.3 CLINICAL PHENOTYPES

The clinical phenotypes include:

a) Age

Older adults have more severe, progressive and less reversible asthma as compared to a younger population with asthma. The mortality of asthma has found to increases with age (37).

b) Gender

The female gender is associated with severe asthma as compared to the male gender. This is more so if the females are obese (38).

c) Race

Asians and black race have increased morbidity and mortality as compared to the other races. This is also attributed to sociodemographic factors and healthcare access. (39).

d) Early and late onset asthma

Asthma is classified as early onset when it commences at 12 years or below and late onset when it commences after 12 years. Early onset asthma is atopic and less severe. It responds well to corticosteroids. Late onset asthma is non atopic, more severe and does not respond well to corticosteroids. It also has a faster decline in lung function (40).

e) Atopy

This is the genetic tendency of an individual to develop allergic diseases such as atopic dermatitis, asthma, allergic rhinitis and allergic conjunctivitis. It is usually associated with increased immune responses to common allergens such as food allergies and inhaled allergens. Immunoglobulin E antibodies are produced in response to low doses of allergens especially proteins (41).

f) Asthma in smokers

Patients who smoke have increased asthma symptoms and poorer quality of life as they have more asthmatic attacks as compared to non smokers. They have reduced lung functions on spirometry. Smokers have more hospital visits than their counterparts and tend to have a higher mortality. Patients who smoke should be encouraged to stop smoking. Maternal smoking in pregnancy increases the risk of developing asthma in the newborn baby (42).

Smokers have decreased histone deacetylase activity which is required for suppression of cytokine production and this may result in corticosteroid resistance in asthmatic patients (43).

g) Premenstrual asthma

It affects about 40 % of women. Female sex hormones are involved but the exact mechanism is unknown. During the luteal phase of the menstrual cycle there is increased airway hyperresponsiveness. There is altered beta 2 adrenoceptor function and regulation in females who have asthma. Use of asthma medication and the combined oral pill or gonadotrophin releasing hormone may be useful in these patients (25).

h) Exercise induced asthma

In this type of asthma symptoms develop with onset or during exercise. It is common in children and adolescents. Exercise induced asthma may cause limitation of daily activities by 30%. There is bronchial airways constriction due to heat and fluid loss resulting from excessive ventilation. Treatment involves use of inhaled corticosteroids. Prevention is carried out by use of short and long term beta 2 bronchodilators and leukotriens before initiating exercise (44).

i) Occupational asthma

This is asthma that results in breathing in chemical gases, fumes and dust at the workplace. It occurs as a result of work exposure. The patient develops symptoms of asthma. The symptoms decrease when the patient is away from work. The prevalence consists of 10% of asthma patients. The agents induce asthma through an immunologic response. Both high and low molecular weight agents have been known to produce IgE antibodies resulting in an immune response. Very low exposure is required to cause an immune response once a person has been sensitized (45).

j) Obesity

This represents a phenotype that is more difficult to control and tends to be less responsive to treatment. Studies published have shown that a minimal reduction in weight can result in improvement of the clinical symptoms and outcomes. These patients do not respond well to corticosteroids (46). There are mechanisms that have been postulated to worsen asthma and they include modification of the mechanical properties of the respiratory system. Obesity is also associated with comorbidities such as GERD and chronic systemic inflammation (47).

The European multicenter study (ENFUMOSA) which was a cross sectional study carried out in 2003. They compared 163 subjects with severe asthma and 158 subjects with controlled asthma. They found that female gender and obesity were associated with severe asthma (38).

k) Aspirin intolerant asthma

This contributes to 5-10% of adults with asthma. It is rare in children but common in non atopic asthmatics. Women have a higher prevalence as compared to men with a ratio of 5.5: 1. In this

type of asthma inflammation is continuous even without the use of the drug. It occurs as a result of overproduction of leukotriens. This type of asthma is severe with patients requiring systemic corticosteroids to control asthma and sinusitis. It is a common cause of life threatening attacks of asthma. NSAIDS and aspirin should be avoided to prevent the asthmatic attacks (48).

i) GERD

The prevalence of GERD in asthma ranges from 18-85%. The distal esophagus may be stimulated by acid leading to bronchoconstriction. This is as a result of reflux that may irritate the airway exacerbating asthma symptoms. It may also increase bronchial reactivity through vagal stimulation. It has also been shown that microaspiration of acid may lead to bronchoconstriction. For patients with difficult to control asthma it may be an exacerbating factor (49). The presence of GERD in asthma is common as the two often coexist.

m) Allergic Rhinitis

Allergic rhinitis is an inflammation of the upper airway. It affects all age groups and races. Allergic rhinitis and asthma are inflammatory disorders. Patients present with sneezing, nasal discharge and nasal congestion. It precedes onset of asthma and is associated with worsening of asthma symptoms and poor quality of life (50).

2.4 INFLAMMATORY PHENOTYPES

a) Eosinophilic phenotype

This phenotype is characterized by elevated levels of eosinophils in induced sputum and bronchial biopsies despite using high doses of corticosteroids (51). Some studies carried out have shown that between half to two thirds of patients with severe asthma have persistent eosinophilia in the main airway (52). There are more symptoms, exacerbations and lower FEV1 values when there are elevated levels of eosinophils.

It is also associated with high levels of sinus disease, involvement of the peripheral airway and remodeling. These patients respond well to monoclonal antibody IL-5 such as mepolizumab. Elevated eosinophils are more prevalent in early onset asthma as compared to late onset irrespective of corticosteroid use (22). The cut off used to determine eosinophilic phenotype is

elevated eosinophils counts within a range of 1-3% (24). There is no consensus on the cut off range of neutrophils and eosinophils for the inflammatory phenotypes.

b) Neutrophilic phenotype

This phenotype is characterized by increased number of neutrophils. The number of eosinophils may be absent or suppressed (51). Elevated neutrophils counts are found in sputum, bronchial tissue and brochoalveolar lavage from patients who are on high doses of corticosteroids. Elevated neutrophils may also be from disease or inflammation.

It is associated with increased numbers of matrix metalloproteinase 9 (19). In some instances neutrophils may be the only cells of inflammation that are left after use of steroids has cleared the eosinophils (53). If neutrophils are elevated with a range between 61-76% in sputum it is classified as Neutrophilic asthma (24).

c) Mixed granulocytic asthma

There is increased numbers of both eosinophils and neutrophils (16). The eosinophils counts are greater than 1-3 % in sputum and neutrophils counts are greater than a range of 61-76 % in sputum.

d) Paucigranulocytic asthma

It has normal levels of eosinophils and neutrophils. Eosinophils counts are less than 1-3% and neutrophils counts are less than 61-76 % in the sputum. There is no thickening of the subepithelial basement or signs of inflammation. This phenotype is associated with stable asthma (19).

2.5 DIAGNOSIS

Previously the diagnosis of asthma was made based on clinical signs. The latest guidelines by GINA in 2014 have laid emphasis on lung function tests to reduce misdiagnosis and over

diagnosis (54). Tests used in the diagnosis of asthma are pulmonary function tests. GINA recommends the use of spirometry with the PEFR being an alternative (10).

Stenton et al in 1993 assessed the value of questionnaires and spirometry in asthma at the workplace. They found that questionnaires had 28% sensitivity and 73% specificity. The sensitivity of pulmonary function tests was 21% with a specificity of 92%. They concluded that caution should be exercised when using questionnaires and pulmonary lung function tests in diagnosing patients with asthma (55).

a) Spirometry

This is the globally recommended test for diagnosing asthma. It is a physiological test that is used for diagnosis of asthma. Spirometry determines airflow obstruction in asthma. A normal spirometry result does not exclude asthma in contrast to COPD. When a patient is suspected of having asthma a bronchoprovocation test may also be carried out for confirmation. It is recommended that spirometry be performed at initial assessment, after treatment and when symptoms have stabilized. Patients on follow up should have spirometry performed every one to two years (56). Spirometry measures lung function, volume and flow of air that is exhaled and inhaled. The flow versus volume provides more information on the initial portion of the FVC maneuver (57). It measures numerous parameters including forced expiratory volume in one second (FEV_1) and Forced Vital Capacity (FVC).

This test provides useful information that is used in the diagnosis of asthma and should form part of the office equipment for diagnosis and management of patients with respiratory pathology (58). The patient takes the deepest breath they can and then exhales into a sensor as hard as possible, for as long as possible preferably six seconds. This test requires a good patient's effort and cooperation. It is usually repeated three times and the best reading is recorded (59). This test is used in adults and children who with encouragement and simple instructions are able to carry out the test (60). It is not suitable for unconscious and heavily sedated patients. The results from spirometry are graded as mild, moderate, severe and very severe obstruction.

FEV1 >80%: Mild obstruction

FEV1 50-80%: Moderate obstruction

FEV1 30-50%: Severe obstruction

FEV1 < 30%: Very severe obstruction

b) Sputum analysis

Induced sputum is the gold standard for classification of inflammatory phenotypes (16). Analysis of sputum is used to identify the various inflammatory phenotypes according to the predominant inflammatory airway cells i.e. eosinophilic, neutrophilic, mixed granulocytic or paucigranulocytic (61). The concentration of inflammatory markers has been found to be higher in sputum as compared to bronchoalveolar lavage fluid thus depicting sputum to have higher airway secretions than bronchoscopy obtained samples (62). Asthma phenotypes are known to be stable. Studies carried out on sputum can be repeated in the short term and long term (63).

Maestrell et al in 1995 carried out a study on comparison of leukocyte count in sputum bronchial biopsies and bronchoalveolar lavage. They found the number of cells of leukocytes, eosinophils, macrophages, neutrophils and lymphocytes were different. It was discovered that the proportion of eosinophils count in the different tissues types was the same when three different techniques were used. This led to the conclusion that sputum analysis may be used for assessment of eosinophils in the airways (64).

Pizzichini et al studied sputum in severe exacerbations in asthma and inflammatory indices after prednisone treatment in 1997. The study population was 10 patients who were followed up for 21 days. They found that the clinical symptoms and blood indices (FEV1, blood eosinophilia, and Serum eosinophilic cationic protein) improved in 24 hours. Sputum eosinophils took 48 hours to decrease. This showed that sputum was a better guide to use in patients who were on follow up for asthma (65).

2.6 STUDY JUSTIFICATION

Asthma is a worldwide problem with increasing prevalence with an estimated 10% of the population being affected. It is a major cause of morbidity and appreciable mortality. It has been noted that not all asthma patients show similar response to all drugs. These variations in response to therapy have put into question the current uniform treatment. Prescription of futile treatments can be minimized and response variability maximized by use of phenotypic patterns that predict response to certain medication.

A few studies in the developed world have suggested that phenotypic asthma treatment is both feasible and cost effective but there are no studies done on asthma phenotypes in Kenya and very few in Africa. We currently do not know the phenotypes of our asthma patients, and whether these fit the global described phenotypes and there is no local data on asthma phenotypes.

This study will serve as a baseline survey of the phenotypes present in asthma patients. Phenotyping asthma will enable individualized treatment of patients resulting in better outcomes and a reduction in cost.

2.7 RESEARCH QUESTION:

What is the prevalence of phenotypes in patients attending the chest clinic at Kenyatta National Hospital?

2.8 RESEARCH OBJECTIVES:

BROAD OBJECTIVE

To determine the prevalence of asthma phenotypes amongst asthma patients attending the chest clinic in Kenyatta National Hospital.

a) SPECIFIC OBJECTIVES

1. To determine the prevalence of inflammatory phenotypes e.g. eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic among asthma patients attending the chest clinic at Kenyatta National Hospital using sputum cytology.
2. To determine the prevalence of clinical phenotypes such as allergic rhinitis, atopy, GERD among asthma patients.

b) **SECONDARY OBJECTIVE**

1. To describe spirometry findings with the different phenotypes.

CHAPTER THREE

3.0 STUDY METHODOLOGY

3.1 STUDY DESIGN

This was a descriptive cross-sectional study.

3.2 STUDY LOCATION

This study was done in Kenyatta National Hospital, the largest national referral facility in Kenya which is a teaching hospital for the University of Nairobi, School of Medicine located in Nairobi. Central, Nairobi and Eastern regions of the country form the main catchment population. It has a bed capacity of 1800. The study was conducted in KNH chest clinic. The hospital runs a chest clinic on Tuesday morning reviewing about 60 patients with approximately 20 asthma patients seen.

3.3 STUDY SUBJECTS

a) Study population

Patients with a chest physician's diagnosis of asthma who attended the chest clinic at KNH

c) Inclusion criteria

1. Patients who met the case definition of asthma.
2. Aged 13 years and above.
3. Patients who were willing to participate in the study by providing an informed consent/assent.

c) Exclusion criteria

1. Contraindication to spirometry such as patients with pulmonary tuberculosis.
2. Patients who were unable to follow instructions for spirometry.

3.4 SAMPLE SIZE CALCULATION

There are an estimated number of 120 asthma patients seen in KNH chest clinic quarterly. A representative sample was drawn from this fixed population and the sample size was obtained using a formula for finite population (less than 10,000). The calculation was as follows:

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1) + Z^2P(1-P)}$$

Where

n' = sample size with finite population correction,

N = size of the target population = 120

Z = Z statistic for 95% level of confidence = 1.96

P = Estimated prevalence of paucigranulocytic phenotype of asthma patients at 6.2% found by Mohamed et al in Egypt.

(The lowest percentage which was of the paucigranulocytic phenotype was used as it was powered to capture a greater proportion of the other phenotypes)

d = margin of error = 3%

$$= \frac{120 \times 1.96^2 \times 0.062 \times 0.938}{0.03^2 (120-1) + 1.96^2 \times 0.062 \times 0.938}$$

n = **81**

A minimum of 81 asthma patients were sampled to estimate prevalence within 3% level of precision.

SAMPLING METHOD

Consecutive sampling method was used.

3.5 CASE DEFINITIONS

1. **Asthma was defined as**

a) Chest physician's previous diagnosis of asthma OR

b) A patient with cough, dyspnoea, chest tightness and wheezing and demonstrated obstruction on spirometry with significant reversibility.

2. **Phenotypes-**

These were classified as clinical and inflammatory.

The study participants were to meet either of the inflammatory or clinical phenotypes.

a) Clinical phenotypes were determined from patient's case notes.

The clinical phenotypes studied included GERD, eczema, food allergy, atopy, and allergic rhinitis.

b) Inflammatory phenotypes were determined by sputum analysis. The eosinophil and neutrophil cut off percentage of 2% and 65% respectively (24).

i) Eosinophilic asthma- Eosinophils in sputum greater or equal to 2%.

ii) Neutrophilic asthma- Neutrophils in sputum greater or equal to 65%.

iii) Mixed granulocytic asthma- Elevated eosinophils greater than or equal to 2% and neutrophils greater than or equal to 65%.

iv) Paucigranulocytic asthma- Normal levels of eosinophils less than 2% and neutrophils less than 65 % in sputum

c) Obesity- Defined as Body Mass Index (BMI) of greater than 30 as calculated using height to the nearest centimeters and weight to the nearest kilograms.

d) Early onset asthma- Defined as asthma diagnosed before the age of 12 years.

e) Late onset asthma- Defined as asthma diagnosed at and after the age of 12 years.

f) Occupational asthma- Defined as asthma due to conditions attributable to work exposures and not to causes outside the workplace.

g) Asthma in smokers- Defined as someone who smoked more than one cigarette daily for more than 1 month.

h) Comorbidities- These were from previous diagnosis and the patients have received treatment in the past. No attempt was made to confirm these comorbid diagnoses. They included atopy, allergic rhinitis, food and drug allergy, GERD and eczema.

3.6 STUDY PARTICIPANT RECRUITMENT PROCEDURE

Files of the patients presenting at chest clinic of KNH were screened by the principal investigator with the help of one research assistant to identify those who had a diagnosis of asthma. The research assistant was a qualified clinical officer from Kenya Medical Training College. The competence of the research assistant was assured by the principal investigator through training prior to the start of the study. The patients' files were reviewed by the principal investigator or research assistant before the start of every clinic to identify the patients that met the case definition of the study

The consenting process was carried out using a consent form as outlined in appendix 1. Eligible patients were taken through the consenting process and patients below the age of eighteen were accompanied by their guardians. The principal investigator explained the details of the study to the patient, the study procedures involved and the benefits for those who participated in the study.

The duration of obtaining consent depended on the patient's comprehension and they were encouraged to ask questions and seek clarification on what they did not understand. A consent form for signing was given to patients who had understood the information given and gave consent. Patients who declined to participate were allowed to do so without any repercussion.

3.7 DATA COLLECTION

a) Clinical methods

The study proforma was used to obtain and record the history of the participants regarding age, gender, marital status, level of education, duration of illness and medication. A focused physical examination that involved a general and respiratory examination on the patients was carried out. The weight and height of the patients was taken. Weight was measured using a digital weighing scale to the nearest kilogram. The height was measured using a heightometer to the nearest centimeters. The Du Bois formula was used to compute the anthropometric measurements into Body Mass Index (BMI).

Spirometry was carried out for all patients.The PI ensured the participant was well seated and relaxed 5 minutes prior to carrying out the test.

b) Spirometry

Standardized procedure was followed to carry spirometry on patients. Adherence to the European Community for Coal and Steel (ECCS/ESR, 1993) acceptability criteria was followed. Spiro Lab III Medical International Research (MIR) spirometer was used. The spirometry readings for analysis that were used are Forced Expiratory Volume in the first second (FEV1) and Forced Vital Capacity (FVC). A spirometry reading of < 70 % depicted obstruction. Patients then inhaled 200 micrograms of salbutamol and performed the spirometry again. The best of three readings was taken and recorded by the respiratory technician.

c) Sputum

1. Sample collection

The sputum collection procedure was explained to the patient by the PI or research assistant and informed consent obtained. The patient gargled water to reduce residual food particles, saliva, mouthwash, drugs and oral bacteria. They also blew their nose to reduce nasopharyngeal discharge. The participant was given a sterile sputum bottle. The patient then placed the open container close to the mouth and then coughed deeply and spit the sputum gently into the container. Patients were encouraged to cough until a satisfactory amount of specimen was

obtained. A sputum specimen of 5 ml was considered adequate. The container was securely closed and the samples were labeled with the patients' study number, age, sex, date and time of collection.

2. Sample transportation and storage

The triple packaging protocol for transport of the sputum specimen to the laboratory was used. The sputum samples were placed in a sputum bottle which was secured tightly and labeled. The container was wrapped with an absorbent material i.e. tissue paper. This was to absorb any accidental leakage from the sputum bottle. This was then placed into a sealed plastic biohazard specimen bag and sealed. Once the sputum samples were collected they were kept in a cooler box with ice packs at temperatures of 4-8° C and this served as a temporary storage to facilitate transport to the laboratory.

Samples were delivered to the UON cytology laboratory at the end of the day after collection. The samples were fixed immediately after delivery to the laboratory.

3. Sputum analysis

Standard operating procedure (SOP) for handling and processing sputum samples were used. The sputum specimen was processed in the laboratory using alcohol to fix the slides. May-Grunwald Giemsa (MGG) stain was used to stain the slides.

4. Reporting of results

The type of cells was determined using an inverted microscope. Eosinophils appeared as cells with pink/orange granulated cytoplasm with two lobed purple nucleuses. The inflammatory phenotypes were determined from the sputum results. The sputum specimen was labeled as eosinophilic when the eosinophils were greater or equal to 2%. Neutrophils appeared as cells with transparent pink/blue cytoplasm and 2-5 bright purple nucleuses. The sputum specimen was labeled as neutrophilic when the numbers of neutrophils was greater or equal to 65%.

3.8 STUDY VARIABLES

a) Independent variables

These included socio-demographic and clinical variables. Which were:

1. Age- Was recorded as the nearest number of years as the period from documented date of birth.
2. Gender- Determined by the observed phenotypical sex that is secondary sexual characteristics of male or female.
3. Duration of illness from diagnosis-Was determined to the nearest year by documented date of when the disease was diagnosed for the first time.
4. Treatment modality – Defined as the drug therapy that was used by the patient and the duration as stated by the patient or obtained from the file.
5. Level of Education- Reported as the highest level of education the patient had acquired as reported by the patient.
6. Marital status- Categorized as single, married, divorced or widowed and was documented as reported by the patient
7. Occupation- Defined as regular activity a person performs for payment that occupies ones time.

c) Dependent variables

1. Inflammatory phenotypes i.e. Presence of eosinophils or neutrophils in the sputum
2. Clinical phenotypes- Atopic Dermatitis, Conjunctivitis, Allergic Rhinitis, Obesity, GERD

3.9 QUALITY CONTROL AND ASSURANCE

The research assistant who assisted in carrying out the research was a registered clinical officer. Before carrying out the study, the principal investigator took the research assistant through the process of consent administration and collection of data to ensure that the proper protocols were adhered to. Throughout the study, the principal investigator supervised the whole process. Standard operating procedures for specimen collection and transport were followed. The specimens were delivered to the laboratory immediately to minimize preanalytical errors.

Sputum specimen collection was carried out according to the recommended procedures in terms of aseptic technique, proper storage and labeling of the specimen. The laboratory tests were carried out in the UON cytology laboratory which undergoes both internal and external quality control measures.

a) Spirometry

Spirometry was carried out by a respiratory physiologist and a respiratory physician reviewed all spirometry tracings. The spirometry device was calibrated daily using a 3 liter calibration needle.

b) Sputum tests

All sputum bottles were labeled with the patients details. The sputum analysis was carried out by a qualified cytology technician. The slides were reviewed by the pathologist.

3.10 DATA MANAGEMENT AND ANALYSIS

Data was coded from data collection tools and then entered in Microsoft Access database. The quality of data was assured at all levels by performing data cleaning during data collection and also at the end of data entry. Cleaned data was then exported to SPSS version 21.0 software for statistical analysis. Descriptive information of the patients was summarized using demographic characteristics. Categorical data was presented using percentages while continuous data was analyzed into means or medians. Prevalence of eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic phenotypes was calculated as proportions and presented with 95% confidence interval. Similarly, clinical phenotypes of asthma patients were presented as percentages. In spirometry Forced Expiratory Volume in one second/ Forced Vital Capacity (FEV1/FVC) less than 70 % was classified as obstruction after the patient had been given an inhaled bronchodilator of 200 mcg of salbutamol. The results from spirometry were graded as mild, moderate, severe and very severe obstructions based on the FEV1 and were presented as percentages.

3.11 ETHICAL CONSIDERATIONS

The study was undertaken after approval by the Department of Clinical Medicine and Therapeutics and the Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee. Enrollment of the patients into the study was voluntary after obtaining a written informed consent. Spirometry was carried out in all asthma patients participating in the study. A sputum specimen was obtained from the patients and thereafter discarded after analysis. There was no additional risk to the patient which was anticipated. Each participant was assigned a study number at enrollment. The number was used to identify patients for matters related to data analysis. Information obtained from the patient was kept confidential. Patients found to have severe asthma were referred to the chest physician.

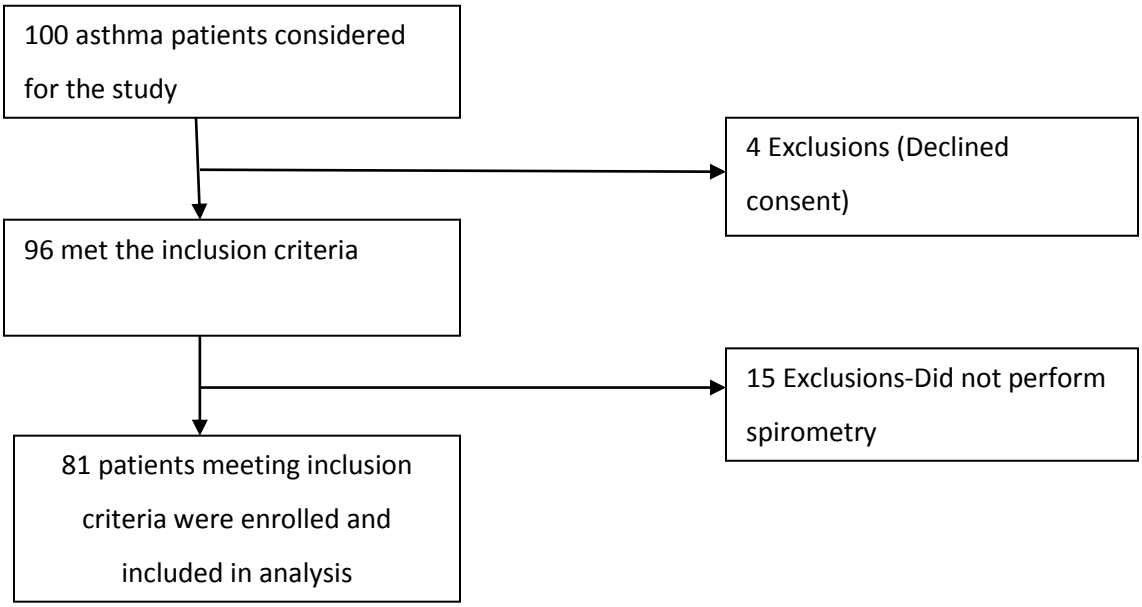
The information collected was stored in a locked cabinet that was only accessible to the Principal Investigator. The information collected was used for the purposes of the study. Upon completion of the study the results were disseminated to the files to aid in patients' management. The soft data will be stored for two years, thereafter it will be destroyed.

CHAPTER FOUR

4.0 RESULTS

During the study period extending from December 2015 to January 2016, one hundred patients being managed for asthma in the chest clinic at KNH were screened for study eligibility and were considered for the study. Nineteen patients were not eligible hence eighty one (81) who met the inclusion criteria were enrolled into the study. Those who were excluded had declined to give consent or did not perform spirometry.

Figure 2: Flow chart showing study enrollment of asthma patients at KNH chest clinic



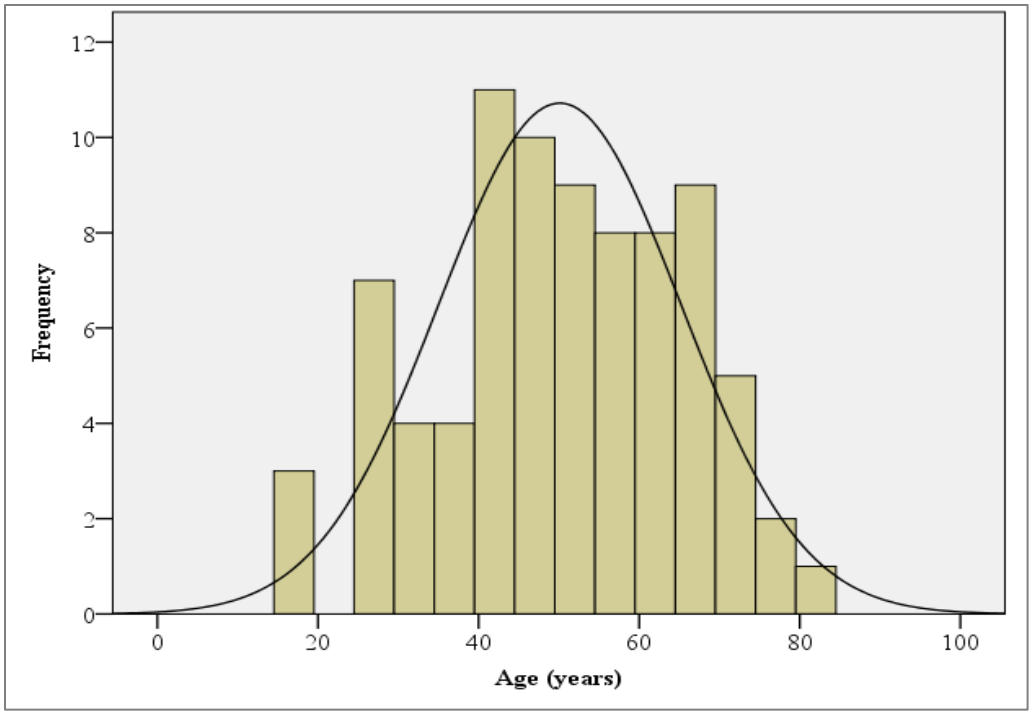
4.1 PARTICIPANTS DESCRIPTION

The mean age of the study participants was 50.1 years (SD 15.1 years) and age ranged between 17 and 80 years. The population was predominantly female at 76.5%, 48.1% had attained secondary education with 11.1% having no education, unemployment level of 49.4% and 59% lived in urban centers. The duration of asthma was 5 to 10 years in 30.9% of the participants and 62% using ICS/SABA combined treatment while 21% were using SABA only. The patients had a mean BMI of 27 kg/m^2 with 25.9% of the patients being obese.

Table 2: Patients characteristics at the KNH chest clinic

Variable	Categories	Frequency (n=81)	Percentage (%)
Gender	Male	19	23.5
	Female	62	76.5
Marital Status	Single	16	19.8
	Married	52	64.2
	Divorced	2	2.4
	Widowed	11	13.6
Level of Education	None	9	11.1
	Primary	24	29.6
	Secondary	39	48.2
	Tertiary	9	11.1
Occupation	Student	2	2.5
	Formal Employment	12	14.8
	Business	27	33.3
	Unemployed	40	49.4
Residence	Urban	48	59
	Rural	33	41
Duration of asthma	<1	11	13.6
	1-5	22	27.1
	5-10	25	30.9
	>10	23	28.4
Asthma treatment	SABA prn	17	21
	ICS+ SABA prn	50	62
	LABA+ICS+SABA prn	14	17
BMI	Obese	21	25.9
	None obese	60	74.1

Figure 3: Age distribution of the study population



4.2 CLINICAL CHARACTERISTICS OF THE ASTHMA PATIENTSMost (93.8%) of the asthma patients who attended the clinic were non-smokers while 6.2% were smokers. Self-reported adherence to medication was high as reported by 75.3% of the patients. A proportion (11.3%) of the female participants reported that their asthma symptoms were worsened by their menses. Exercise exacerbated asthma symptoms in 70.4 % of patients. There was no change in asthma symptoms in 29.6% of the patients with exercise. The cost of asthma medication was mainly met by the patients themselves (58%) while 4.9% were covered by insurance.

Table 3: Clinical characteristics of the asthma patients

Variable	Frequency (n=81)	Percentage (%)
Smoking	5	6.2
Drug Adherence	61	75.3
Severity of symptoms with Menses (n=62)	7	11.3
Asthma Exacerbation during exercise	57	70.4
Obtaining medication		
Self	47	58
Employer/Insurance	4	4.9
Parent	5	6.2
Child	10	12.3
Others	15	18.5

Spirometry findings

Spirometry revealed that majority (53.1%) of the patients had moderate obstruction and 40.7% had mild obstruction post bronchodilation.

Table 4: Spirometry findings of the study participants

Spirometry **FEV1**	**Pre-BD** **(n=81) Percentage (%)**	**Post-BD** **(n=81) Percentage (%)**
Mild (>80%)	27(33.3)	33(40.7)
Moderate (50-80%)	40(49.4)	43(53.1)
Severe (30-50%)	12(14.8)	5(6.2)
Very severe (<30%)	2(2.5)	0(0.0)

4.3 PREVALENCE OF INFLAMMATORY AND CLINICAL PHENOTYPES

Majority (84%) of asthma patients had paucigranulocytic phenotype. The other inflammatory phenotypes were mixed granulocytic (8.6%) and neutrophilic (7.4%). However, eosinophilic phenotype was not identified in this population of patients. The most common clinical phenotypes identified in this study included allergic rhinitis and GERD in 76.5% and 54.3% of the patients respectively. Food allergy was the least clinical phenotype at 11.1%

Figure 4: Prevalence of inflammatory phenotypes

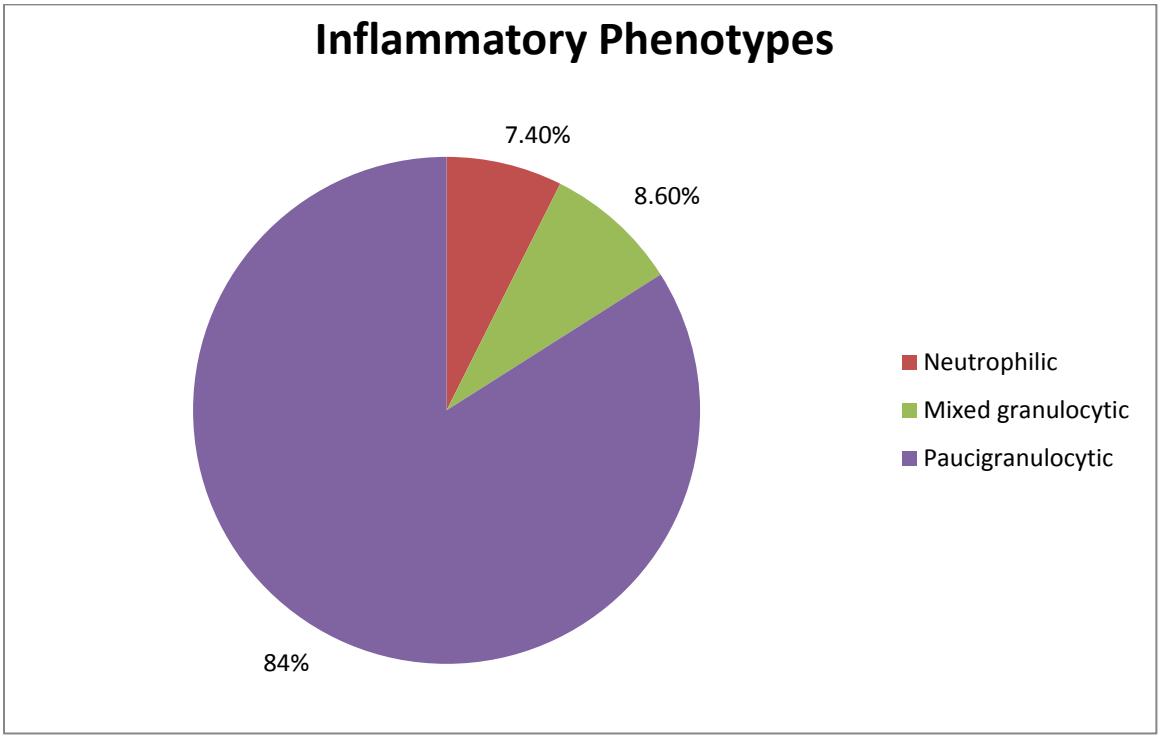
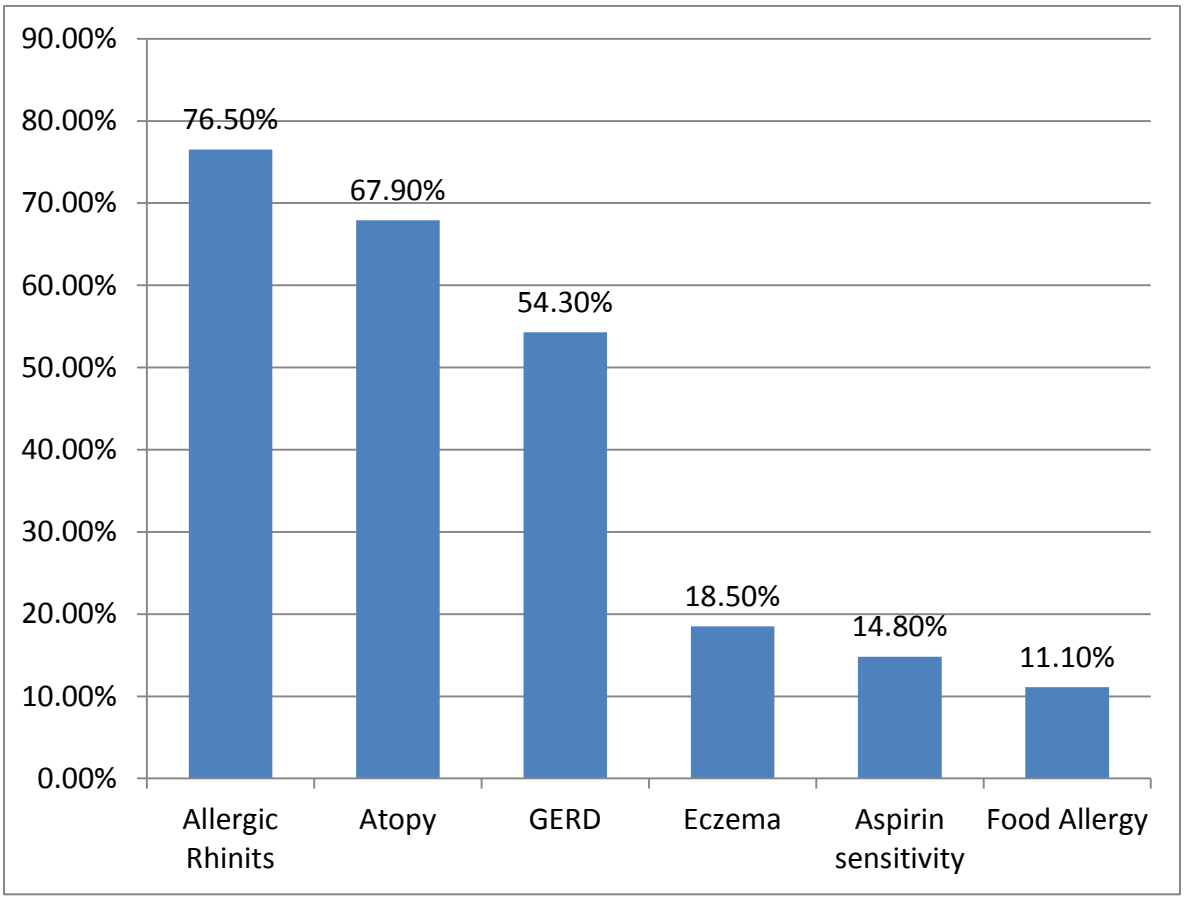


Figure 5: Prevalence of clinical phenotypes

The study population had more than one comorbid condition and this resulted in an overlap of the clinical phenotypes.



4.4 ASSOCIATIONS BETWEEN FEV1 AND ASTHMA PHENOTYPES

No significant association was found between the inflammatory and clinical phenotypes and FEV1. (Table 5, 6)

Table 5: Association between inflammatory phenotypes and FEV1

FEV1 / Inflammatory phenotypes	Mild/Moderate obstruction	Severe/Very severe obstruction	P Value
Neutrophilic	5 (83.3%)	1 (16.7%)	0.333
Mixed granulocytic	7 (87.5%)	1 (12.5%)	
Paucigranulocytic	56 (82.4%)	12 (17.6%)	

Table 6: Association between clinical phenotypes and FEV1

FEV1 / Clinical phenotypes	Mild/Moderate obstruction	Severe/very severe obstruction	P value
GERD	36 (81.8)	8 (18.2)	0.542
Eczema	13 (86.7)	2 (13.3)	0.088
Food allergy	7 (77.8)	2 (22.2)	0.717
Atopy	48(87.3)	7 (12.7)	0.484
Aspirin sensitivity	10(83.3)	2 (16.7)	0.720
Allergic rhinitis	48(77.4)	14 (22.6)	0.072

CHAPTER FIVE

5.0 DISCUSSION

The prevalence of inflammatory and clinical phenotypes of asthma patients in our setting has for the first time, been illustrated by our findings.

Asthma patients were fairly middle aged population with our study showing a mean of 50.1 years with the youngest patient being 17 years and the oldest 80 years. Most of the patients we studied were females at 76.5% and this may reflect the fact that women have a higher health seeking behavior. Previous studies have shown that women asthmatics have better health seeking behavior (66).

Asthma patients in this study were mainly non-smoking with 93.8%. This was comparable to a previous study by Mohamed et al where 71.3% of the asthma patients were non-smokers (24) Smoking has been shown to result in a lower FEV1 in asthma patients (67). Since smoking was rare in our study population there was little chance that that the phenotypes were significantly altered by smoking.

Prevalence of paucigranulocytic phenotypes was high at 84% among the asthmatic patients. Paucigranulocytic phenotype has been found to be the most common phenotype in stable patients (68). The patients in the study had mild asthma with a mean FEV1 of 70.2%. We analyzed spontaneous sputum samples where the patients voluntarily coughed up sputum. In induced sputum hypertonic saline is used in the form of nebulization resulting in production of sputum from the airways. Cell viability and cellular yield is lower in spontaneous sputum samples than in induced samples (69). This may have resulted in low cell yield in our study.

A retrospective study carried out by Schleich et al in 2006 in Belgium reported that 40% of the asthma patients had paucigranulocytic phenotype. A study population of 508 patients was analyzed. The participants comprised of a middle aged population with a mean age of 52 years with a female predominance. Majority of the patients at 73% underwent sputum induction. In this study the study participants had well controlled asthma (4). Wang F. et al in a case control study carried out in Australia in 2011 reported the prevalence of paucigranulocytic phenotype at 51.7%. The close similarity to our prevalence may have been due to the shared attributes of the study population with our patients (69). Our findings differed from those of a cross-sectional

study carried out by Mohamed et al in 2013 among 80 outpatients' asthma participants in Egypt where 6.2% of the patients had paucigranulocytic phenotype. The difference in prevalence may have been due to the use of sputum induction and the younger population with a mean age of 32 years that was used in the Mohamed et al study (24).

Mixed granulocytic phenotype in our study was in 8.6% of the patients; findings which were similar to the 10% prevalence reported in a cross-sectional study by Murthy M. et al in 2012 (77). This may have been due to the similar characteristics of the participants to our study population. The prevalence of mixed granulocytic phenotype was lower than 31% reported in a case control study by Simpson et al in 2006 that studied 93 asthma patients in Australia. The difference could be explained by the fact that the study used a population with a lower mean age and a higher number of patients who were ex smokers at 39%. The study also used a lower cut off to determine the inflammatory phenotype with neutrophilic at or greater than 61% and eosinophilic greater than 1% (19). In our study we used a higher cut off of 65% and 2% respectively.

Neutrophilic phenotype was identified in 7.4% of the patients we studied. Similar findings were reported in a case control study carried out by Romagnoli et al in 2002 including 43 asthma patients where 5.7% of the patients had neutrophilic phenotype. The study population was similar to patients recruited in our study comprising of middle aged asthma patients who were non-smokers and had moderate obstruction from their spirometry findings (25). However higher prevalence of neutrophilic phenotype has been reported elsewhere such as in a case control study by Pin et al in 1992 among 17 asthma patients in Canada, 23.5% had neutrophilic phenotype. The patients were induced sputum and were poorly controlled (27). The higher prevalence could be explained by the participants having poorly controlled asthma. In their study induced sputum was used to analyze the inflammatory phenotypes. This may explain the differences in the prevalence rates of the neutrophilic phenotype.

In our study we did not report any eosinophilic phenotype. A low prevalence of 2.5% of eosinophilic phenotype was also reported in a case control study by Romagnoli et al in 2002, which included 43 outpatients' asthma participants. The cohort in Romagnoli study had mild asthma as depicted in the spirometry findings (25). Higher prevalence was reported in a cross-sectional study by Mohamed et al in Egypt in 2013 where out of the 80 outpatients asthma participants studied, 63.8% was positive for eosinophilic phenotype (24). The difference in the eosinophilic phenotype prevalence rate might be due to variation in the attributes of the study participants and the use of induced sputum to determine the inflammatory phenotype.

The study by Mohamed et al reported that asthma patients who have been on ICS had reduced eosinophil counts compared with patients receiving other forms of treatment. Our study cohort had been on ICS and this may have attenuated airway inflammation thus decreasing eosinophils. In addition, eosinophils may have decreased due to the fact that the patients in our study had well controlled asthma. Eosinophilic phenotype, patients tend to have more symptoms, exacerbations and lower FEV1 (68). The difference in prevalence rate may be due to variations in the study subjects and different study design used in the studies. In this study, we analyzed spontaneous sputum compared to the other studies which analyzed induced sputum. The viability and quality of cells has been found to be higher in induced sputum samples. The presence of mucus secretion along the airways for a long duration of time may result in fewer viable cells and the inability to distinguish the different types of cells in spontaneous sputum (69). This may explain why majority of the patients were found to have paucigranulocytic phenotype and the lack of eosinophilic phenotype.

The study participants were able to produce sputum. Induced sputum is the preferred method. However, it is associated with the risk of bronchoconstriction if hypertonic saline is used in the process. This is due to activation of mast cells or sensory nerve endings. Geographic differences between different locations may have resulted in differences with the inflammatory phenotypes. This is demonstrated in the studies carried out in the different parts of the world where there was no similarity in the prevalence of the inflammatory phenotypes.

About three quarters (76.5%) of the asthma patients had allergic rhinitis. This high prevalence was comparable to 70% reported in a study carried out by Ankan B et al in 2013 in India in which 160 asthma participants were reviewed (71). Presence of allergic rhinitis in the asthmatic

patients necessitates the use of drugs such as antihistamines, montelukast and inhaled nasal sprays. Controlling of allergic rhinitis results in better control of asthma and relieves patients' discomfort (72).

The prevalence of atopy in our study was 67.9%. This was similar to a study by Murthy M et al in 2012 in United Kingdom in which 62% of their study population had atopy. The similarities in the prevalence could have been due to the shared attributes of the study participants(73). The high prevalence of atopy could be due to exposure of the asthma patients to allergens. Atopy has been found to be a risk factor for development of asthma. Exposure to allergens such as house dust mites, cats and dogs may result in development of atopy (74).

The prevalence of GERD was reported to be 54.3%. Our findings were similar to a study by Ankan B et al in 2013 in which 55% had a diagnosis of GERD (71). Julian et al in 2005 in United Kingdom carried out a cross-sectional study on 52 asthma patients in an outpatient clinic and reported a higher prevalence of 75% of the participants having GERD diagnosis. The high prevalence in this study could have been due to the diagnosis of GERD having being made by use of a 24 hour dual probe PH monitoring which may have identified more patients with GERD. Their study population consisted of difficult to control asthma patients (49). There was a high prevalence of clinical phenotypes such as allergic rhinitis, atopy and GERD in our study population. These comorbidities may affect asthma control and thus should be identified and treated.

The finding of 0% eosinophilic phenotype in a population with a high prevalence of allergic rhinitis and atopy may have due to the methodology, where spontaneous sputum rather than induced sputum that has high yield of cells was used in the study. The patient population used in our study had mild asthma and were well controlled.

Inflammatory and clinical phenotypes were not significantly associated with spirometry findings. However, this analysis was inconclusive because our study utilized a crossectional study design hence the likelihood of having small numbers of participants with little capacity to test associations. Similar findings were reported in a study by Ankan B in India who did not find any association between the asthma phenotypes and spirometry findings (71).

5.1 LIMITATION

Recall bias as patients were required to remember events that occurred several years back. As a result patients may have forgotten important details in their history e.g. duration of illness.

Sputum samples analyzed in the study were spontaneous rather than induced with the latter found to have a higher yield of cells. The use of spontaneous sputum may have resulted in a lower yield of cells as they have prolonged residence in the airway thus resulting in decreased viability of the cells. Induced sputum specimens have mobilization of newer cells in the airways resulting in a higher yield of cells in the sputum.

The clinical phenotypes were based from a previous diagnosis and no attempt was made to confirm this diagnosis. Primary errors in diagnosis could have influenced these results.

5.2 CONCLUSION

The study demonstrates a high prevalence of the paucigranulocytic phenotype among the asthma patients. This prevalence was substantially higher than that demonstrated in other studies on inflammatory phenotypes. Our findings are in keeping with the fact that majority of the patients in this study had mild to moderate asthma as demonstrated from the spirometry findings. The paucigranulocytic phenotype is the most common phenotype in patients with stable asthma. Among the clinical phenotypes allergic rhinitis was the predominant clinical phenotype with a high prevalence.

5.3 RECOMMENDATION

The findings of this study show no benefit in routine phenotyping of asthma patients and continued use of GINA treatment steps is the best to manage our patients. Most of the patients in the study had mild to moderate asthma and sputum was spontaneous rather than induced. We recommend further studies in severe asthma population using induced sputum.

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6 APPENDICES

6.1 CONSENT INFORMATION FORM

Study title-Prevalence of asthma phenotypes in patients attending chest clinic at Kenyatta National Hospital

Introduction

My name is Dr Rebecca Karanja. I am a post graduate student in the department of internal medicine at the University of Nairobi. The purpose of this consent form is to give you information regarding the study so that you can decide whether to participate in the study. It will also enable you to understand what the study is about. You may ask questions about the research.

Purpose of the study

The purpose of the study is to find out the different types of asthma. These are the patients attending the chest clinic at KNH. Finding out the different types of asthma will enable better treatment of patients.

Benefits for the participant

You will not be charged for the tests. The information gathered will be shared with your doctor to aid in better treatment of your illness.

Risks

You may experience some shortness of breath or dizziness after the spirometry procedure. This will however disappear after a few minutes. You will be required to answer the questions which may be personal but this will help in strengthening the study.

Procedure

If you agree to participate in the study you will be asked a few questions on your medical history about your illness. You will undergo a focused physical examination before starting the study. We will perform a lung function test (spirometry) that will enable us to find out how your lungs are functioning. You will also be requested to give sputum. This will be examined in the laboratory to find out the type of cells present to determine the type of asthma that you have.

This will enable us to treat you according to the type of asthma you have. The sputum samples will be discarded afterwards and no other studies will be carried on it

Confidentiality

The information we obtain from you will be treated with utmost confidentiality. You will be assigned unique numbers linked to your name. Thus your name and file number will not appear on any data form or specimen.

Voluntary Participation

Participation is voluntary in this study. Your treatment will not be affected if you do not participate in the study. You are allowed to withdraw from the study or decline participation without loss of benefit or penalty.

If you have any questions you can contact:-

1) The Chairman, KNH/UON – Ethics and Research Committee
   P.O BOX 20723-00202, Nairobi
   Tel. 020 2726300 ext 44355

2) Dr Rebecca Karanja
   P.O BOX 7457-00200, Nairobi
   Tel 0721407666

CONSENT TO PARTICIPATE IN THE STUDY

I have read and understood the information in the consent form and it has been explained to me. My questions have been answered. I am also aware that participation is voluntary and I can withdraw from the study at any time without consequences. I have agreed to participate in the study.

Name of the Participant/Guardian_____ Date_____

Signature of Participant/Guardian_____

I confirm that I have explained the details of the research to the participant.

Researcher's Name _____ Date

Signature of Researcher_____

ASSENT FORM (age 13-18)

Study title-Prevalence of asthma phenotypes in patients attending chest clinic at Kenyatta National Hospital

Introduction

My name is Dr Rebecca Karanja. I am a post graduate student in the department of Internal Medicine at the University of Nairobi.

Purpose of the study

We want to find out the different types of asthma in patients attending the chest clinic at Kenyatta National hospital. This will enable us to treat people with asthma better.

Benefits

You will not pay any money for the test. The information we obtain will be shared with your doctor to help in better treatment of your illness.

Risks

You may feel some shortness of breath or dizziness after the lung function test has been carried out. This will however disappear after a few minutes. We will also ask you some questions which may be personal but this will help in making the study better.

Procedure

If you agree to take part in the study you will be asked a few questions about your illness. You will also be examined before starting the study. We will perform a lung function test that will enable us to find out how your lungs are working. You will also be requested to give sputum. This will be examined in the laboratory to find out the type of cells present in the sputum. This will enable us to treat you according to the type of asthma you have.

Confidentiality

When we complete the study we will write a report about what we have learned. Your name will not be included in the report.

Voluntary Participation

You do not have to be in the study if you do not want to be in it. After we begin the study and you do not want to take part in it any further, it is fine to withdraw. We have informed your parents/guardian about the study.

1) The Chairman, KNH/UON – Ethics and Research Committee
   P.O BOX 20723-00202, Nairobi
   Tel. 020 2726300 ext 44355

2) Dr Rebecca Karanja
   P.O BOX 7457-00200, Nairobi
   Tel 0721407666

If you agree to take part in the study, please sign your name.

Name of the Participant_____ Date_____

Sign your name _____

I have been informed about the study my child will take part in,

Name of the Parent/Guardian_____ Date_____

Signature of Parent/Guardian_____

I confirm that I have explained the details of the research to the participant.

Researcher's Name _____ Date

Signature of Researcher_____

6.2 STUDY PROFORMA

PREVALENCE OF PHENOTYPES OF ASTHMA PATIENTS ATTENDING CHEST CLINIC AT KENYATTA NATIONAL HOSPITAL

BIODATA

Study number……

Name (initials)……

Physical address……

Date of Enrollment……...

SOCIAL DEMOGRAPHIC DATA

1. Age (in years)…….

2. Gender *(tick one)*

A) Male……. B) Female…….

3. Marital status *(tick one)*

A) Single….C) Divorced……..

B) Married…… E) Widowed……..

4. Level of education *(tick one)*

A) None……. C) Secondary……..

B) Primary……. D) Tertiary……

5. What is your occupation?

A) Formal employment C) Farming

B) Business D) Others (specify)……

MEDICAL HISTORY

6. Do you smoke?

A) Yes B) No

7. When was the diagnosis of asthma made?

A) < 1 year C) 5-10 years

B) 1-5 years D) >10 years

8. How many exacerbations/ attacks have you had in the last one year?

9. Other Comorbid conditions

A) GERD D) Food Allergy

B) Allergic Rhinitis E) Aspirin sensitivity

C) Eczema F) Atopy

10. Are the asthma symptoms worse during your menses? *(Females)*

A) Yes B) No

11. Do you develop asthma symptoms during exercise?

A) Yes C) No

12. Which medications have you been using?

A) SABA …….

B) ICS/SABA……...

C) Others……..

13. How long have you been on the medication?

A) < 1 year C) 5-10 years

B) 1-5 years D) >10 years

14. Are you adherent to your medication?

A) Yes B) No

15. Who buys your medication?

A) Self D) Child

B) Employer/Insurance E) Others (specify)……

C) Parent

PHYSICAL EXAMINATION

Anthropometric data

A) Weight…… B) Height…….

BMI…….

SPIROMETRY

PreBD: FVC……% FEV1……% FEV1/FVC ratio……

PostBD: FVC……% FEV1……% FEV1/FVC ratio……...

Reversibility……..

FEV1 80%: Mild obstruction
FEV1 50-80%: Moderate obstruction
FEV1 30-50%: Severe obstruction
FEV1 < 30%: Very severe obstruction

SPUTUM RESULTS

THE PREVALENCE OF ASTHMA PHENOTYPES OF ASTHMA PATIENTS ATTENDING CHEST CLINIC AT KENYATTA NATIONAL HOSPITAL

BIODATA

Study Number……..

Lab Number…….

Sex……

LABORATORY RESULTS

Sputum Analysis

A) Eosinophilic
B) Neutrophilic
C) Mixed Granulocytic
D) Paucigranulocytic

Eosinophilic- Eosinophils greater than or equal to 2 %

Neutrophilic- Neutrophils greater than or equal to 65%

Mixed Granulocytic- Eosinophils greater than or equal to 2% and neutrophils greater than or equal to 65%

Paucigranulocytic- Eosinophils less than or equal to 2% and neutrophils less than or equal to 65%

6.3 DEFINITION OF VARIABLES

A) BODY MASS INDEX

$$BMI = \frac{\text{Body Weight (Kg)}}{\text{Height (Meters)}^2}$$

WHO Classification	Associated risks
BMI between 18.5 and 25 : normal weight	Normal
BMI between 25 and 30 : overweight	Average
BMI between 30 and 40 : obesity	Important
BMI above 40 : morbid obesity	Severe

6.4 PROCEDURES

DETAILED SPIROMETRY PROCEDURE

1. Patients were advised to seat down comfortably in the chairs provided.
2. Patients took the deepest breath they could, for as long as possible, preferably for 6 seconds.
3. They then breathed into a disposable turbine that was connected to a spirometer.
4. Soft nose clips were used to prevent air escaping through the nose.
5. Spirometry was performed using Spirolab III spirometer.
6. The best of three readings was recorded according to the American Thoracic standards.

DETAILED SPUTUM PROCESSING PROCEDURE

The smears were fixed to the slides using methanol and were then air dried. A Shpandon Cytospin machine was used to cytospin the specimen and concentrate the cells. The slides were stained using MGG stain in the UoN cytology laboratory.

METHOD FOR MAY-GRUNWALD GIEMSA STAINING

a) The air dried smear specimen was fixed in methanol for 10-20 minutes
b) Then stained with May-Grunwald working solution for 5 minutes
c) Stained with Giemsa working solution for 12 minutes
d) Washed with clean buffered water for 2 and 5 minutes
e) The slides were dried in an upright position at room temperature
f) The slides were mounted with a cover slip

6.5 ETHICAL APPROVAL LETTER FROM CHEST CLINIC KENYATTA NATIONAL HOSPITAL

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/500

9th December, 2015

KENYATTA NATIONAL HOSPITAL APPROVED 09 DEC 2015 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

Dr. Rebecca Njeri Karanja
Reg. No. H58/68804/2013
Dept. of Clinical Medicine and Therapeutics
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Karanja,

Revised research proposal: The Prevalence of Asthma Phenotypes in Patients Attending the Chest Clinic at Kenyatta National Hospital (P583/09/2015)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above proposal. The approval periods are 9th December 2015 – 8th December 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chair, KNH-UoN ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
Supervisors: Dr. Jared Mecha, Dr. George Nyale, Prof. Kirana M. Bhatt, Prof. Lucy Muchiri

6.6 STUDY APPROVAL LETTER

KENYATTA NATIONAL HOSPITAL
P.O. BOX 20723, 00202 Nairobi

Tel.: 2726300/2726450/2726550
Fax: 2725272
Email: knhadmin@knh.or.ke

Ref: KNH/AD-MED/42B/VOL.1

Date: 16th December, 2015

Dr. Rebecca Njeri Karanja
Department of Clinical Medicine & Therapeutics
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Karanja

RE: APPROVAL TO CONDUCT STUDY IN KNH, MEDICINE DEPARTMENT

Following approval of your study by the KNH/UoN ERC and completion of the KNH study registration form, permission is hereby granted for you to collect data from Medicine Department to enable you complete your research on study titled: *"The prevalence of asthma phenotypes in patients attending the chest clinic at Kenyatta National Hospital"*.

Kindly liaise with the Senior Assistant Chief Nurse, Medicine Department for facilitation. By a copy of this letter, the Senior Assistant Chief Nurse, Medicine Department is informed and requested to facilitate.

DR. BERNARD GITURA
AG. ASSISTANT DIRECTOR, MEDICINE

Cc. Senior Assistant Chief Nurse, Medicine