PREVALENCE OF INFLUENZA VIRUS INFECTION IN ASTHMATIC CHILDREN PRESENTING WITH AN ACUTE EXACERBATION AT KENYATTA NATIONAL HOSPITAL.

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A dissertation presented in part fulfilment for the degree of Masters in Medicine (Paediatrics) at the University of Nairobi.

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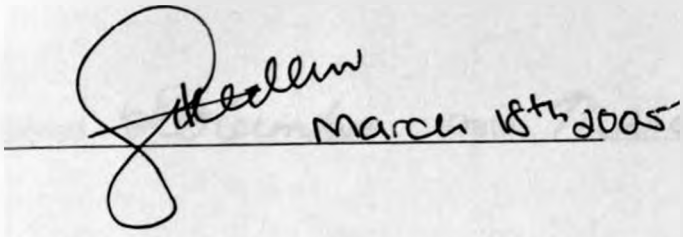
Declaration

I certify that this is my original work and has not been presented for any academic program in any other academic institution.



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Supervisors Declaration

I hereby certify that this dissertation has been submitted for examination with my approval.

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Dedications

To my parents, in memory of my late father, my mentor.

To my wife, for her patience and support.

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There are many people who have been involved, either directly or indirectly, in making successful the completion of this dissertation. A few however cannot go without mention: -

- All my teachers, past and present.
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List of Abbreviations

CDC	Center for Disease Control
CI	Confidence Interval
IFAT	Immunofluorescent Antibody Test
KNH	Kenyatta National Hospital
K.E.M.R.I	Kenya Medical Research Institute
Mmed	Masters in Medicine
OR	Odds Ratio
RPM	Revolutions Per Minute
RBCs	Red Blood Cells
RNA	Ribonucleic Acid
USA	United States of America
WHO	World Health Organisation

SUMMARY

Background

There is limited local data on the prevalence of influenza viral infection in children and no local data on the prevalence of influenza infection in asthmatic children. In temperate countries where influenza infection is prevalent, there is clear evidence of association between influenza and asthma exacerbation. Influenza virus infection is preventable in high-risk individuals by vaccination. Determination of prevalence of influenza virus infection in high-risk children such as asthmatics is important to eventually guide in local policy on prevention of influenza infection.

The main purpose of this study was to determine the prevalence of influenza virus infection and various strain-types in children with acute asthma exacerbation. It was hoped that this information would provide the baseline data for research in determining association between influenza virus infection and asthma exacerbation.

Methods

Over a 5-month period, 259 patients with acute asthma exacerbation were identified using the WHO clinical criteria for asthma at the paediatric filter clinic of Kenyatta National Hospital, Nairobi. Virological diagnosis of the influenza virus and strain-type was based on immunofluorescence studies of nasopharyngeal aspirates obtained from the asthmatic patients in acute exacerbation.

Results

The study subjects' median age was 2 years, (range 1 to 12 years), with 65.6% of the children aged less than 2 years. Of the patients recruited, 151 were males and 108 females, giving a male to female ratio of 1.4:1.

Thirty point one percent of the study population was exposed to passive cigarette smoke in their households, while 58.3% lived in one-bedroom houses. Twenty one point six percent of the subjects had a family history of asthma, while charcoal was the main source of cooking fuel, used in 54% of the homes. Mild exacerbation was the most common type of exacerbation (87.6%). There were no children with severe exacerbation Wheezing was a common presenting symptom during an exacerbation (90.7%), and 15.4% of asthmatics had a high-grade fever (temperature $\geq 38.5^{\circ}C$).

Forty-three children were found to have influenza virus in their nasopharyngeal aspirates, giving a prevalence of 16.6%, and all influenza virus isolates in this group of asthmatics were of strain-type B.

Conclusions and Recommendations

The prevalence of influenza viral infection was 16.6% (95% CI, 12 - 21%) among asthmatic children with acute exacerbation presenting at Kenyatta National Hospital and all influenza virus isolates were of strain-type B.

We recommend that an ongoing local surveillance for. and strain typing of influenza virus infection be continued especially among high-risk groups such as asthmatic children as this would determine the local influenza infection pattern. We also recommend that further research be done to evaluate association between influenza virus infection and asthma exacerbation.

1 BACKGROUND

1 1 Introduction

The magnitude of influenza virus infection in Kenya is generally unknown even though CDC reports that influenza virus infection in the tropics is a year round problem (1). No data showing evidence and distribution of the disease is available locally. In many countries antibiotic therapy still forms an integral part of management of asthma exacerbation though viral infections are the main culprits (2-5). In temperate countries where influenza is prevalent, influenza associated morbidity has cost the American and European economies a lot in medical related costs (1,6-10). In these areas, influenza has also played a major role in asthma exacerbation (2,7,8,11). Where vaccination against influenza virus has been used, studies have shown a decrease in influenza-related morbidity and economic gain (1,12-16). This information is from influenza infection in the general population, little is available on the prevalence of infection in the asthmatic population.

In Kenya, like in many other areas of the world therefore, data on the prevalence of influenza virus in asthmatic children is lacking hence the need to explore the problem in our set-up. It was hoped that this information would provide the baseline data for research in determining association between influenza virus infection and asthma exacerbation.

1 2 Literature Review

1 2 1 Influenza

Influenza is a major cause of morbidity and mortality in the world (6), however its effect in the Kenya population are not known. It is an acute respiratory illness caused by influenza A or B or C viruses, which are members of the orthomyxoviradae family of viruses. They are enveloped RNA viruses. Infection occurs mainly in the winter months in temperate countries, that is, countries with distinct winter months, whereas in the tropics, influenza infection occurs through out the year (1).

Influenza infection is a highly contagious disease that passes from person to person. The transmission of the virus is direct via airborne droplets. Large amounts of virus are present in respiratory secretions of infected persons, and are spread through sneezing, coughing and talking. Indoor crowding facilitates spreading of the virus. The contagious nature of the disease was exemplified in a case in 1977 during a commercial flight lasting four and half-hours, where a person suffering from influenza contaminated 72% of the other passengers. Spreading of the virus was facilitated by the closed circuit system used for the ventilation of the aircraft (17).

The incubation period is 1-4 days with an average of 2 days. Uncomplicated influenza illness is characterised by the abrupt onset of constitutional and respiratory signs and symptoms e.g. fever, myalgia, severe malaise, non-productive cough, sore throat and rhinitis. Illness typically resolves after several days for most persons, although cough and malaise can persist for 2 weeks or more. In some persons, influenza can exacerbate

underlying medical conditions such as pulmonary or cardiac disease or lead to secondary bacterial pneumonia or primary influenza viral pneumonia (18,19).

Diagnostic methods of identifying the influenza virus include direct detection of virus or viral protein in clinical samples, (for example nasopharyngeal aspirates), isolation of viable virus in cell culture, and detection of influenza virus specific immunoglobulins. The direct immunofluoresent test offers a rapid sensitive and specific method for the direct detection of influenza viruses A and B. The test utilizes species-specific monoclonal antibodies conjugated to fluorescein isothiocyanate to detect epitopes of influenza virus glycoproteins and fusion proteins specific to either influenza virus type A or type B. In one study conducted in five trial centres looking at the "IMAGEN" direct immunfluorenscence testing kit by DAKO Limited, sensitivity and specificity against the gold standard cell culture, was 96.2% and 100% respectively, and cross reactivity with other viruses low (20). The test does not detect antibodies of the influenza virus in serum.

Influenza is a major cause of morbidity and mortality in the developed world (6). It is not only a major cause of school absenteeism but is also estimated to cost the American economy over $12 billion and to cause 10,000-40,000 deaths annually. Studies looking at direct medical and medically related transportation costs incurred by patients in long-term facilities as a result of influenza like illnesses, showed high figures; an example is in the epidemics of 1970-78 in the U.S. where over $300 million was spent per epidemic. A substantial percentage of the patients were not immunised and were mainly children and old people (7-10,12). Otitis media and pneumonia are other complications of influenza

virus infection in children. Acute otitis media may be seen in up to 25% of culture-documented influenza. Unusual clinical features of influenza include acute myositis, myocarditis and toxic shock syndrome seen in influenza B. Influenza infection is also particularly severe in children with underlying cardio-pulmonary disease including congenital and acquired valvular disease, cardiomyopathy, broncho-pulmonary dysplasia and asthma (21).

As alluded to above, the economic impact of influenza infection on the society is enormous and the expected impact in a resource poor setting such as the Kenyan one may indeed be higher. Unlike the temperate areas where influenza infection is seasonal, in the tropical and sub-tropical areas, the virus is reported to be present all year round, and influenza outbreaks may be long and irregular or sometimes have no discernible limits at all (1). This has led to the recommendation that persons at high-risk of complication of influenza and who are not vaccinated should receive the vaccine before travelling to the tropics (1).

Influenza may be prevented through chemoprophylaxis using specific antiviral drugs; amantadine and rimantidine, which inhibit replication but are only effective against type A viruses. Influenza may also be prevented by vaccination, which is recognised as the only effective way to prevent influenza in high-risk groups. Currently available vaccines are 70-90% effective in preventing clinical illness when the circulating virus is homologous to the vaccine strain (2,11,22). Some examples of the clinical efficacy of the influenza vaccine are demonstrated in the study of influenza seasons of 1990-93 in the U.S.A. where

Vaccination was associated with 39-54% reduction in mortality from influenza related causes during the study periods (1). This observation was also seen during the influenza outbreaks of 1982-83 and 1985-86 in Canada where vaccination prevented 32-39% of hospital admissions for pneumonia and 15-34% of respiratory illnesses during the two outbreaks. Vaccination was 43-65% effective in preventing deaths associated with either condition (12). Also in Japan during the 1992-93-influenza season, a total of 137 asthmatic children with a mean age of seven years were enrolled. Protective efficacy by the vaccine was found to be as high 67.5% despite poor match between the virus and the vaccine strain (13).

Three types of trivalent vaccines are present; whole virion, split virion, and sub-unit vaccines. All influenza vaccines in routine use are killed or 'inactivated' so that they remain non-infective but retain their antigenic properties. Although all vaccines are similar in haemagglutin content, they differ in preparation, reactogenicity and immunogenecity (21).

1.2.2 **Asthma**

Asthma is the most common chronic disease in childhood. Between 5-15% of the paediatric population develop the disease (23). It is characterised by symptoms of cough, wheezing, dyspnoea, chest tightness (which occur in paroxysms and are usually related to specific triggering factors), airway hyper-responsiveness to a variety of stimuli, and reversible airway narrowing (23). Symptoms of asthma are due to airway obstruction, which results from cumulative effects of smooth muscle constriction of the small and large

airways. wall oedema. intraluminal mucous accumulation, and inflammatory cell infiltrates into the submucosa and basement thickening.

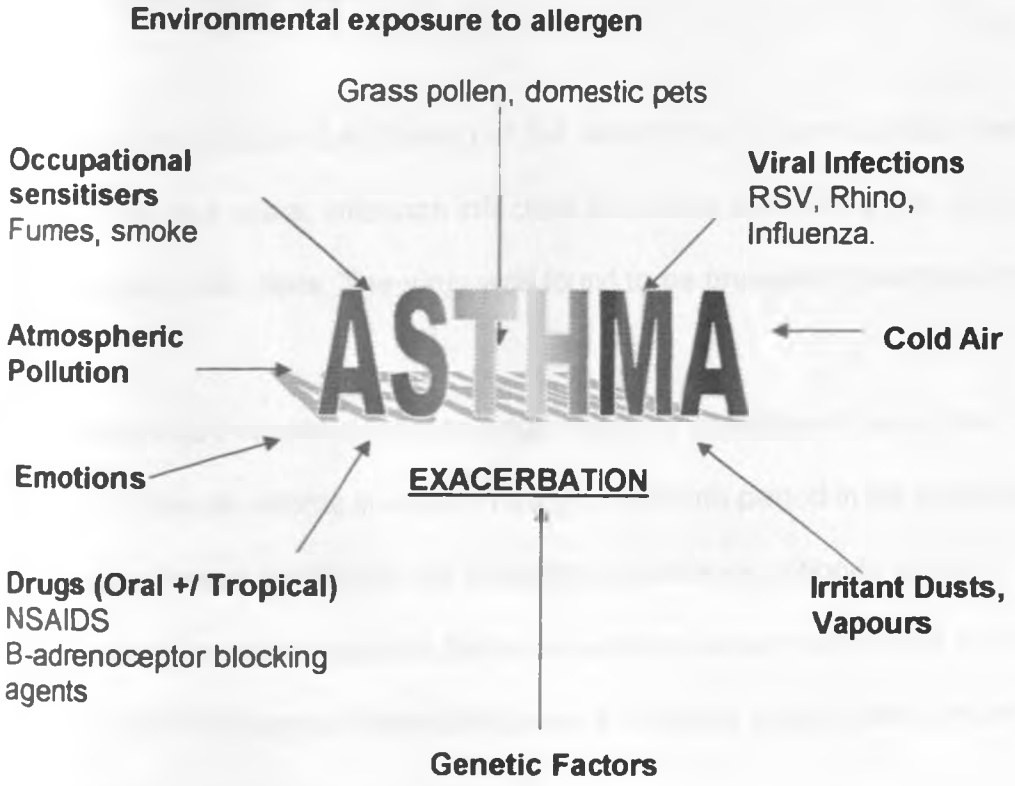
Data from the CDC in America has shown that the prevalence of asthma has increased (6). The prevalence has ranged from 1.2% in Scandinavian countries to 19% among children in New Zealand, while in the developing countries the prevalence of childhood asthma ranges from 0.01-3.3% in rural Africa to 9.8 -11.4% in urban Africa. The prevalence in many parts of Africa seems to have increased considerably, a phenomenon that may be explained by urbanisation and the accompanying life styles (24-27). In Kenya, local studies suggest a prevalence of about 11% (25).

The vast economic and social toll which asthma cumulatively exacts is illustrated by the fact that 2/3 of the children with asthma suffer noticeable disability, ten million school days are missed each year due to the disease, childhood asthma is a major cause of parental work absenteeism and an estimated one billion dollars is spent per year for children with asthma (6).

There are no Kenyan studies that exhaustively look at the role of infective agents in asthma attacks. Data from the temperate areas however suggests that viral infections are some of the most important triggering factors of asthma in most infants and young children (21). Early in life, respiratory syncitial virus (RSV) and para-influenza virus are most often involved; in older children rhinovirus has been implicated. Influenza virus infection assumes importance with increasing age (21). In the temperate areas, clustering

of attacks from fall to spring suggest a viral induced phenomena (2,4,11,19,23-28). Previous studies show that viral infections increase airway reactivity, probably by stimulation of afferent vagal receptors of the cholinergic system in the airway, and exacerbate asthma in children and thus accounting for 26-42% of childhood asthma episodes (21,29). During the high prevalence season of viral infection, up to 85% of the childhood asthma exacerbation is associated with viral infections. Thus, viral infections are one of the major precipitants of childhood asthma (3,4,18,30). Other triggers of asthma exacerbation are as illustrated below (figure 1)

Fig 1 Triggers of Asthma



1.3 Influenza and Asthma

There is paucity of data on the possible triggers of asthma exacerbation in children locally. Literature from the temperate countries suggests that influenza virus infection may trigger off an acute exacerbation of asthma that may result in hospitalisation hence causing substantial morbidity in children. Such information about influenza virus infection and its effects on asthmatic children in Kenya and in Africa as a whole is lacking. Kenya Medical Research Institute is undertaking a surveillance study of influenza in the general population (both adults and in children) in Kenya since 2001. The project has received to date about 100 samples of which 10% have tested positive for influenza by immunoflourescence antibody test; the number out of these who were asthmatics and it's effects on them is not known (31).

A 1985 study by Wafula et al, looking at the aetiology of acute respiratory infections in children under five years, influenza infection accounted for about 2.2% of the acute respiratory tract infections. The virus was found to be prevalent throughout the year (32).

The Nigerian study comes closest to addressing the objective of our study. It looked at microbial inciters of asthma in children over a 16 month period in 86 known asthmatics and influenza A was isolated by the immunoflourencence antibody test in 21.8% of the asthmatics with an acute episode. Bacterial isolates were only found in 3% of the aspirates. The mean age of the subjects was 27 months (range from 6 months to 12 years) and 48% were 5 years or under (5).

In a similar study by Pizzichini et al, where 8 asthmatics and 9 healthy (non-asthmatic) subjects were recruited on day 4 of a cold, influenza viral infections were confirmed in 6 of the 8 asthmatics and 6 out of the 9 healthy subjects. Compared to the healthy subjects and that one asthmatic patient without influenza infection, majority of the asthmatics not only had an objective exacerbation of asthma but also had more severe inflammatory response as was seen by the marked leucocytosis in their sputum. Influenza infection caused only "flu" like symptoms in healthy individuals, implying an exaggerated inflammatory response in those asthmatics infected with influenza (33).

In another study conducted in Japan where a total of 137 children aged 2-4 years with moderate to severe asthma were recruited, it was seen that majority of the asthmatics who were not vaccinated had influenza isolates during an exacerbation compared to those who were vaccinated who not only had less severe forms of exacerbation but also had other aetiologies attributed to their exacerbation (34).

These and other data suggest that a definite relationship does exist between influenza virus infection and asthma exacerbation in asthmatics (2,7-11).

We searched for direct evidence of the efficacy of the influenza vaccine in preventing asthma exacerbation in prospective randomised controlled studies but most data was inconclusive. Most of the evidence is in the form of health reports from different organisations recommending the vaccine in this sub-set of the population. A few studies

ke that from Japan (34) above, and that of a population based retrospective study in the U S. that looked at the vaccine safety data base of 3 influenza seasons showed that the use of the vaccine was associated with decrease in the rate of asthma exacerbation. The latter study looking at 1-6 year old asthmatic children showed that the risk of asthma exacerbation was decreased by 22-41% following vaccination in the 3 seasons (35).

1.4 Study Justification

There is limited data on the prevalence of influenza viral infection in children and no local data on the prevalence of influenza infection in asthmatic children. In temperate countries where influenza virus infection is prevalent, there is clear evidence of association between influenza and asthma exacerbation. Influenza virus infection is preventable in high-risk individuals by vaccination. There was therefore need to determine the prevalence of influenza virus infection and various strain-types in children with acute asthma attack in our set-up as this information would provide the baseline data for research in determining association between influenza virus infection and asthma exacerbation which would lead to local policy on prevention of influenza virus infection in asthmatic children.

1.5 Study Objectives

1. To determine the prevalence of influenza virus infection among asthmatic children presenting at Kenyatta National Hospital with acute asthma exacerbation.

2. To determine the strain-type of the influenza viral isolates from asthmatic children presenting to Kenyatta National Hospital with acute asthma exacerbation.

2 METHODS

2.1 Study Design

This was a hospital based cross-sectional study.

2.2 Study Site

The study was based at Kenyatta National Hospital's paediatric filter clinic, which is Kenya's largest referral hospital. The hospital is located in the capital city, Nairobi. Apart from attending to referral patients, the hospital also acts as a primary hospital serving many inhabitants of Nairobi, mainly from a poor socio-economic background. The study was conducted over a 5 months period between the months of May and October 2003.

2.3 Study Population

All asthmatic children aged between 1-12 years (both years inclusive) presenting with acute asthma exacerbation were included in the study.

2.3.1 Inclusion Criteria

Included were all patients aged between 1-12 years whose parents or guardians had given consent and had presented with any of the symptomatology of asthma having occurred more than once at any time during a 3 month period prior to presentation to the study site and having had responded favourably after treatment with broncodilators or and

anti-inflammatory drugs on a previous occasion. These subjects were considered to fit the criteria of an asthmatic patient.

2 3 2 Exclusion Criteria

Excluded were all children whom the diagnosis of asthma could not be adequately established from previous history.

2 4 Definitions

2 4 1 Asthma

Asthma was defined as a chronic inflammatory condition with reversible airway obstruction characterised by recurrent episodes of wheezing, often with cough, which responds to treatment with bronchodilators and anti-inflammatory drugs (21). Chronicity was taken as a time frame of 3 months' duration or longer as per the US National Centre for Health Statistics (36). Reversibility of airways was determined from previous history determined from the questionnaire as having had responded favourably by either reduction or cessation of the symptoms of asthma exacerbation usually common to that particular patient after use of a bronchodilator such as salbutamol or after use of anti-inflammatory drugs such as steroids. It was not based on the response to treatment of the current episode of exacerbation or respiratory function tests. Wheezing was defined as musical sounds heard on auscultation, which tend to be polyphonic (varied pitch) usually on expiration but can be both on inspiration and expiration heard either by placing the ear next the subjects' mouth during quiet respiration or heard by use of the stethoscope. Recurrence was considered as having occurred more than once.

2.4.2 Acute Asthma Exacerbation

Acute asthma exacerbation was defined as a history of acute onset of symptoms of wheeze, cough, difficulty in breathing, chest tightness as reported by the asthmatic patient or parent/guardian of the asthmatic patient.

2.5 Sampling Methods

The principal investigator would present himself to the study site on average 4 hours (usually 2 hours in the morning and 2 hours in the evening) per day on any day of the week. All patients with respiratory complaints during this time period were identified sequentially initially by clinicians (doctors or clinical officers) in the paediatric filter clinic. The clinicians varied depending on their duties at the hospital. All subjects who had presented with wheezing or features suggesting an acute asthma attack were then handed over to the principal investigator usually on their way to the nebulisation room, who then further screened them for eligibility. Only those subjects who qualified as per the above inclusion criteria were then recruited for the study. Clinical response to nebulisation while at the study site was monitored by the primary clinician.

2.6 Sample Size Estimation

Sample size was calculated according to the following formula (37):

$$N = \frac{Z\alpha^2 pq}{d^2}$$

Where:

N = the desired sample size.

$Z\alpha$ = the standard normal deviate at the required confidence interval.

p = the proportion in the target population estimated to have the characteristic being measured.

q = 1-p.

d = the level of statistical significance set.

In this study, at 95% confidence interval, Z statistic at 1.96, while p is 20% based on the estimated prevalence from a similar Nigerian study (5). This was because the Nigerian study was the only African study that attempted to address the issue in question and also, that it was performed on the same economically deprived background as ours. The level of statistical significance for this study was $\pm$ 5% (0.05). Using the above assumptions, the minimum sample size is 246.

2.7 Study Procedures

Names and numbers of the potential subjects were recorded and then handed over to the principal investigator who reviewed each patient's eligibility for the study and consent sought from parent/guardian if they qualified. All subjects identified were then interviewed and examined and relevant data collected using a questionnaire.

Nasopharyngeal aspirates were obtained using a soft catheter and a mucous trap. One of the catheters was introduced into the nasopharynx through one of the nostrils. To ensure proper insertion of the catheter, either the parent/guardian or a nurse, often assisted in securing the child to reduce excessive movements by the child. The distance from the nose to the nasopharynx was estimated by approximating the distance from the angle of the jaw to the mouth. A large 50 millilitres syringe was used to apply the suction pressure.

After secretions were collected in the mucous trap, the catheter was gently removed and about two millilitres of phosphate buffered solution added to the secretions. The contents were shaken gently and then injected into the virus transport medium. The specimen was labelled and transported in a vaccine carrier to the K.E.M.R.I. virology laboratory where it was processed on the same day the specimen was collected. The laboratory diagnosis of influenza was based on the direct immunofluorescent antibody test. The kit used at the K.E.M.R.I laboratory was made by Dako Limited, United Kingdom and was supplied by Aventis Pasteur limited.

2.8 Laboratory Procedures

The laboratory diagnosis of influenza was based on the direct immunofluorescent antibody test (IFAT). IFAT was performed using species-specific monoclonal antibodies conjugated to fluorescein isothiocyanate.

Nasopharyngeal secretions were prepared by washing with phosphate buffered saline three times and by centrifugation at 3000 rpm at room temperature, and cells from the nasopharyngeal aspirate suspended in small volumes of the phosphate buffered saline and applied on multi-spot slides. The slides were dried by a stream of cool air at room temperature where they were then fixed in cold acetone for 10 minutes at 4°C if they were to be used on the same day or - 40°C for future use. Slides were stained with specific antiserum for Influenza A and B and incubated for 30 minutes at 37°C in a humidified chamber. Excess antiserum was removed by rinsing and then washing in 3 changes of phosphate buffered saline for 10 minutes each time. Slide spots were stained with relevant influenza specific (A or B) fluorescein-labelled antiserum diluted to optimal concentration followed by incubation for 30 minutes at 37°C in humidified chamber. Slides were washed again as in the step above. The slides were then dried, mounted in glycerol and examined under x40 objective lens with the fluorescent microscope. A positive diagnosis was made If cells in the fixed stained specimen showed specific fluorescence to either influenza A or B virus reagent, that is, a characteristic bright apple-green colour seen within the cytoplasm and the nucleus of the cell, which contrasts with the red background staining uninfected cells. A negative diagnosis was made when the fixed specimens did not exhibit fluorescence with either reagent.

2.9 Data Management and Analysis

Data was coded and then stored and analysed using the Statistical Packages for Sciences Software (SPSS) version 10.0. Descriptive statistics of the study, that is, the median and age ranges of the population were determined. The age distribution was then expressed in graphic form. Prevalence of influenza virus was computed by dividing the number of positive isolates with total number of subjects sampled and this expressed in percentage form. The prevalence of the strain-type was computed by dividing the positive isolates for that particular strain divided by the total number of positive isolates and then this expressed in percentage form. Frequency distribution tables for the socio-demographic and clinical characteristics were also determined. Associations between influenza virus infection and the socio-demographic and clinical characteristic were explored using Chi-square test and Odds ratios for dichotomous and categorical values.

2.10 Ethical Consideration

Approval from the Kenyatta National Hospital ethical committee was sought prior to starting the study.

Informed consent was obtained from the parent/guardian of the child being recruited. This involved signing a form after explanation by the investigating officer about the details of the study. The explanation included facts and basis of the study, the risks and benefits of the study, and confidentiality and voluntary nature of the study. The contact address of the investigating officer was also forwarded to the parent/guardian of the child in case

he/she needed further details about the study or wished to withdraw from the study. Investigations were done at no cost to the patient.

The risks to the child participating in the study were minimal. No obvious trauma was experienced from the process of sample collection in any of the patients, though some discomfort was felt.

Efforts were made to reduce risk of transmission of infection among the patients, from patient to the investigating officer and vice versa and among the laboratory staff. Standard (hand washing), contact (gloves), and airborne (masks) precautions were maintained whenever collecting and handling specimens. A separate catheter and mucous extractor were used for each patient.

To avoid contamination and confusion of samples, specimens were sealed, labelled and then stored in a cooler box before being transported to the laboratory.

Results of the laboratory analysis were stored in a floppy diskette and hard-drive of the investigating officer's computer only so as to maintain confidentiality. This information was made available to the parent/guardian/caretaker at any time during the study period and was communicated to them in absolute confidence.

3. RESULTS

The study was carried out between the months of May and October 2003. The months of May to August comprise the coldest months in Nairobi, while October is the beginning of the 'short' rainy season. 2072 patients with respiratory complaints were screened aged 1-12 years from the paediatric filter clinic. Of these, 259 satisfied the inclusion criteria and were recruited into the study.

3.1 Socio-economics and Demographics Factors

The study subjects' median age was 2 years, ranged from 1 to 12 years, with 65.6% of children aged less than 2 years (Table 1). Of the patients recruited, 151 were males and 108 females, giving a male to female ratio of 1.4:1.

Thirty point one percent of the study population was exposed to passive cigarette smoke in their households, while 58.3% lived in one-bedroom houses. Twenty one point six percent of the subjects had a family history of asthma, while charcoal was the main source of cooking fuel, used in 54% of the homes (Table 1). Mild exacerbation was the most common type of exacerbation (87.6%). There were no children with severe exacerbation. Wheezing was a common presenting symptom during an exacerbation (90.7%), and 15.4% of asthmatics had a high-grade fever (temperature $\geq 38.5^{\circ}C$) (Table 2).

Fig 2 Age Distribution of the Study Population (N = 259)

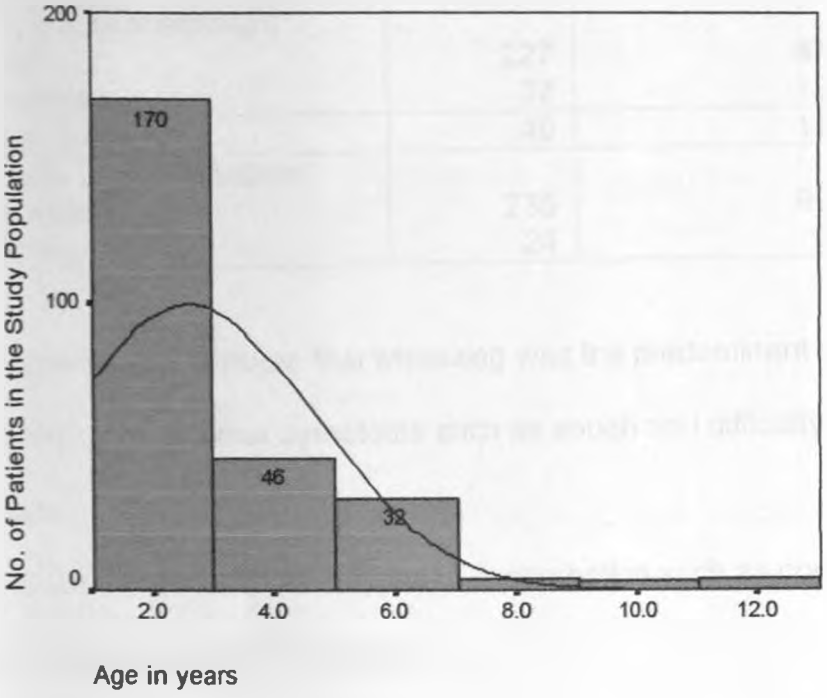


Table 1. Descriptive Characteristics of the Study Population

Characteristic (N=259)	Number	Percentage
Age ≤ 2 years	170	65.6
Age > 2 years	89	34.4
Sex:		
Male	151	58.3
Female	108	41.7
Exposure to cigarette smoke	78	30.1
Rooms in the house:		
One bedroom	151	58.3
Family history of asthma	56	21.6
Cooking fuel:		
Charcoal	189	73
Kerosene stove	70	27

Table 2 Clinical Characteristics of the Study Population

Characteristics (N=259)	Number	Percentage
Type of exacerbation:		
Mild	227	87.6
Moderate	32	12.4
High grade fever	40	15.4
Profile of exacerbation:		
Wheezing	235	90.7
Others	24	9.3

Wheezing in table 2 denotes that wheezing was the predominant symptom during an exacerbation though other symptoms such as cough and difficulty in breathing may also be present.

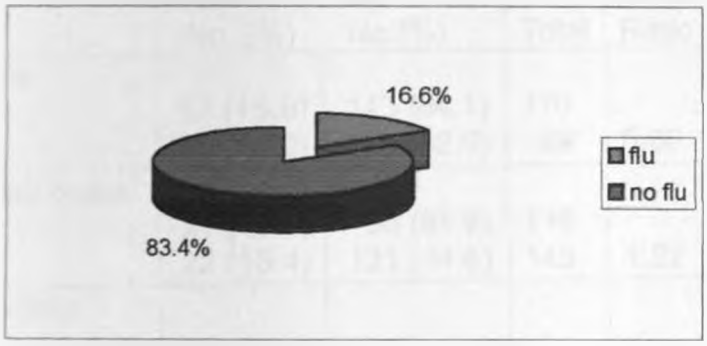
Others in table 2 denotes other types of exacerbation such as cough and difficulty in breathing with absence of wheezing.

3.2 **Prevalence of Influenza Virus**

Of the 259 patients recruited for the study, 43 were found to have influenza virus in their nasopharyngeal aspirates giving a prevalence of 16.6%, (95% CI, 12.1-21.1)(figure 3). The median age of the patient with the influenza virus was 2 years (range 1 and 9 years). The influenza virus prevalence was 15.9% in the age group ≤ 2 years and 18% in the age group > 2 years. The influenza virus was not isolated in patients aged 8 to 12 years of age. There were more males (58.1%) than females (41.9%) with the influenza virus. The difference was not statistically significant (P value 0.98)

All influenza virus isolates in this group of asthmatics were of strain-type type B.

Fig 3 Prevalence of Influenza virus



3.4 Exploring Associations between Influenza Virus Infection and Other Factors

The presence of influenza virus was cross tabulated against age, exposure to cigarette smoke at home, number of rooms in the house, severity of exacerbation, presence or absence of high grade fever and type of cooking fuel at home and odds ratio computed and it's significance sought. There appeared to be a tendency towards significant association between presence of fever and influenza virus infection in asthmatic children with acute exacerbation (OR 13.2 (95% CI, 3.62-51.50) and $p < 0.001$). In all the other descriptive and clinical characteristics, no significant association was seen (Table 3).

Table 3 Exploring Associations between Presence of the Influenza Virus and some of the variables

Characteristics N=259	Presence of Influenza Yes No. (%)	No No.(%)	Total	Odds Ratio	95% CI	P value
Age in years						
< 2 years	27 (15.9)	143 (84.1)	170			
> 2 years	16 (18.0)	73 (82.0)	89	0.86	0.42-1.80	0.67
Rooms in the house						
One	21 (18.1)	95 (81.9)	116			
> One	22 (15.4)	121 (84.6)	143	1.22	0.60-2.46	0.56
Cigarette smoke exposure						
Yes	15 (19.2)	63 (80.0)	78			
No	28 (15.5)	153 (84.5)	181	1.30	0.61-2.74	0.46
Cooking fuel						
Charcoal	30 (15.9)	159 (84.1)	189			
Kerosene	13 (18.6)	57 (81.4)	70	0.83	0.38-1.81	0.60
Exacerbation						
Moderate	8 (25.0)	24 (75.0)	32			
Mild	35 (15.4)	192 (84.6)	227	1.83	0.69-4.72	0.17
Fever						
Yes	34 (85.0)	6 (15.0)	40			
No	9 (4.1)	210 (95.9)	219	132.22	36.22-515.02	< 0.001

DISCUSSION

Hitherto, influenza viral infection has been perceived to be erratic and of little consequence in tropical Africa (1,38). This infection has been viewed as a problem mainly of temperate countries. Our data demonstrates a high prevalence of almost 17% among Kenyan asthmatic children in exacerbation at Kenyatta National Hospital. Our prevalence compares to those described in the paediatric populations in temperate countries, which range from 10-40% (40) and is similar to the Nigerian prevalence of 20% in a comparable paediatric population (5). Our finding demonstrates that asthmatic children in Nairobi presenting with acute asthma attacks at Kenyatta National Hospital are as susceptible to influenza virus infection as are other children.

During the season in which the study was conducted a single viral strain-type (influenza strain-type B) was isolated from the children. From this finding we can forecast a good response to vaccination should it be recommended by later studies as this is one of the strain-types in the current influenza vaccine. However because of the limited time period in which the study was conducted, only a year round surveillance study would be able to be more informative as to whether this is the predominant strain-type locally.

An ongoing national influenza surveillance study similarly reports strain-type B as the predominant strain-type, accounting for more than 90% of the isolates so far collected (31). Our findings therefore concur with the strain pattern being observed nationally. Our study seemed to differ greatly from those of other African countries. In Nigeria, influenza

strain-type A was the only strain-type identified in a study of asthmatic urban children (5). This difference in strain-type was also seen in the Influenza outbreak of Madagascar (38). The difference in the strain pattern with that of the Nigerian study may be attributed to the geographical location of Nigeria, with Europe to its north and the Americas to its west. This is because, though, Europe has both strain types A and B, it is mainly strain-type A that has been blamed for the numerous influenza epidemics observed in these regions (38), hence frequent movements to and from these temperate areas might have resulted in easy contagion of this particular strain. The Nigerian study was also conducted in a different year (1993) from our study. With the year-to-year antigenic changes that are characteristic of this virus, this could also be a possible explanation for different strain patterns observed between this and the Nigerian study.

Regarding the socio-demographic profile of our study population, the majority of asthmatics identified were young toddlers (mean age of 2.6 years). This observation was similar to that of the Nigerian study (mean age 2.3 years) (5). This finding suggests that asthma sets in at an earlier age in both the asthmatic children presenting at Kenyatta National Hospital as well as the Nigerian paediatric populations, an observation that is in keeping with the trend in the rest of he world (21).

In a child presenting with acute asthma and pyrexia, our data suggests that a temperature of greater than 38.5°C might be a pointer towards a possible influenza viral infection. This has been seen in other studies as well, where high-grade fever was associated with viral infection during an acute asthmatic attack (4,5,8,18,21). In the Nigerian study, there was

significant association between high-grade fever and influenza virus infection. Our inability to rule out other infections (mainly attributed to financial constraints in providing kits for laboratory identification of other agents) down plays this observation in our study. It is however prudent that the clinician faced with such a child considers the possibility of influenza viral infection as a one of the possible causes of exacerbation.

The study had a few limitations; for example we would have liked to have explored more conclusively, seasonality and prevalence of co-infection with other agents, however due to financial limitations this was not possible. One study limitation noted, was our inability to conclusively rule out bronchiolitis as cause of wheeze in our study. However, based on our stringent clinical inclusion criteria, mis-classification of bronchiolitiis as asthma exacerbation was probably rare. This is because though recurrence of bronchiolitis is rare (hence recurrent wheezing episodes unlikely to be attributed to bronchiolitis), there is still that remote possibility that a known asthmatic too may suffer from an acute episode of bronchiolitis. Response to treatment could have guided us in differentiating the two, as an asthmatic patient would show a favourable response when put on bronchodilators while a patient with bronchiolitis would not. We also acknowledge that spending approximately 4 hours for screening purposes per day might be a potential source of bias and some patients might have been missed by the principal investigator

We conclude that the prevalence of influenza virus infection among asthmatic children with acute exacerbation presenting at Kenyatta National Hospital is as high as that seen

n the paediatric population in the temperate countries (40), and that during this study eriod, strain-type B was the prevalent strain.

We recommend that local regular surveillance for, and strain typing of influenza virus nfection be continued especially among high-risk groups as this would determine the ocal influenza infection pattern throughout the year. This is not only because the prevalence rate observed in our study was similar to that seen in the paediatric population n the temperate countries but also that data from these countries indicate that influenza nfection may be responsible for increased morbidity in susceptible groups (1, 9-12, 37), hence the need for the regular surveillance of influenza infection patterns in high-risk groups locally.

We also recommend that further research be done to evaluate association between influenza virus infection and asthma exacerbation.

CONCLUSIONS

The prevalence of influenza viral infection among asthmatic children with exacerbation presenting at Kenyatta National Hospital was 16.6%.

All influenza virus isolates from asthmatic children presenting at Kenyatta National Hospital were of strain-type B.

6 RECOMMENDATIONS

We recommend that local surveillance for, and strain typing of influenza virus infection be continued especially among high-risk groups such as asthmatic children as this would determine the local influenza infection pattern.

We also recommend that further research be done to evaluate association between influenza virus infection and asthma exacerbation.

8 APPENDICES

Appendix I Written consent form

Prevalence of influenza in asthmatic children with exacerbation

Investigators
Dr Twahir Hemed

24 hour contact telephone number: Dr Twahir Hemed, 0722821712.
Ethical Review Committee Chairperson: Professor K.M. Bhatt, 726300 Nairobi.

We are asking you to allow your child to be in a research study. The purpose of this consent form is to give you the information you will need to help you decide whether your child should be in this study or not. Please read this form carefully. You may ask questions about what we will ask your child to do, the risks, the benefits, your child's rights as a volunteer, or anything else about the study or this form that is not clear. When all your questions have been answered, you can then decide on behalf of your child to be in the study or not. This process is called "informed consent". We will give you a copy of this form for your records.

Introduction.

When children with asthma are infected with the influenza virus, they have a tendency to get more serious and more frequent symptoms of asthma hence requiring more medical attention in hospital outpatient and inpatient wards. This translates to more costs to you the parent or guardian of the child with asthma. We need to therefore learn how many asthmatic children like yours, suffer from the influenza virus. We need to study secretions obtained from an area called the "nasopharynx" which is between the throat and the nose of your child, during an attack of asthma.

The purpose of this study is to investigate influenza infection among asthmatic children with acute attack. This will help us to determine the magnitude of influenza infection in a susceptible population such as the asthmatic children, which in turn might help us decide on whether to study the effects of the virus in this population and eventually effectiveness of the influenza vaccine in preventing asthma attacks. All this at the end will translate into formulation of policies concerning prevention of infection of this virus in asthmatic children, as a vaccine is available in the country.

Purpose and benefits.

By allowing your child to participate in the study, you will help us determine the magnitude of the problem in our set-up and help us to make decisions concerning your child and others like yours in the future. We will do tests to see if your child has the influenza virus during an asthma attack. Your child will be given treatment for the asthma attack as is available at Kenyatta National hospital. Treatment for the influenza virus if found in your

child's secretions, is expensive and not easily available at Kenyatta National Hospital and is useful in only one influenza virus strain-type. We will however share information about your child's test with you and will advise you on the use of the influenza vaccine, which may prevent similar future infections in your child.

How a child can get influenza infection.

Influenza virus is found in the secretions of the respiratory tract in an infected person. It is transmitted from one person to another by spread of droplets during coughing or sneezing.
Normal children may get the normal "flu" symptoms, however asthmatic children infected with the virus may get more frequent and more severe asthma attacks.

Procedures

At the time you enrol your child in the study, we will ask you questions about your child's medical history and about yourself and family. Some of the questions are of personal nature. You are free to refuse to answer any questions you do not wish to answer. We will collect samples from your child's "nasopharynx" and will consist about a half a teaspoonful of secretions. We will use an instrument called a mucous extractor that will involve inserting a small tube through the nostril of your child. The procedure should take not more than one minute to perform. The test will be done at no cost to you.

Risks and discomforts of being in the study.

The study involves information that may be embarrassing to you. Obtaining secretions from your child is not a painful process though some discomfort is expected. We will use a sterile "mucous extractor" for your child hence the risks involved is minimal, which may include trauma to the nasopharynx and infection.

Other Information.

Information about your child's diagnosis is confidential and will kept in a floppy diskette and hard-drive in a computer in a locked office. Information about your child's diagnosis and his/her participation will be available to you and the study team but not to anyone outside the study.

You may refuse to participate or may withdraw from the study at any time without penalty or loss of benefit to which your child is entitled.

Do you have any questions? Do you agree to participate?

Signature of investigator. Printed Name. Date

Parent's/Guardian's signature

The study described above has been described to me, the parent/guardian of _____. I have had a chance to ask questions. I have been told that if I have future questions about the study or my child's rights as a subject, I can ask the investigator above. I have been told that I am free to withdraw my child from the study at any time.

.....

Signature of parent/guardian Printed name Date

Appendix II

II.a Guide to Rates of Breathing in Awake Children

Age	Normal rate
0-2 months	< 60/min
2-12 months	< 50/min
1- 5 years	< 40/min
6-12 years	< 30/min

II.b Guide to Normal Pulse Rates in Children

Age	Normal rate
0-2 months	120-160/min
2-12 months	110-150/min
1- 5 years	100 -120/min
6-12 years	70-110/min

Appendix III Classification of Severity Asthma (39)

Clinical features before treatment	Symptoms	Night time symptoms
Mild intermittent	Symptoms ≤ x 2 a week. Exacerbation brief. Intensity may vary.	≤ 2 times a week
Mild persistent	Symptoms > x 2 a week but < 1 time a day. May affect activity.	> 2 times a month
Moderate persistent	Daily symptoms. Daily use of β 2 agonist. Activity affected, ≥ 2 x a week, lasts long.	> 1 time per week
Severe persistent	Continual symptoms. Limited physical activities. Frequent exacerbation.	Frequent

Appendix IV Classification of Severity of Acute Asthma Exacerbation (39)

	Mild	Moderate	Severe	Imminent respiratory failure
Symptoms.				
Breathlessness	While walking	While talking (infant-softer shorter cry; difficulty in feeding	While at rest (infant stops feeding)	
	Can lie down	Prefers sitting	Sits upright.	
Talks in..	Sentences	Phrases	Words	
Alertness.	May be agitated	Usually agitated	Usually agitated	Drowsy or confused
Signs.				
Respiratory rate.	Increased	Increased	Increased	May be decreased
Use of accessory muscles; sub-costal recessions.	Usually not.	Commonly.	Usually.	Paradoxical thoraco-abdominal movement.
Wheeze.	Moderate, often only end expiratory.	Loud, throughout exhalation.	Usually loud, throughout inhalation and exhalation.	Absence of wheezing.
Pulse/min.	<100	100-200	>120	Bradycardia (<50)

The feature in the most severe category of classification indicated the severity of asthma exacerbation in that patient.

Appendix V Clinical data sheet

Demographic Data (tick where appropriate)

Date _____/_____/_____

Study No: _____

Hospital No: _____

Name (Initials may do). _____

Gender: 1. Male_____ 2. Female_____ Temperature:_____

Age: _____ years Respiratory rate:_____

Profile of Typical Exacerbation.

Cough _____
Chest Tightness _____
Wheezing _____
Difficulty in breathing _____
Other _____

Rooms in House: (one, two-three, ≥ three)
_____

Type of cooking fuel: (charcoal, stove, gas)
_____

Exposure to cigarette
Smoke: _____

Family history of asthma. Yes_____ No_____

- Severity of exacerbation:
  - Mild_____
  - Moderate_____
  - Severe_____
  - Imminent respiratory failure_____

- Severity of asthma:
  - Mild intermittent_____
  - Mild persistent_____
  - Moderate persistent_____
  - Severe persistent_____

- Any other comments?

_____
_____
_____

Signature of investigating officer: _____

Date: _____/_____/_____

Appendix VI Approval Letter from Kenyatta National Hospital's Ethical Committee

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P O Box 20723, Nairobi

Tel 726300-9
Fax 725272
Telegrams: "MEDSUP", Nairobi.
Email **KNHplan@Ken.Healthnet.org**

Ref: KNH-ERC/01/1792 **Date: 22 May 2003**

Dr Twahir Hemed
Dept. of Paediatrics & Child Health
Faculty of Medicine
University of Nairobi

Dear Dr. Twahir,

RESEARCH PROPOSAL "THE PREVALENCE OF INFLUENZA VIRUS INFECTION IN ASTHMATIC CHILDREN AT KENYATTA NATIONAL HOSPITAL" (P21/3/2003)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** the revised version of your above cited research proposal.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication

Yours sincerely,

DR. LUCY MUCHIRI
For: SECRETARY, KNH-ERC

Cc Prof. K.M. Bhatt, Chairperson, KNH-ERC
The Deputy Director (C/S), KNH
The Dean, Faculty of Medicine, UON
The Chairman, Dept. of Paediatrics, UON
CMRO
Supervisors: Dr. E. Obimbo, Dept. of Paediatrics, UON
Dr. J.M. Chakaya, KEMRI

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