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Serum 25-hydroxyvitamin d levels in children with mild and severe atopic asthma

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SERUM 25-HYDROXYVITAMIN D LEVELS IN CHILDREN WITH MILD AND SEVERE ATOPIC ASTHMA

By

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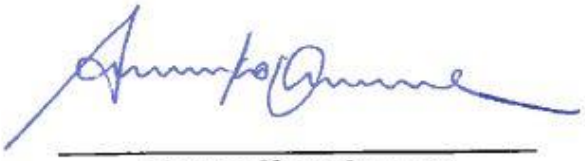

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DEDICATION

I dedicate this thesis to my parents, Mr Lawrence Masaku and Dr Margaret Masaku. I thank them for all their hard work and for giving me the best life and educational opportunities. They are my role models, and I appreciate and love them dearly.

To my sisters Rachael, Rabecca and Ruth Masaku, you continue to inspire me to be my best.

ABSTRACT

Background

Asthma is a chronic respiratory illness affecting an estimated 334 million people worldwide, including 14% of the total paediatric population. It results in significant morbidity, and therefore the etiologies and risk factors associated with asthma are of great interest. Serum 25-hydroxyvitamin D (25(OH)D) has substantial immunomodulatory effects on the innate immune system. It reduces the probability of viral respiratory infections and asthma exacerbations by potentiating the anti-inflammatory action of corticosteroids.

Objectives

This study was conducted to determine if there was a difference in serum 25-hydroxyvitamin D levels between children with mild and severe atopic asthma and explored the correlation between various comorbidities and severity of asthma in children.

Methods

The study design was cross-sectional. Recruitment was done at the Aga Khan University Hospital, Nairobi (AKUHN). Participants were classified as having either mild or severe asthma as per the Global Initiative for Asthma **(**GINA) guidelines of 2019 (1). The analysis was stratified based on the severity of asthma (mild and severe) and compared using chi-square for categorical variables while continuous data were compared using the Mann-Whitney test. Multivariable analysis was employed to ascertain the association between severity of asthma and various comorbidities with a statistically significant p-value being less than 0.05.

Results

Seventy children were recruited into the study with a median age of 5 (IQR: 3-7) years. The median (IQR) serum 25(OH)D levels in the mild and severe asthma categories were estimated as 26.8(23.5-32.9) and 24.6(21.0-31.8) ng/mL, respectively. This difference was not statistically

significant. In the multivariable analysis; family history of asthma, BMI, age and allergic conjunctivitis were not significantly correlated to severe asthma.

Conclusion

The level of serum 25(OH)D was not significantly associated with the severity of asthma. No association between known comorbidities and asthma severity was found.

LIST OF ABBREVIATIONS

AKUHN	Aga Khan University Hospital in Nairobi
GINA	Global Initiative for Asthma
ICS	Inhaled corticosteroid
LTRA	Leukotriene receptor antagonists
LABA	Long-acting beta-adrenoceptor agonist
FEV1	Forced expiratory volume in one second
C-ACT	Childhood Asthma Control Test
25(OH)D	25-hydroxyvitamin D

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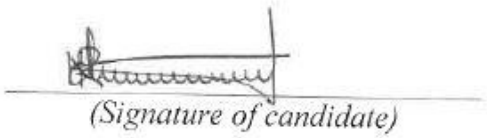
I am eternally grateful to all the participants and their guardians who allowed me to recruit them for this study.

Lastly, I want to thank the Almighty God, who granted me the strength to pursue my studies

Thank you all

DECLARATION

I declare this dissertation does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university and that to the best of my knowledge it does not contain any material previously published or written by another person except where due reference have been made in the text.



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CHAPTER ONE: INTRODUCTION

1.1 Background of the study

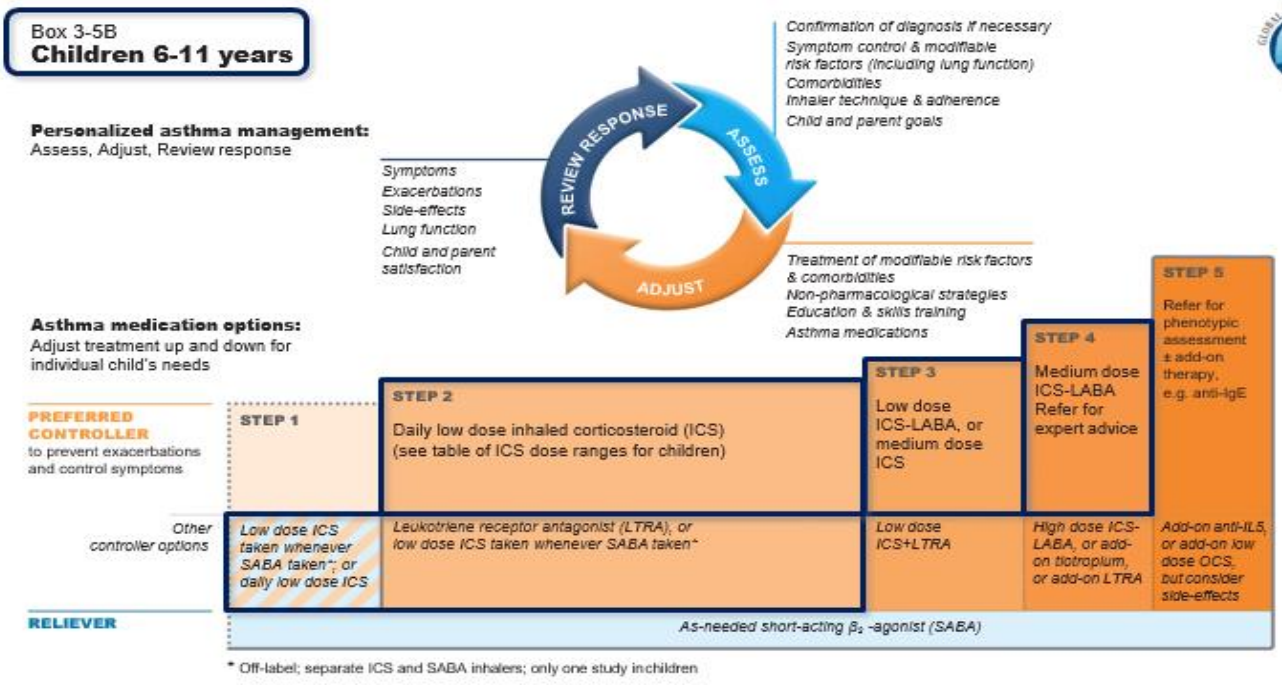
Asthma is a heterogeneous illness associated with chronic inflammation of the airway. It is characterised by a history of symptoms in the respiratory tract such as a cough, chest tightness, wheeze, shortness of breath. These symptoms differ in intensity and duration (1). It is estimated that about 334 million people worldwide are affected by asthma, including 14% of children. As a chronic condition, the prevalence of asthma worldwide is high. Its prevalence has worsened in the previous several years, leading to a worsening of the health burden globally (2, 3). There is an estimate that by the year 2025, approximately 400 million people worldwide will have asthma (4).

Atopic asthma tends to occur before the age of 6 years. It is linked to atopy, which is a genetic tendency to be sensitised to allergens and have immune responses, which are exaggerated to common allergens in the environment. There is a connection with severe hyper-responsiveness of the bronchi, which continues into adulthood (5).

The significant morbidity associated with asthma results in days lost both from school and work, economic losses due to absenteeism, and leads to distress among patients due to their quality of life being affected. Due to these factors, identification of causes and related comorbidities of asthma becomes necessary and of importance.

The National Heart, Lung and Blood Institute (NHLBI) in 2007 came up with the diagnosis and management of asthma guidelines (3). Wheezing in high-risk children is the primary symptom, and the guidelines require 1 major criterion or 2 minor criteria to be fulfilled for diagnosis.

The guidelines by the Global Initiative for Asthma (GINA) (1) are used widely in the management of asthma. A stepwise management plan is shown in figure 1 below.



Courtesy of the GINA global strategy for asthma 2019

Figure 1: GINA guidelines 2019 on stepwise asthma management

The GINA guidelines of 2019 provide a stepwise plan for treatment of asthma based on how severe the patient's condition is. In the management of mild asthma which is step 1 and 2, a leukotriene receptor antagonist (LTRA) or low-dose inhaled corticosteroids (ICS) is used as a controller. Step 3 for the management of moderate asthma uses a long-acting beta-adrenoceptor agonist (LABA) and low-dose ICS or LTRA and low-dose ICS or medium-dose ICS. Finally in step 4 and 5, severe asthma is managed with medium-dose ICS and a LABA or high-dose ICS and a LABA or tiotropium or LTRA.

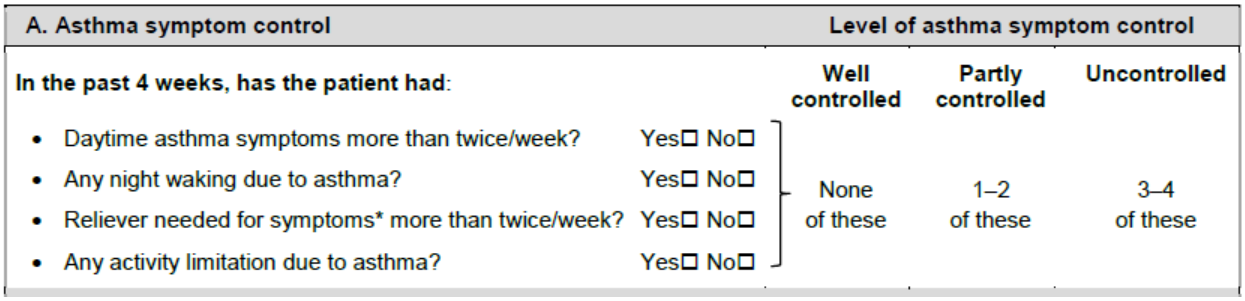
Inhaled corticosteroid use as per the GINA guidelines can be used as low, medium or high doses. Budesonide and fluticasone are commonly used inhaled corticosteroids, and their doses are outlined in table 1 below.

Inhaled corticosteroid	Low dose	Medium dose	H.0igh dose
Budesonide	100 - 200 mcg	>200 – 400 mcg	> 400 mcg
Fluticasone	100 – 200 mcg	>200 – 400 mcg	>400 mcg

Table 1: Inhaled corticosteroid use in asthma

Assessment of asthma control while on follow up is also delineated in the GINA guidelines.

Level of asthma control is assessed by asking four questions, as outlined in figure 2 below. Control is classified as either uncontrolled, partly controlled or well controlled.



A. Asthma symptom control		Level of asthma symptom control		
In the past 4 weeks, has the patient had:		**Well controlled**	**Partly controlled**	**Uncontrolled**
• Daytime asthma symptoms more than twice/week?	Yes☐ No☐	None of these	1–2 of these	3–4 of these
• Any night waking due to asthma?	Yes☐ No☐			
• Reliever needed for symptoms* more than twice/week?	Yes☐ No☐			
• Any activity limitation due to asthma?	Yes☐ No☐			

Courtesy of the GINA global strategy for asthma 2019

Figure 2: Asthma symptom control; GINA guidelines 2017

25-hydroxyvitamin D is both a hormone and a nutrient which is soluble in fat. Its primary source is by natural production by the skin. This production occurs after exposure to the sun and then undergoes metabolism by 2 significant steps to become biologically active. Cholecalciferol is converted to 25-hydroxycholecalciferol by 25-hydroxylase in the liver. 25-hydroxycholecalciferol is then converted to 1,25-hydroxycholecalciferol by the action of 1-alpha-hydroxylase in the

kidney. The biologically active form of vitamin D is 1,25-hydroxycholecalciferol. Vitamin D can be found as supplements and foods that are fortified. 25(OH)D is found in circulation, and its serum level reflects the status of vitamin D storage in the body (6).

Research in the last several years has identified vitamin D receptors (VDRs) in almost all body cells. These receptors have been found on several immune cells, for example, dendritic cells, activated T- and B-cells, macrophages and monocytes. This has resulted in postulations about the crucial role of vitamin D in the regulatory functions of the immune system. The immunomodulatory effects that vitamin D has on these cells of the innate immunity are potent (7). Low vitamin D levels may lead to atopy development as it affects Th1 and Th2 cytokines (8). The risk of viral respiratory infections in asthmatic patients has been shown to be reduced by vitamin D. This is important as viral infections are important triggers for asthma exacerbations (9). Corticosteroids are the most efficacious asthma controllers, and their anti-inflammatory action is potentiated by vitamin D (10). In response to an infection, it is thought that the production of antimicrobial peptides like cathelicidin involved in Toll-like receptor signalling, is up-regulated by vitamin D (11).

Serum 25-hydroxyvitamin D levels in the body are determined by the quantity and quality of sunshine exposure, dietary intake and any comorbidities. Vitamin D is synthesised after exposure to the sun and from dietary and supplement intake. These factors lead to differences in vitamin D levels in different populations worldwide, including within sub-Saharan Africa (12). These differences are mainly due to the vast variations in skin exposure to sunlight, dietary supplementation, the effectiveness of production in the skin and fortification policies in different regions (13).

The prevalence of serum 25-hydroxyvitamin D deficiency around the world has been noted to be high. Serum 25-hydroxyvitamin D deficiency prevalence is highest in countries around the Persian Gulf, and this is despite having adequate sunlight. Studies have revealed that adolescent girls in Saudi Arabia and Iran have serum 25-hydroxyvitamin D deficiency rates of approximately 80% and 70%, respectively (14). A study in children 9 -12 years old conducted in Iran noted that 72.4% of the participants had 25(OH)D deficiency (15). Studies in Africa show that 25(OH)D levels

ranging from deficiency to sufficient levels indicating that even within Africa, there is no homogeneity and therefore, levels need to be considered within each local context (16).

Serum 25-hydroxyvitamin D reference ranges have been delineated by the Institute of Medicine and the Endocrine Society, as shown in Table 2 below (17).

Serum 25-hydroxyvitamin D level	**ng/mL**	**nmol/L**
Deficiency	<20	<50
Insufficiency	20 - <30	52.5 – 72.5
Sufficiency	30 – 150	75 - 375

Table 2: Serum 25-hydroxyvitamin D reference ranges

1.2 Literature Review

Research has been done worldwide to look at serum 25(OH)D levels in asthmatic paediatric patients. The purpose of these studies is to find a link between levels of 25-hydroxyvitamin D and the control of asthma symptoms in these patients. Chinellato in 2011, investigated seventy-five children in Italy aged between five to eleven years who had asthma. The outcome measures explored were spirometry and level of asthma control. Of the 75 patients, 40 (53.3%) showed deficient levels (<20 ng/mL), 28 (37.3%) had insufficient levels (between 20 and 30 ng/mL) and only 7 (9.4%) patients had sufficient serum 25-hydroxyvitamin D levels (at least 30 to 40 ng/ml) (18). 25-hydroxyvitamin D levels were higher in the well-controlled asthmatic patients contrasted to the patients in the partially-controlled group. They concluded that lower 25(OH)D levels were related to poorer control of asthma (18).

In addition to asthma control and severity, correlation with markers of allergy has been investigated. Brehm conducted a cross-sectional study involving 616 asthmatic children. They looked at asthma exacerbations and levels of serum total and specific IgE. The results showed that of the participants, 152 (24.6%) had insufficient levels 20 to 30 ng/mL, with 21 (3.4%) having deficient 25(OH)D levels below 20 ng/mL and the rest had sufficient levels above 30 ng/mL. They noted that 25-hydroxyvitamin D levels were inversely proportional to both eosinophil count and total IgE count, which are both markers of allergy. A strong inverse association between levels of circulating 25-hydroxyvitamin D and asthma severity and markers of allergy was found (19). A significant variation in 25-hydroxyvitamin D levels between patients with mild and severe asthma and healthy controls was noted. In severe asthmatics, reduced 25-hydroxyvitamin D levels with a noted increase in the need for controller inhaled corticosteroids has been shown.

A study in Egypt of 100 paediatric patients, 60 of whom had asthma, and 40 healthy controls were undertaken. Their outcome measures were asthma control and corticosteroid use. The study showed that among the asthmatics, 45% had serum 25-hydroxyvitamin D deficiency with 55% having insufficient levels, while in the control group 37% had insufficient levels, and 62.5% had sufficient levels. They demonstrated a correlation that was significant between 25(OH)D deficiency and the severity of asthma. No relationship between sun exposure and levels of 25-hydroxyvitamin D was found (20). They noted a correlation in the reduced levels of 25-hydroxyvitamin D and the need for higher corticosteroid use. 25-hydroxyvitamin D levels were also reduced in asthmatic patients with concomitant allergic rhinitis. They concluded that reduced levels of 25(OH)D were associated with worsening severity of asthma, higher corticosteroid use and more inadequate asthma control (20).

Some studies have found no correlation between levels of 25(OH)D and the severity of asthma. Krobtrakulchai in Thailand looked at asthma exacerbation, pulmonary function and inhaled corticosteroid dose among 125 asthmatic children. They found that 19.2% of the patients were serum 25-hydroxyvitamin D deficient, while 44.8% were insufficient. The study did not reveal any significant differences in the inhaled-corticosteroid (ICS) dose, rates of emergency department visits or hospitalisation in the three classifications of 25-hydroxyvitamin D. Pulmonary function,

use of anti-inflammatory drugs and rates of asthma exacerbations were not significant either. The serum 25(OH)D deficient patients were older and had a reported delay in the onset of asthma compared to the patients with insufficient or sufficient levels. No significant correlation was determined in 25-hydroxyvitamin D levels and pulmonary function or required doses of inhaled corticosteroids (21).

In 2012, Neagu conducted a study among 52 children with asthma in the US, with outcome measures being asthma severity and control. They found that 40% of the participants had deficient 25(OH)D levels, while 44% had insufficient levels (22). No correlation between 25-hydroxyvitamin D levels and the severity of asthma, asthma control or FEV1 was found (22).

Despite conflicting proof of the correlation between levels of 25(OH)D and control of asthma in different regions, some body of work has demonstrated that vitamin D supplementation has an impact on asthmatic patients. A trial was conducted in 2014 by Yadav in 100 children in India. One group received 60,000 IU/month of serum 25-hydroxyvitamin D for six months, while a placebo was given to the second group. They studied asthma exacerbations and the frequency of casualty department visits. Their conclusion was that serum 25-hydroxyvitamin D had a definitive place in the treatment of moderate to severe persistent asthma (23).

A trial conducted in 2016 by Tachimoto, compared vitamin D3 supplements with a placebo for two months in Japanese school-going children with asthma. Outcome measures studied were changes in control of asthma as classified by GINA guidelines and the C-ACT. They concluded that serum 25-hydroxyvitamin D supplementation given in low doses over a short term duration added to regular treatment might lead to an improvement in asthma control in school-going children (24).

Studies done have shown differing results regarding the association between the control of asthma and levels of 25-hydroxyvitamin D in different regions. While this is appreciated, evidence exists showing the part that vitamin D plays in the treatment of asthma as an adjunct.

This study sought to look at our local situation in regards to 25-hydroxyvitamin D and asthma severity. Additionally, we sought to establish whether there was an association between the two. Establishing this would help us determine whether there is a potential role in our local context for considering vitamin D supplementation in our current management of asthmatic children.

Obesity is considered to be a comorbidity associated with asthma. It is linked to worsening morbidity in asthmatic patients. In obesity, pulmonary mechanics are altered associated with obstruction in the lower airways and lower lung volumes in asthmatic children. Obese children have reduced vitamin D levels due to its reduced bioavailability compared to non-obese children. The decreased bioavailability of vitamin D is likely due to its storage in adipose tissue as opposed to an absolute deficiency of 25(OH)D. Lautenbacher found no variation in levels of 25(OH)D in a study comparing obese and normal weight asthmatic children. The severity of asthma in these obese children was concluded to be associated with the pulmonary mechanisms mentioned earlier (25).

A systematic review concluded that levels of 25(OH)D were not linked to the presence nor development of allergic rhinitis (26) A study conducted by Dogru compared 25(OH)D in participants who had allergic rhinitis and those with non-allergic rhinitis. The mean levels in those groups were found to be reduced, in contrast to those in the control group. No correlation was deduced between the severity or duration of allergic rhinitis and levels of serum 25-hydroxyvitamin D (27).

1.3 Study Justification

There is a paucity of data in Sub-Saharan Africa about levels of 25(OH)D in paediatric patients who have atopic asthma. The purpose of this study was to look at our local population of asthmatic children and compare levels of 25-hydroxyvitamin D in those with mild and severe disease. The results of the study may, therefore, inform practice and local protocols on possible interventions as part of the management of asthmatic children. It is especially important since research has shown that sufficient levels of 25(OH)D improve the control of asthma and help reduce the need for inhaled corticosteroid.

CHAPTER TWO: STUDY MATERIALS AND METHODS

2.1 Research Questions

Is there a difference in the levels of serum 25-hydroxyvitamin D between children with mild atopic asthma and those with severe atopic asthma?

2.2 Objectives

2.2.1 Primary objective

To determine whether there is a difference in the levels of serum 25-hydroxyvitamin D between children with mild atopic asthma and those with severe atopic asthma.

2.2.2 Secondary Objective

To determine the association between various comorbidities and the severity of asthma in children

2.3 Methodology

2.3.1 Study Design

Cross-sectional study design.

2.3.2 Study variables

2.3.2.1 Independent variables

Independent variables included socio-demographic and clinical characteristics including sex, age and family history of asthma.

2.3.2.2 Dependent variables

Serum 25-hydroxyvitamin D level.

2.3.3 Study Site

AKUHN serves both the population within Kenya and from the neighbouring countries. It is accredited by Joint Commission International and offers both inpatient and outpatient paediatric services. Patients are seen in the paediatric casualty, the specialist clinics, the Doctors' Plaza clinics and in the wards. The AKUHN laboratory has ISO 15189:2012 and College of American Pathologists accreditation.

2.3.4 Study Population

Included patients aged between 1 – 18 years diagnosed with asthma in the paediatric casualty, the specialist clinics, and the Doctors' Plaza clinics and the paediatric wards.

2.3.4.1 Inclusion criteria

Patients ages 1 to 18 years diagnosed with asthma

2.3.4.2 Exclusion criteria

Patients currently on oral vitamin D supplementation

Patients with a history of receiving vitamin D supplementation in the previous 3 months

Patients on follow up for kidney or liver disease

Patients on follow up for parathyroid or bone disease

Patients on treatment that alters vitamin D pharmacokinetics such as anticonvulsants, antiretrovirals or tuberculosis treatment.

2.3.5 Sample size

$$n = \frac{2(Z_{\frac{\alpha}{2}} + Z_{\beta})^2 \sigma^2}{d^2}$$

Where; $Z_{\alpha/2}$ is the critical value of the normal distribution at α/2 (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96)

$Z_{1-\beta}$ is the critical value of the normal distribution at β (e.g. for a power of 90%, β is 0.1 and the critical value is 1.28)

σ^2 is the population variance of 99.2 ng/ml

d is the effect size of 9.47 ng/ml

A minimum sample size of 24 in each of the two categories was determined as sufficient to detect a difference in mean vitamin D levels between children with mild asthma using independent t-test. The calculation assumed an effect size of 9.47 ng/ml with a confidence level of 95%, 90% power, and population variance of 99.2 and standard deviation of 9.96 ng/ml between the groups. A

sample size of 70 distributed equally among the two groups was used due to the number of testing kits batch available.

2.3.6 Sampling procedure

Asthmatic patients in the clinical areas were reviewed and assessed for eligibility for the study. If eligible, they were placed into 2 categories as either mild or severe asthmatic. The researcher explained the study objective to the guardian accompanying the participant. Written consent was obtained after which recruitment into the study was done. A questionnaire was administered to the guardians, blood samples were drawn and *vacutainer*s clearly labelled. Sequential sampling was employed until the desired number of participants were recruited.

2.3.7 Research tools

A questionnaire was utilised for data collection

2.3.8 Data collection procedures

The purpose and goals of the study were explained to the guardians of the patients enrolled in the study in detail, ensuring that medical jargon was not used. A questionnaire was administered face-to-face to obtain socio-demographic data, medical history and determine any risk factors that they may have had. Blood sampling was discussed with both the patient and the guardian, as well as any discomfort and distress that could arise due to the procedure. Participants were reassured that although the procedure could cause distress, it would not be life-threatening. Sample collection was done by the researcher who took the utmost care to make the experience bearable. If the participant's parent or guardian declined phlebotomy, they were at liberty to withdraw without any consequences or compromise to their care. Blood samples collected were processed at the AKUHN biochemistry laboratory. Samples were run as they were received at the laboratory. The 25-

hydroxyvitamin D was measured using the Cobas e 601 analyser (Roche, Mannheim, Germany), which utilises the electrochemiluminescence assay principle. The assay is enrolled for external quality assurance with the College of American Pathologists with excellent performance. The results obtained were interpreted as per the earlier determined values.

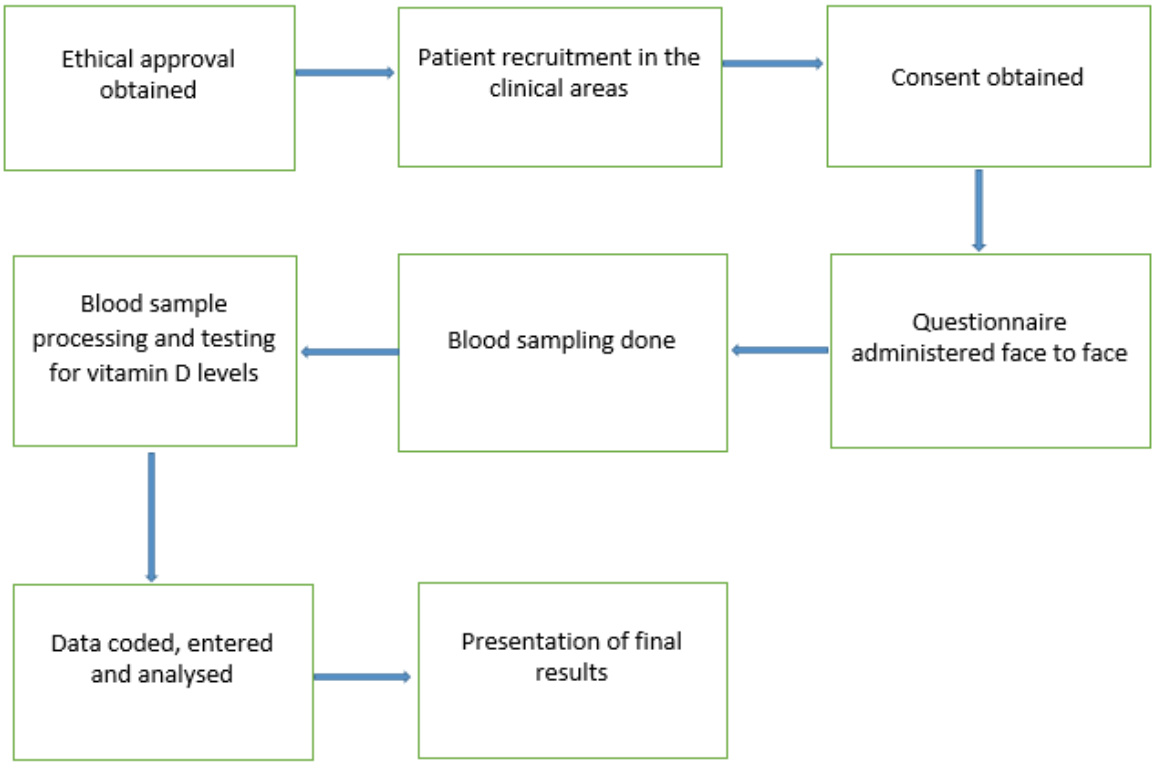


Figure 3: *The study process*

2.3.9 Data Management

All study questionnaires were examined for completeness and accuracy manually and prior to entry into Microsoft Excel spreadsheet. All forms were identified using unique serial numbers without any personal identifiers. The questionnaires were stored in a lockable cabinet and the electronic data was password protected with access granted to the study researcher, PI and other supervisors.

2.3.10 Data Analysis

Levels of serum 25-hydroxyvitamin D were classified as either deficient (<20ng/ml), insufficient (20 - 30 ng/ml) or sufficient (>30ng/ml). Different clinical and socio-demographic characteristics of the study population were described using frequencies and percentages. Continuous data were described using summary measures of median (interquartile range). The analysis was stratified to asthma severity (mild and severe) and compared using chi-square for categorical variables while continuous data were compared using the Mann-Whitney test. The association between severity of asthma and comorbidities was determined using binary logistic regression. A p-value of less than 0.05 was considered statistically significant. SPSS version 23 was used for all analyses.

2.3.11 Ethical considerations

The institutional ethics and research committee (IERC) in AKUHN approved the study. Since this study involved minors ethical considerations that were necessary to consider were beneficence, with the application of evidence in their care that was specific to them as children. Respect for their privacy and confidentiality was of utmost importance. They were involved in the consenting process based on their age and understanding. Consent was acquired from the guardians. Their concerns were adequately addressed for them to make an informed choice about whether to participate or not. In addition, the same was explained to the participants and assent sought depending on their age and level of understanding. This included what the study sought to do, explaining how the study would benefit them and other children with a similar condition. The fact that blood was to be drawn and what they would experience when this is done, was explained. The child was encouraged to ask questions about the process. Participants were given the option to withdraw at any stage from the study. The guardians did not incur any cost to have the test done.
Once the results were ready, the guardians were informed of the results through contacts that were obtained during the recruitment process. The results were explained to the guardians, including the need for vitamin D supplementation where applicable. For the patients with 25(OH)D deficiency, a prescription for vitamin D supplementation was offered either as an oral formulation or intramuscular injection.

CHAPTER THREE: RESULTS

Characteristics of the study participants

Seventy children were enrolled in the study with a median age of 5 (IQR: 3-7) years. The median BMI was estimated to be 15.9 (IQR: 14.5-18.5) kg/m^2 and median (IQR) of 25.9(21.9-32.1) ng/mL for 25-hydroxyvitamin D levels. There was an equal number of males and females, of whom 46(65.7%) had a family history of asthma. Most of the children had a history of eczema and allergic rhinitis, while a history of allergic conjunctivitis and food allergy was not common among the children. There was also an equal number of children who had mild and severe asthma. 35 (50%) of the children had insufficient serum 25-hydroxyvitamin D levels, 24(34.3%) had sufficient levels, and 11(15.7%) had deficient +serum levels. This information is summarised in table 3 below.

Table 3: Clinical and demographic characteristics of the children

Characteristics	Frequency (%)	Median(IQR)
Age (years)		**5(3-7)**
BMI (kg/m^2)		**15.9(14.5-18.5)**
Vitamin D levels (ng/mL)		**25.9(21.9-32.1)**
Sex		
Male	35(50.0)	
Female	35(50.0)	
Family history of asthma		
Yes	46(65.7)	
No	24(34.3)	
Eczema		
Yes	42(60.0)	
No	28(40.0)	
Allergic rhinitis		
Yes	47(67.1)	

No	23(32.9)
Allergic conjunctivitis	
Yes	21(30.0)
No	49(70.0)
Food allergies	
Yes	22(31.4)
No	48(68.6)
Severity of asthma	
Mild	35(50.0)
Severe	35(50.0)
Serum levels	
< 20	11(15.7)
20-30	35(50.0)
> 30	24(34.3)

Table 4 shows a stratified analysis of the demographic and clinical characteristics with the severity of asthma. The median (IQR) of levels of 25(OH)D in the mild and severe classes were estimated to be 26.8 (23.5-32.9) and 24.6 (21.0-31.8) ng/mL respectively and were not significantly different in either group. In addition, there was no significant variation in BMI and age in the two groups (p-value>0.05). None of the factors was significantly correlated with the severity of asthma at the univariate level; however, BMI, family history of asthma and allergic conjunctivitis met the cut-off defined as p-value< 0.3 for inclusion into the multivariable logistic regression analysis in addition to age. Age was incorporated in the model even though it did not meet the cut-off since other literature had shown that it is clinically relevant in explaining the outcome of interest.

Table 4: stratified analysis of clinical and demographic characteristics of the children

	Severity of Asthma		All children(N=70)	p-value mild vs severe
	Mild(n=35)	Severe(n=35)		
	n(%)	n(%)	N(%)	
Characteristics				
Age (years)	4(3-6.5)	5(3-10.5)	5(3-7)	0.312
BMI (kg/m^2)	15.3(14.3-16.9)	16.6(14.8-19.7)	15.9(14.5-18.4)	0.091
Vitamin D levels (ng/mL)	26.8(23.5-32.9)	24.6(21-31.8)	25.9(21.9-32.1)	0.561
Sex: n (%)				
Male	16(45.7%)	19(54.3%)	35(50.0%)	0.633
Female	19(54.3%)	16(45.7%)	35(50.0%)	
Family history of asthma: n (%)				
Yes	19(54.3%)	27(77.1%)	46(65.7%)	0.078
No	16(45.7%)	8(22.9%)	24(34.3%)	
Eczema: n (%)				
Yes	22(62.9%)	20(57.1%)	42(60.0%)	0.807
No	13(37.1%)	15(42.9%)	28(40.0%)	
Allergic rhinitis: n (%)				
Yes	25(71.4%)	22(62.9%)	47(67.1%)	0.611
No	10(28.6%)	13(37.1%)	23(32.9%)	
Allergic conjunctivitis: n (%)				
Yes	8(22.9%)	13(37.1%)	21(30.0%)	0.297
No	27(77.1%)	22(62.9%)	49(70.0%)	
Food allergies: n (%)				
Yes	10(28.6%)	12(34.3%)	22(31.4%)	0.797

No	25(71.4%)	23(65.7%)	48(68.6%)	
Serum levels: ng/mL				
< 20	4(11.4%)	7(20.0%)	11(15.7%)	
20-30	19(54.3%)	16(45.7%)	35(50.0%)	0.584
> 30	12(34.3%)	12(34.3%)	24(34.3%)	

The 25(OH)D levels were computed against the number of participants as a whole and stratified as mild and severe asthma. The most substantial proportion of the participants had insufficient levels of 25(OH)D, as seen in figure 4 below. 24% of all 70 participants had sufficient levels of 25(OH)D, while 50% had insufficient levels, and 15.7% had deficient levels.

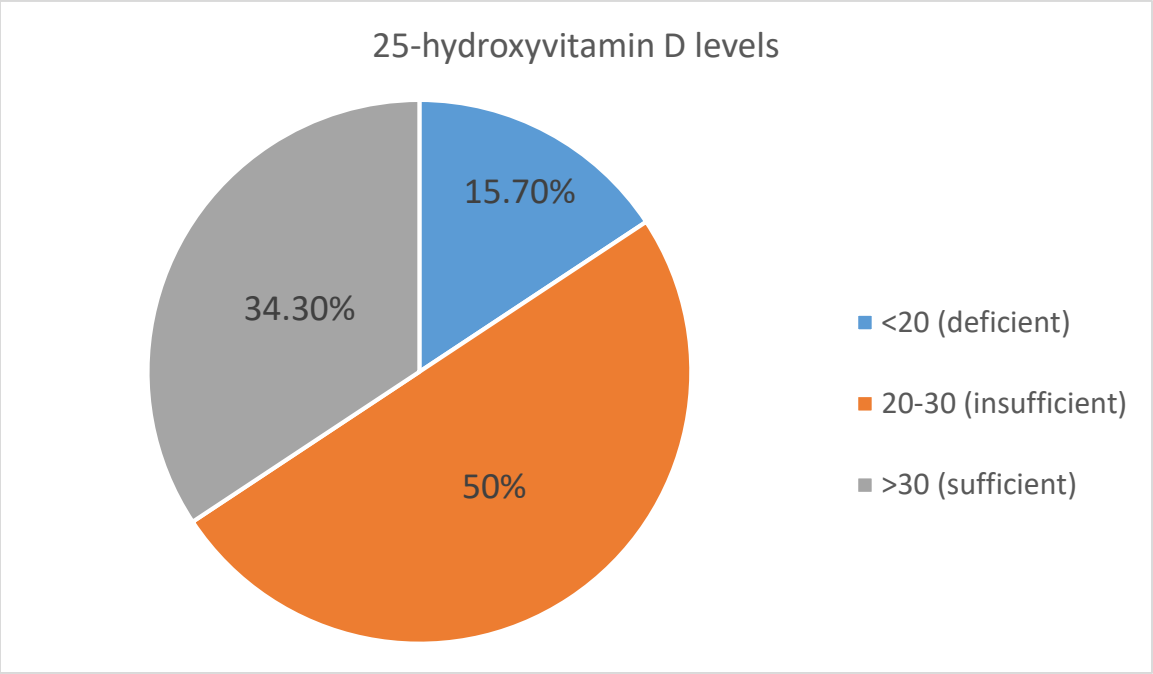


Fig 4: Serum 25-hydroxyvitamin D levels in all the participants

The levels of 25(OH)D were then computed according to asthma severity (mild and severe asthma). Levels in the 2 groups were analysed, and the proportions in the two groups found to be similar, as seen in figure 5 below. The number of patients with insufficient levels was noted to be highest in both mild and severe asthma groups at 54.3% and 45.7% respectively. The sufficient levels were equal in both groups, while the deficient levels were 11.4% for mild and 20% for the severe asthma group.

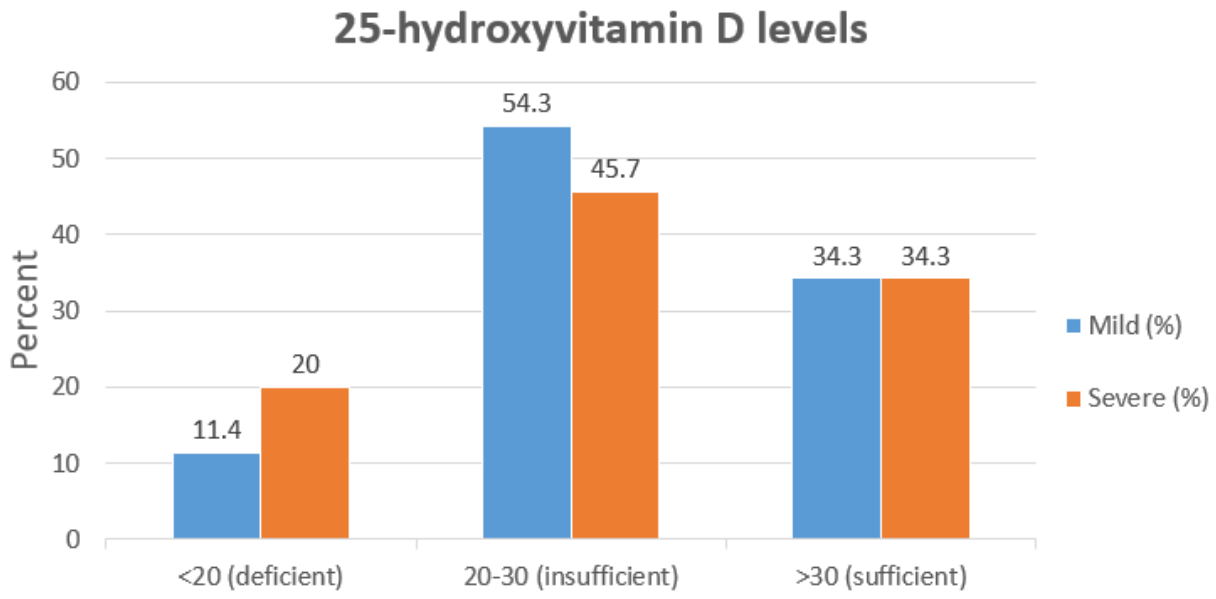


Fig 5: Serum 25-hydroxyvitamin D levels in the participants stratified as mild and severe asthma

A box and whisker plot compared levels of 25-hydroxyvitamin D between the mild and severe asthma patients, as seen in figure 6 below. The interquartile range (IQR) of vitamin D levels in the mild group was 23.5-32.9ng/mL and a median of 26.8 ng/mL. In the severe asthma group, the IQR was 21.0-31.8 ng/mL with a median of 24.6 ng/mL. No significant variation in the mild and severe asthmatic groups was found.

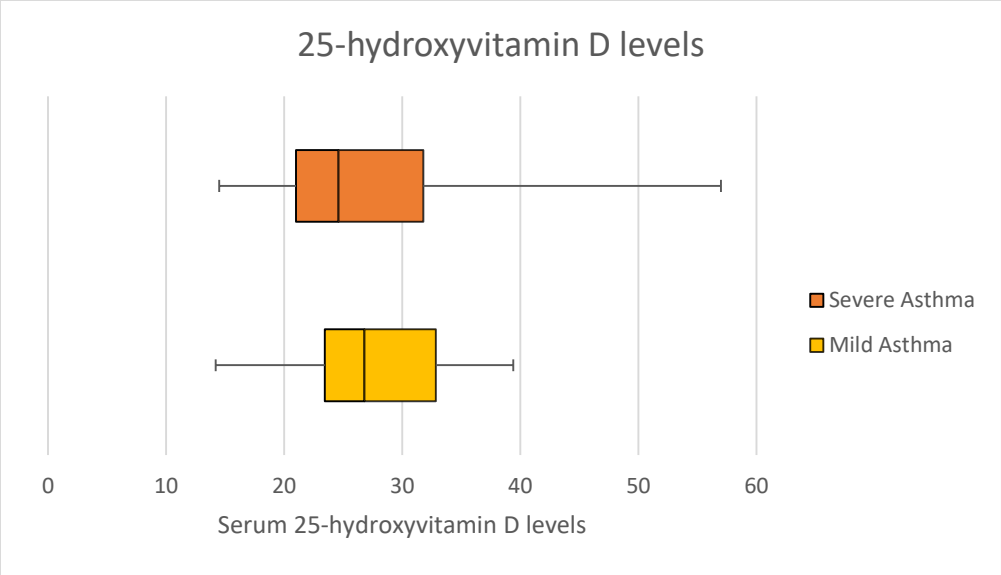


Fig 6: Mean 25-hydroxyvitamin D levels in the mild and severe asthma groups

Multivariable analysis

In the multivariable analysis, the odds of severe asthma increased with age, BMI, family history of asthma and allergic conjunctivitis; however, none of these was significantly different/associated with severe asthma. The odds of severe asthma increased with a unit increase in age (Adjusted OR=1.022; 95%CI: 0.866-1.214) and BMI (adjusted OR: 1.109; 95%CI: 0.932-1.335). Being born in a family with a history of asthma increased the odds of severe asthma by 2.5 times compared to children born in families without a history of asthma (adjusted OR: 2.548; 95%CI: 0.840-8.304) and those who had allergic conjunctivitis had 1.8 times higher odds of having severe asthma compared to those without allergic conjunctivitis (adjusted OR: 1.8; 95%CI: 0.572-5.851). This information is shown in table 5 below.

Table 5: Multivariable analysis of risk factors of severe asthma

Characteristics	Crude OR(95%CI)	Adjusted OR(95%CI)
Age (years)	0.532(0.210-1.298)	1.022(0.866-1.214)
BMI (kg/m^2)	1.123(0.958-1.332)	1.109(0.932-1.335)
Family history of asthma		
Yes	2.842(1.036-8.298)	2.548(0.840-8.304)
No		Reference
Allergic conjunctivitis		
Yes	1.994(0.711-5.867)	1.800(0.572-5.851)
No		Reference

Correlation analysis

The relationship between age and levels of 25(OH)D and also levels of 25(OH)D and BMI using a correlation matrix plot was examined (Figure 7). There was a significant negative Pearson correlation between Vitamin D levels and age of the child (r=-0.43, p-value=0.0002); however, a non-significant negative correlation between 25-hydroxyVitamin D and BMI(r=-0.16, p-value=0.1909) was noted.

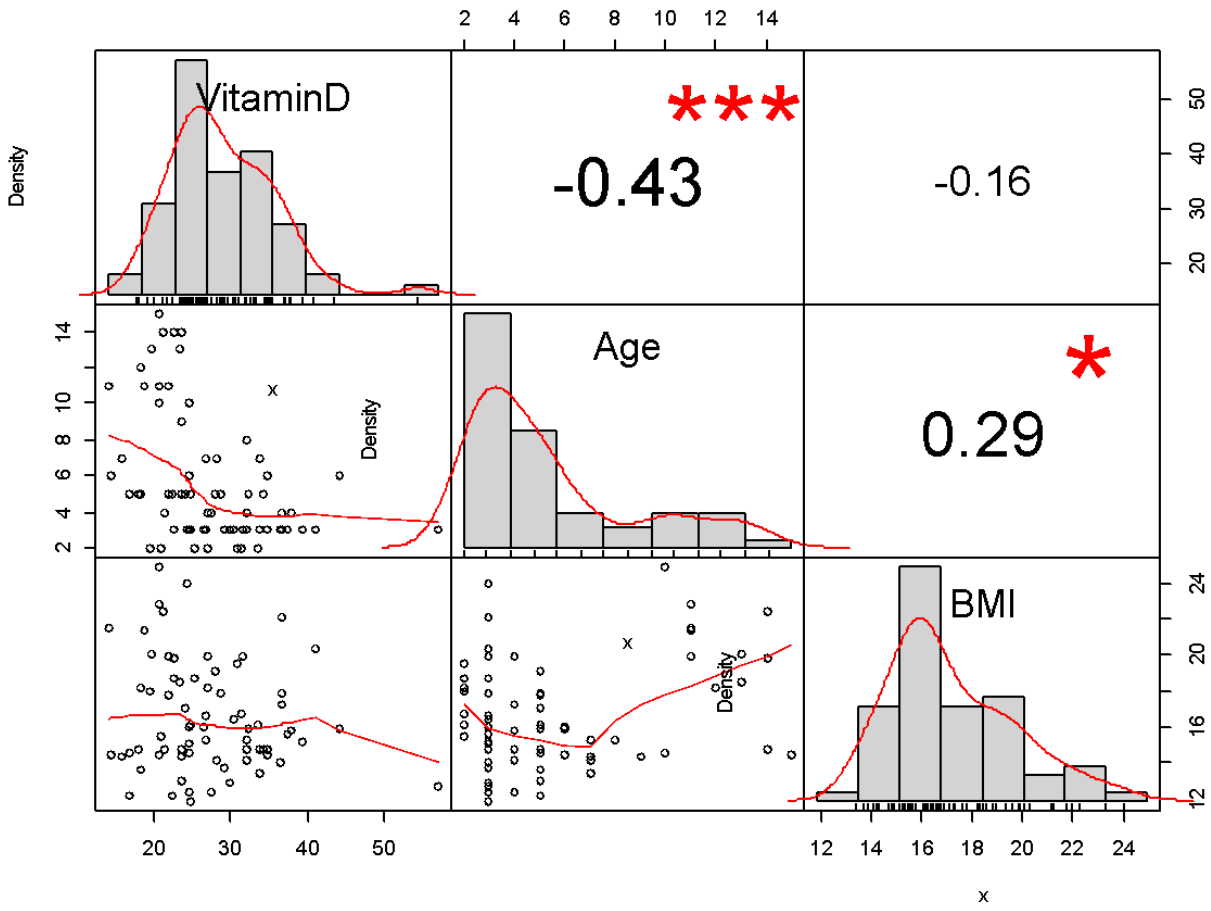


Figure 7: Correlation matrix for Vitamin D, BMI and Age

CHAPTER FOUR: DISCUSSION

The present study classified the level of asthma as mild or severe based on GINA guidelines on stepwise management of asthma. The levels of serum 25-hydroxyvitamin D were categorised as deficient (< 20 ng/ml), insufficient (20-30 ng/ml) and sufficient (> 30 ng/ml).
The results revealed that 65.7% of all the participants were vitamin D deficient and insufficient. This is similar to the prevalence (60%) demonstrated in healthy black adults in a study done at the Aga Khan University Hospital, Nairobi (28).

No significant variations in 25(OH)D levels between asthma severity (mild and severe) were found, which is inconsistent with the results of the majority of the other studies done. Of the patients with mild asthma, 11.4% had deficient levels of 25(OH)D as contrasted with 20% who had severe asthma. Chinellato found a positive association between 25-hydroxyvitamin D levels and asthma control They found that 53.5% of the patients had deficient levels, 37.3% had insufficient levels and patients with sufficient levels were only 9.4% (18). According to Gupta, children with moderately controlled or controlled asthma had higher levels of 25-hydroxyvitamin D compared to those with severe refractory asthma (29). Similarly, Mohamoud found a significant relationship in the severity of asthma and levels of 25(OH)D deficiency (20).

This difference in outcome compared to a majority of the other studies could be due to several factors. The serum 25-hydroxyvitamin D reference ranges used in this study and in clinical practice have not been specifically developed for our local population or region. Studies done previously have noted that serum 25-hydroxyvitamin D levels are affected by geographical, environmental and genetic factors (12, 13, 16). This could, therefore, affect the results of this present study. Another factor that might explain this difference would be based on the sample size. Given that studies from different populations were used to calculate the sample size, it is possible that based on those normed levels that the sample size used could have been too small to detect a significant difference.

Several studies have had comparable results to this present study. These studies were done in different geographic regions of the world with different climates. One was done in North America

while the other was in Asia. Behavioural and dietary factors in the different regions could explain the outcomes of these studies. Krobtrakulchai found that among 125 asthmatic children, 19.2% had 25(OH)D were deficient, while 44.8% had insufficient levels. No differences in levels 25-hydroxyvitamin D and asthma severity were found (21). Naegu found that 40% of the participants had deficient 25(OH)D levels, while 44% had insufficient levels. No correlation between levels of 25-hydroxyvitamin D and asthma severity or asthma control was found (22).

This study explored some of the comorbidities associated with asthma. BMI, age, family history of asthma, allergic rhinitis, eczema, food allergies and allergic conjunctivitis. This study did not find any significant differences between the two groups in all the factors explored. One explanation for this is that the study was not powered to show significance in these factors. Several studies done to look at these factors did not find any association with the level of serum 25-hydroxyvitamin D. In the multivariable analysis, having a family history of asthma was noted to increase the odds of severe asthma by 2.5 times compared to children born in families without a history of asthma (adjusted OR: 2.548; 95%CI: 0.840-8.304). The p-value was >0.05, and the odds ratio 95% confidence interval crossed 1, proving not to be statistically significant.

Lautenbacher found no variation in levels of 25(OH)D comparing obese and normal weight asthmatic children. Asthma severity among the obese participants was concluded to be associated with the pulmonary mechanisms causing lower airway obstruction rather than low 25(OH)D (25). Dogru looked at 25-hydroxyvitamin D in participants with allergic rhinitis and with non-allergic rhinitis. No correlation in levels of 25-hydroxyvitamin D, and the severity or duration of allergic rhinitis was noted (27). These are similar findings to this study, where the participants had similar levels of allergic rhinitis in both mild and severe asthma groups.

Diet is an essential factor that plays a role in the 25(OH)D levels. Of note is that few sources of vitamin D occur naturally. Sources with notable amounts of vitamin D are mostly of animal origin. These foods include oil-rich fish like tuna and salmon, egg yolk and liver and kidney (30). Many Kenyans do not have access to these foods due to their cost and availability. This, therefore, reduces the reliance on dietary intake as a significant source of vitamin D in our population. In the

present study, 25(OH)D levels could not be predicted using questions about sun exposure and vitamin D rich diet intake.

CHAPTER FIVE : CONCLUSIONS

This study looked at children with mild and severe asthma and found no statistically significant difference in the serum levels of vitamin D. Various comorbidities associated with asthma were also found not to be significantly different in the two groups.

CHAPTER SIX: RECOMMENDATIONS

6.1 Recommendations

From this study we recommend;

1. Routine screening of 25(OH)D in severe asthmatic patients is not beneficial for the routine care of the patients in the absence of clinical features of hypovitaminosis D.

2. Studies to determine the prevalence of serum 25-hydroxyvitamin D deficiency in a healthy paediatric population should be undertaken. This study only looked at a specific population of children with asthma without including healthy controls. Looking at a healthy cohort would provide more information regarding normal vitamin D levels.

3. Given that this was a cross-sectional study, long-term follow-up studies on the effect of trends through time of 25(OH)D levels in asthmatic children may provide more information about this association in our population.

6.2 Study limitations

1. This study did not have a control arm consisting of healthy children which have allowed for a comparison of 25(OH)D levels in healthy and asthmatic children.

2. This study was not powered for the secondary analysis. Conclusions about these findings cannot be made.

6.3 Dissemination of findings

The findings of the individual levels of 25-hydroxyvitamin D levels were explained to the guardians of the patients. Results were presented at a departmental meeting to members of faculty and residents. They will further be presented at the faculty academic research meeting.

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REFERENCES

1. Asthma GIf. 2018 GINA report, global strategy for asthma management and prevention. 2018.
2. Ellwood P, Asher M, Beasley R, Clayton T, Stewart A, Committee IS. The International Study of Asthma and Allergies in Childhood (ISAAC): Phase Three rationale and methods [Research Methods]. The International Journal of tuberculosis and lung disease. 2005;9(1):10-6.
3. National Heart L, Institute B. National Asthma Education and Prevention Program. Expert panel report 3: guidelines for the diagnosis and management of asthma: full report 2007. http://www nhlbi nih gov/guidelines/asthma/asthgdln pdf. 2007.
4. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. World Allergy Organ J. 2014;7(1):12.
5. Statistics A. American Academy of Allergy, Asthma, and Immunology. 2012.
6. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. The Journal of nutrition. 2005;135(2):317-22.
7. Lin R. Crosstalk between Vitamin D metabolism, VDR signalling, and innate immunity. BioMed research international. 2016;2016.
8. Bantz SK, Zhu Z, Zheng T. The role of vitamin D in pediatric asthma. Annals of pediatrics & child health. 2015;3(1).
9. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and respiratory infections: the vitamin D hypothesis. Allergy. 2012;67(1):10-7.
10. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. Journal of Allergy and Clinical Immunology. 2010;126(1):52-8. e5.
11. Prentice A. Vitamin D deficiency: a global perspective. Nutrition reviews. 2008;66(suppl_2):S153-S64.

12. Mogire RM, Mutua A, Kimita W, Kamau A, Bejon P, Pettifor JM, et al. prevalence of vitamin D deficiency in Africa: a systematic review and meta-analysis. The Lancet Global Health. 2020;8(1):e134-e42.
13. Lucas R, McMichael T, Smith W, Armstrong BK, Prüss-Üstün A, Organization WH. Solar ultraviolet radiation: global burden of disease from solar ultraviolet radiation. 2006.
14. Mithal A, Wahl DA, Bonjour J-P, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis international. 2009;20(11):1807-20.
15. Zahedirad M, Nikooyeh B, Neyestani TR. The epidemic of poor vitamin D status among 9-12 years old children in Tehran, 2008, using HPLC: need for an urgent Action. Nutrition and Food Sciences Research. 2015;2(3):15-20.
16. Prentice A, Schoenmakers I, Jones KS, Jarjou LM, Goldberg GR. Vitamin D deficiency and its health consequences in Africa. Vitamin D: Springer; 2010. p. 505-28.
17. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline (Journal of Clinical Endocrinology and Metabolism (2011) 96,(1911-1930)). Journal of Clinical Endocrinology and Metabolism. 2011;96(12):3908.
18. Chinellato I, Piazza M, Sandri M, Peroni D, Piacentini G, Boner AL. Vitamin D serum levels and markers of asthma control in Italian children. The Journal of pediatrics. 2011;158(3):437-41.
19. Brehm JM, Celedón JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. American journal of respiratory and critical care medicine. 2009;179(9):765-71.
20. El-Menem MA, Al-Aziz MA, El-Guindy WM, El Banna N. The frequency of vitamin D deficiency among asthmatic Egyptian children. Egyptian Journal of Pediatric Allergy and Immunology (The). 2013;11(2):69-74.
21. Krobtrakulchai W. The effect of Vitamin D status on pediatric asthma in a university hospital, Thailand. Journal of Allergy and Clinical Immunology. 2012;129(2):AB78.
22. Neagu F. Hypovitaminosis D is very frequent but not associated with asthma control in a low-income pediatric population seen in an allergy and immunology clinic. Journal of Allergy and Clinical Immunology. 2012;129(2):AB153.

23. Yadav M, Mittal K. Effect of vitamin D supplementation on moderate to severe bronchial asthma. The Indian Journal of Pediatrics. 2014;81(7):650-4.
24. Tachimoto H, Mezawa H, Segawa T, Akiyama N, Ida H, Urashima M. Improved control of childhood asthma with low-dose, short-term vitamin D supplementation: a randomised, double-blind, placebo-controlled trial. Allergy. 2016;71(7):1001-9.
25. Lautenbacher LA, Jariwala SP, Markowitz ME, Rastogi D. Vitamin D and pulmonary function in obese asthmatic children. Pediatric pulmonology. 2016;51(12):1276-83.
26. Kim YH, Kim MJ, Sol IS, Yoon SH, Park YA, Kim KW, et al. Vitamin D Level in Allergic Rhinitis: A Systemic Review and Meta-Analysis. Journal of Allergy and Clinical Immunology. 2016;137(2):AB94.
27. Dogru M, Suleyman A. Serum 25-hydroxyvitamin D3 levels in children with allergic or non-allergic rhinitis. International journal of pediatric otorhinolaryngology. 2016;80:39-42.
28. Kagotho E, Omuse G, Okinda N, Ojwang P. Vitamin D status in healthy black African adults at a tertiary hospital in Nairobi, Kenya: a cross-sectional study. BMC endocrine disorders. 2018;18(1):70.
29. Gupta A, Sjoukes A, Richards D, Banya W, Hawrylowicz C, Bush A, et al. Relationship between serum vitamin D, disease severity, and airway remodelling in children with asthma. American journal of respiratory and critical care medicine. 2011;184(12):1342-9.
30. Buttriss J. Vitamin D: Sunshine vs diet vs pills. Nutrition bulletin. 2015;40(4):279-85.

APPENDICES

Appendix I: QUESTIONNAIRE

1.	Code number	_____
2.	Age in years	_____
3.	Sex	Male / Female
4.	Body Mass Index	_____
5.	Family history of asthma	Yes / No
6.	History of eczema	Yes / No
7.	History of allergic rhinitis	Yes / No
8.	History of allergic conjunctivitis	Yes / No
9.	History of food allergies	Yes / No
10.	Total steroid use per day	_____

Appendix II: INFORMED CONSENT FORM

Study Title: Serum 25-hydroxyvitamin D levels in children with mild and severe atopic asthma

Primary researcher: Dr Rhoda Ndinda Masaku
Primary supervisor: Dr Adil Waris

My name is Rhoda Ndinda Masaku a Masters student in Paediatrics and Child Health, Aga Khan University. A research on the levels of serum 25-hydroxyvitamin D in children with mild and severe asthma here in Aga Khan University Hospital, Nairobi is being conducted. You will be given information, after which you will be invited to participate in this research. You do not have to decide today whether or not you will take part in this research. Feel free to ask questions and seek clarification as we go along.

Purpose of the research

Asthma is a common chronic condition that children suffer from in our country and worldwide. As clinicians, we are always for ways to ensure that this condition is well controlled so that our patients are able to live a life as normal as possible. Serum 25-hydroxyvitamin D level is one of the factors that have been shown to have some effect on the control of asthma in children. By establishing whether this true for us locally, we will be able to identify and further develop interventions to assist patients in controlling the symptoms that they face.

Type of research intervention

This research will involve your participation by answering a questionnaire and blood being drawn for laboratory assessment of serum 25-hydroxyvitamin D.

Risks

The questionnaire will involve answering some personal information and medical history. The drawing of blood for the lab assessment will involve a needle prick which may cause pain and discomfort.

Benefits

You will benefit from this study, as the results of the serum 25-hydroxyvitamin D levels will be shared with you and you will be offered a prescription for vitamin D supplementation if the serum 25-hydroxyvitamin D levels are low. This is expected to improve their asthma control and help reduce the dose of the required corticosteroid. The supplementation will, unfortunately, not be paid for by this particular research. In addition, the information obtained will be used in developing evidence-based strategies that will help in our current practice in the management of asthmatic patients.

Confidentiality

The information obtained during the interview will only be accessible to the primary researcher and two supervisors. Any information about you will have a number on it and not your name. Data will be stored safely in a locked cabinet and will only be accessible to the primary researcher and the supervisors.

Voluntary participation

Your participation in this research is entirely voluntary. You are free to withdraw from this research at any point during the research. Should you choose not to participate in this research, your decision will not in any way affect the services that you are receiving in this centre. If you are willing to participate in this study, please sign below to show that you have understood and have consented to participate.

Consent agreement

I have read the above information, or it has been read to me. I have had the opportunity to ask questions, and my questions have been answered satisfactorily. I agree to be a participant in this study

Name of participant_____

Signature of participant_____

Date_____

Statement by the researcher

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands. I confirm that the participant had an opportunity to seek clarification and that their questions were answered accurately to the best of my ability. I confirm that the individual has not been coerced into giving consent and that the consent has been given freely and voluntarily.

Name of researcher_____

Signature of the researcher_____

Date_____

For further clarification, contact the primary researcher or supervisor:

Primary Researcher

Dr Rhoda Ndinda Masaku

Department of Paediatrics and Child Health

Aga Khan University. PO Box 30270-00100, Nairobi

Telephone 0727523270. Email: rhoda.masaku@aku.edu

Primary supervisor

Dr Adil Waris

Department of Pediatrics and Child Health

Aga Khan University, PO Box 30270-00100, Nairobi

Telephone 0733725972. Email: adil.waris@aku.edu

If you have any questions related to the ethics of this study, please contact:

The Secretariat, Ethics Review Committee Aga Khan University, P. O. BOX 30270-00100, Nairobi Telephone 0203662148, Email: research.supportea@aku.edu

Appendix III: ASSENT FORM

Study Title: Serum 25-hydroxyvitamin D levels in children with mild and severe atopic asthma

Primary researcher: Dr Rhoda Ndinda Masaku
Primary supervisor: Dr Adil Waris

Hello, my name is Dr Rhoda Masaku.

We are doing a study to learn about vitamin D in children with asthma, like yourself.
We are asking you for your help because we want to learn if your vitamin D level affects your asthma.
If you agree to be in our study, we will ask you to allow us to take some blood from you and take it to the laboratory for testing.
What we learn in this research will help us know how to help you and other children who also have asthma better. It helps us know if you need medication to increase your vitamin D or not.
It is possible you will feel pain when we take your blood but will be very gentle to ensure that you don't feel a lot of pain.
Being in the study is up to you, and no one will be upset if you don't agree to it, or if you change your mind later.

Child's Signature _____ Date _____

Investigator's Signature _____ Date _____