VITAMIN D LEVELS IN CHILDREN WITH OBSTRUCTIVE ADENOTONSILLAR DISEASE AT THE KENYATTA NATIONAL HOSPITAL

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H58/6837/2017

A proposal submitted in partial fulfillment of the requirements for the award of Master of Medicine degree in otorhinolaryngology/head and neck surgery in the University of Nairobi

March 2022

STUDENT'S DECLARATION

I, the undersigned hereby declare that this proposal is my original work and has not been presented for the award of degree in any other university nor has it been submitted for publication in any journal. Where I have used another person's work, I have carefully acknowledged and referenced.

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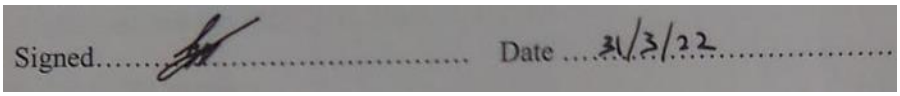

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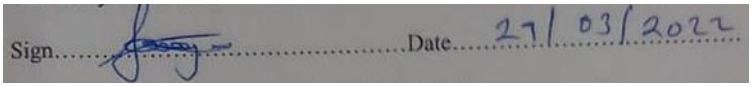

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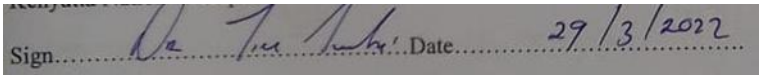

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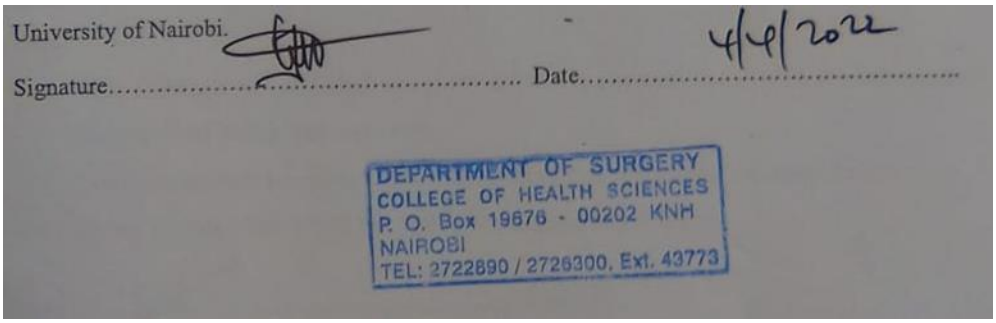

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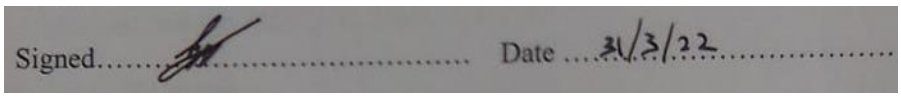

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List of Abbreviations

$1,25(OH)_2D_3$	1,25- dihydroxycholecalciferol
$25(OH)D_3$	25- hydroxycalciferol
AMC	Antimicrobial peptides
APC	Antigen Presenting Cell
BMI	Body Mass Index
EPS	Extracellular polymeric substance matrix
KNH	Kenyatta National Hospital
Ng/ml	Nanograms per Milliliter
Nmol/l	Nanomoles per Litre
NK	Natural Killer cell
PTH	Parathyroid hormone
Th1	T-helper cell type 1
Th2	T-helper cell type 2
URTI	Upper Respiratory Tract Infection
VDR	Vitamin D Receptor

ABSTRACT

Background: Adenotonsillar disease is a common problem for which children undergo surgery. Vitamin D plays an essential role in immunity, deficiency of which has been linked to disease in these lymphoid organs

Objective: To determine Vitamin D levels in patients with obstructive adenotonsillar disease scheduled for surgery at the Kenyatta National Hospital

Study Design and Setting: This was a case control study that was conducted at Ear, Nose and Throat clinic, well-baby and pediatric surgical unit in Kenyatta National Hospital

Methodology: Eighty children under 12 years were recruited into the study comprising 40 cases and 40 controls. Demographic data including age, gender, weight, height and BMI were recorded. Venous blood was obtained for measurement of Vitamin D levels. The process was repeated for the age and sex matched controls

Data Analysis and Results: Prevalence of Vitamin D deficiency between cases and controls was analyzed and presented as percentages with odds ratio and p-values calculated for significance. Spearmans rank correlation test was used to analyze the relationship between specific risk factors and development of Vitamin D deficiency. A P value of <0.05 for a 95% confidence interval was considered significant

Results: There were 70% males and 30% females with a mean age of 4.4+/-1.6 years. Vitamin D deficiency was found in 31.3% of the study population, 42.5% of the cases and 20% of the controls (OR=3.0(95%CI: 1.1-8.0), **p=0.05**). Age (r= -0.39, p=0.01), weight (r= -0.34, p=0.03), height (r= -0.39, p=0.02) and BMI (r= -0.35, p=0.03) were inversely associated with Vitamin D levels amongst the cases

Conclusion: Children with obstructive adenotonsillar disease were three times more likely to be Vitamin D deficient with older age, increased height and obesity being risk factors

CHAPTER ONE- INTRODUCTION

1.1 BACKGROUND

Adenotonsillar hypertrophy is a common condition in children leading to upper airway obstruction which may also cause sleep disordered breathing (1). Adenotonsillectomy is the most frequent elective procedure performed in Kenyan Ear, Nose and Throat/Head and Neck (ENT/HN) units, mainly for upper airway obstruction and recurrent tonsillitis (2,3). Surgery is one of the important treatment modality, but can be associated with significant morbidity and very rarely mortality, hence, there may be significant advantage in searching for alternative medical therapeutic options that will reduce the burden of the disease.

Vitamin D is a fat-soluble vitamin which is required for an appropriate immune response, thus its deficiency may result in an increased risk of infections and inflammation of the upper and lower airway (4). Deficiency of vitamin D can also increase the risk of other acute and chronic otolaryngologic disorders, such as asthma, chronic rhinosinusitis with nasal polyps, and recurrent otitis media (5). Recurrent tonsillitis which can be due to biofilms can be prevented by having adequate levels of vitamin D (6).

1.2 Adenotonsillar disease and its impact on pediatric health

Adenotonsillar disease is a disorder that is commonly due to chronic inflammation caused by a persistent bacterial infection which can lead to hypertrophy of the lymphoid tissue that consequently leads to upper airways obstruction (7). There are over 6000 children with adenoid or tonsillar hypertrophy seen in ENT clinic in Kenyatta National Hospital annually (1). The

disease affects them in several ways such as sleep apnea leading to neurocognitive impairment. Even more serious complications have been implicated such as pulmonary hypertension (1). Recurrent use of antibiotics alters the microbiota which has an effect on the development of the immune system and also can cause antibiotic resistance. The disease also significantly depresses various measures of quality of life and is equivalent to or slightly worse than rheumatoid arthritis in terms of quality of life (8). Premature children have less time to accumulate Vitamin D through the placenta and deficiency may predispose them to adenotonsillar disease.

1.3 Vitamin D and its role in immunity

When T-lymphocytes and antigen-presenting cells that express vitamin D receptor (VDR) are exposed to bacterial cell wall components, vitamin D plays an important role in immunity by activating several cytokines and increases lysosomal activity thereby enhancing the chemotactic and phagocytic activity of the antigen presenting cells. It also induces differentiation of monocytes to macrophages and stimulates production of antimicrobial peptides such as *Cathelicidin* and *beta-defensin* which are bactericidal. Macrophages also have 1-alpha hydroxylase and therefore are able to produce the biologically active form $1,25(OH)_2D_3$. At the same time, Vitamin D also favors production of Th2 cytokines such as interleukin 4, 5 and 10, therefore skewing towards a Th2 cell response that is more protective. Activation induced cell death via the Fas/FasL system is also altered since 1,25(OH)2D3 inhibits Fas-L expression by activated T-lymphocytes affecting overall survival (9,10).

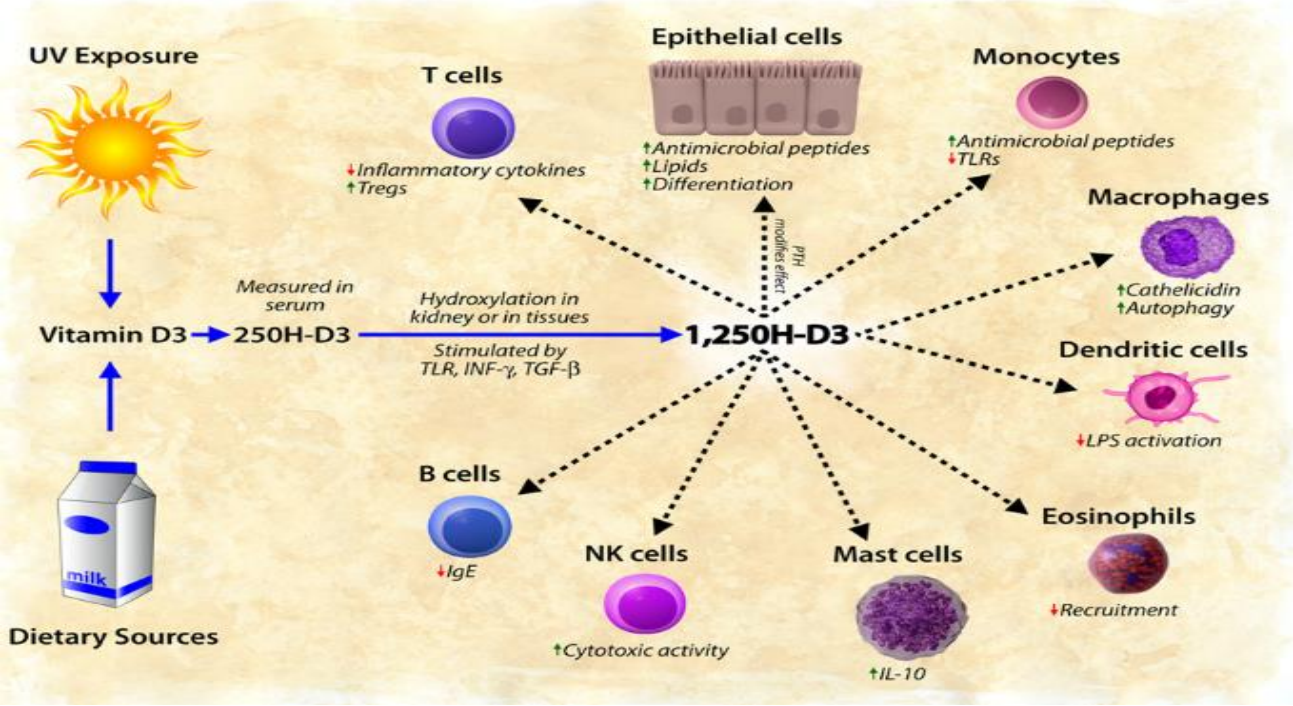


Figure 1- Role of Vitamin D in immunity. Image courtesy of Muehleisen and Gallo (11)

1.4 Vitamin D, Biofilms and Adeno-tonsillar disease

Low vitamin D levels interferes with the normal immune cell function of the adenoid and tonsils, which can lead to formation of biofilms. These are microorganism communities that form an irreversible bond to a surface and are encased in a self-produced extracellular polymeric material. (EPS) (12). The close proximity allows more conjugative exchange of genetic material leading to altered gene expression patterns that greatly reduce their rates of metabolism and cell division. Hence, antibiotics that target highly metabolic and reproducing bacteria are rendered less efficacious. The bacteria also communicate by ‘quorum sensing’ whereby once a threshold number of bacteria are reached, certain coordinated processes like the production of EPS are made sufficient. The EPS is mainly made up of polysaccharide biopolymers along with proteins, glycoproteins, glycolipids and extracellular DNA with the bacteria embedded in it. This matrix

provides mechanical stability while protecting its inhabitants against external predators such as antibiotics, antibodies and biocides. When biofilms are found on or in mucosal surfaces, the EPS matrix is largely composed of host cells and proteins (7, 10).

Vitamin D stimulates production of Cathelicidin which when cleaved releases a peptide called LL-37 that is bactericidal and in vitro, LL-37 is able to prevent formation and also breakdown Pseudomonas aeruginosa biofilms (10).

Approximately 74% of patients with recurrent tonsillitis showed the presence of mucosal biofilms in a study done by Chole and Faddis (13). Chronic otitis media and chronic rhinosinusitis are caused by biofilms found on the adenoid, that act as a bacterial reservoir (14).

Typical bacteria detected in biofilms from otolaryngologic patients include Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, , and Haemophilus influenzae; however, normal flora too can produce biofilms (7) (15).

1.5 Clinical presentation and diagnosis of adenotonsillar disease

Adenoids and palatine tonsils form part of the Waldeyers ring of lymphoid tissue in the posterior wall of the nasopharynx and lateral walls of the oropharynx respectively (3). They contain immune cells that react upon exposure to antigens within the aero-digestive tract. The nasopharynx is a box like space that functions to resonate speech, drain the Eustachian tube middle ear complex and act as a conduit for nasally inspired air, thus hypertrophied adenoids within this space may manifest as mouth breathing, nasal obstruction, rhinorrhea, hyponasal speech and otitis media (16). Paradise criteria, which include at least seven episodes within the

past year, at least five episodes annually for two consecutive years, or at least three episodes annually for three consecutive years, in a patient with a sore throat and pain when swallowing, and one of the secondary criteria, can be used to diagnose recurrent tonsillitis. Secondary criteria include a body temperature of more than 38.3°C, tonsillar exudate, cervical adenopathy, or a group A beta-hemolytic streptococci culture which is positive (7). Assessment of tonsil size is done using the Brodsky scale which grades the tonsils based on the percentage of patent oropharyngeal airway between the palatine tonsils, and grades 3 and 4 are defined as hypertrophied tonsils.

Table 1- Brodsky scale. Courtesy of Brodsky L. (16)

Grade of tonsils	Description
0	Tonsils in fossa
1	Tonsils occupying up to 25% of the airway
2	26-50%
3	51-75%
4	More than 75%

1.6 Clinical presentation and diagnosis of Vitamin D deficiency

Conversion of 7 dehyrocholesterol in skin to cholecalciferol occurs after exposure to Solar ultraviolet B radiation. This is converted to calcidiol (25(OH)D3) in the liver and subsequently to its active form called calcitriol (1,25(OH)2D3) in the kidneys. Vitamin D deficiency may lead to several chronic diseases. It can affect intestinal calcium absorption and affect the growing skeleton leading to rickets or osteomalacia. It can also impair intracellular calcium metabolism in muscle cells and lead to myopathy. There is also evidence that Vitamin D has a protective role in certain auto-immune diseases like rheumatoid arthritis and inflammatory bowel disease by inhibiting TNF-alpha and promoting IL-10, respectively. It has also been viewed as an anti-tumour substance by inducing apoptosis in many malignant cells and has also shown protective effects against cardiovascular diseases (17). Serum calcitriol levels is not a very reliable indicator of Vitamin D status because levels may be normal or higher due to secondary hyperparathyroidism and also due to its short half-life (3-6 hours) as opposed to 25(OH)D3 whose half-life is about 20 days (6). On the other hand, low levels of active vitamin D may also be observed due to insufficient substrate availability for renal 1-alpha hydroxylase, reduced number of viable nephrons thus reduced 1-alpha hydroxylase levels, high serum concentrations of fibroblast growth factor-23 and inflammatory cytokines (17, 18), hence, circulating 25(OH)D3 levels closely reflect the skin and dietary levels of Vitamin D.

The Institute of Medicine defines vitamin D deficiency as 25(OH)D3 levels of less than 20ng/ml, and insufficiency as levels of 21-29ng/ml (19). However, laboratory measurements have shown that parathyroid hormone levels start rising at 25(OH)D3 levels of about 30-34nmol/l (12ng/ml) (20), hence, the 2016 Global Consensus Recommendations on prevention and management of

nutritional rickets (20) which are similar to the 2011 recommendations from the Pediatric Endocrine Society (21), define Vitamin D sufficiency as 20 to 100 ng/mL (50 to 250 nmol/L), Vitamin D insufficiency as 12 to 20 ng/mL (30 to 50 nmol/L) and Vitamin D deficiency as <12 ng/mL (<30 nmol/L) in healthy children and adolescents.

CHAPTER TWO- LITERATURE REVIEW

Globally, the prevalence of deficiency of Vitamin D varies because of differences in risk factors like skin pigmentation, sun exposure and dietary intake of vitamin D. However, more than a third of the world's population have Vitamin D levels of less than 20ng/ml (22).

Vitamin D deficiency has been linked to infectious diseases prevalent in the African continent. It has been estimated that one in five people in Africa have inadequate 25(OH)D3 levels (threshold of <12ng/ml) with a higher prevalence seen in newborns, urban populations and in northern and southern Africa (23). Several studies have also linked Vitamin D deficiency to adenotonsillar disease leading to recurrent infections and obstructive symptoms.

Worldwide, the prevalence of adeno-tonsillar disease varies. The prevalence of adenoid hypertrophy was reported as 7.7% in children aged 6-12 years in a Nigerian study (24). Santos studied children aged 6-13 years found a prevalence of 2nd and 3rd degree adenoid hypertrophy at 49.4%, whereas Aydin reported a prevalence which was higher at 66.4% in children aged 5-14 years in Turkey (25, 26). Oburra et al found adenotonsillectomy to be the most common otorhinolaryngologic procedure performed in three Nairobi hospitals (2). These studies demonstrate the prevalence of adeno-tonsillar disease in children.

Kagotho et al did a cross sectional study amongst healthy black African adults in Aga Khan University Hospital in Nairobi and found that 17.4% were Vitamin D deficient with levels

<20ng/ml. However, if a cut-off of <30ng/ml was used then 60.1% would be deficient (27). Toko et al recorded 74.4% insufficiency and 30% deficiency of plasma 25(OH)D levels measured in cord blood in newborns in rural western Kenya, while 14% of children without rickets or malnutrition had levels less than 20ng/ml in a study done by Jones et al in Nairobi (28, 29).

Bartley et al looked at Vitamin D levels in patients attending general otolaryngologic clinic in South Auckland and found that 58% of patients had levels less than 20ng/ml while 2% had levels less than 7ng/ml (associated with osteomalacia) (30).

Martineau et al carried out a meta-analysis involving 25 randomized controlled trials. This showed that Vitamin D supplementation provided protection against upper respiratory infections. There was a greater protective effect when patients received daily or weekly Vitamin D without boluses, especially when 25(OH)D3 levels were less than 10ng/ml as opposed to more than 10ng/ml (31). These studies show that Vitamin D deficiency is highly prevalent in the diseased and healthy populations, and supplementation may be beneficial.

A study done by Reid et al (2010) to estimate the prevalence of 25(OH)D3 deficiency in children aged 4-16 years who were undergoing adenotonsillectomy for sleep apnea and/or recurrent tonsillitis had their 25(OH)D3, Iron and Zinc levels measured. 15.6% were Vitamin D deficient with levels less than 20ng/ml whereas 78% had levels less than 30ng/ml, a level which is associated with an increased incidence of upper respiratory tract infections (URTI). Vitamin D levels were also noted to be inversely related to BMI and tonsil size (32).

Shin et al (2018) conducted a retrospective cross-sectional study using 88 children to see if there is an association between Vitamin D deficiency and sleep disordered breathing due to adenotonsillar hypertrophy.

The mean 25(OH)D3 level was lower in the cases (18.4ng/ml in children with adenotonsillar hypertrophy, 18.7ng/ml in adenoid hypertrophy group and 19.4ng/ml in tonsillar hypertrophy group) then the control group (22.5ng/ml). An inverse relation was noted between serum 25(OH)D3 levels and age, height, tonsil size and adenoidal nasopharyngeal ratio (ANR). Of note was also that children who were Vitamin D deficient showed higher frequencies of adenoid and/or tonsillar hypertrophy compared to those who were Vitamin D sufficient (5).

Yildiz et al (2012) did a retrospective case control study to see if Vitamin D has a role in recurrent tonsillopharyngitis. 84 children with recurrent tonsillitis and 71 healthy children between 2 and 10 years were assessed. Serum 25(OH)D3 was measured using ELISA and Vitamin D receptor gene polymorphism using PCR. The mean serum 25(OH)D3 level in the study group was 57.2 ng/ml whereas in the control group it was 77 ng/ml and this difference was significant ($p<0.01$). Four children in the study group had levels less than 20ng/ml while none had levels below 20 ng/ml in the control group. There was no significant difference in Vitamin D receptor gene polymorphism between the two groups (6).

Pirzadeh et al (2019) did a case control study on 200 children admitted to the ENT clinic to determine whether low vitamin D levels were associated with tonsillar hypertrophy. 100 of them

had tonsillar hypertrophy grade 3 or 4 while the other 100 were healthy without previous surgery or tonsillar hypertrophy. The mean vitamin D levels in males was noted to be significantly higher than females (23.22 ng/mL vs 19.36 ng/mL), and in the case group was significantly lower compared to controls (19.21 ng/mL vs 24.45 ng/mL), however, no correlation was found between Vitamin D levels and tonsil size (33).

In the above review of relevant literature, it is evident that Vitamin D contributes to adenotonsillar disease through its effect on the immune system, hence, knowledge of the same in the local setup will play a pivotal role in public health measures which could reduce the burden of disease. It may also open new treatment options before surgery.

2.1 Study Justification

Obstructive adenotonsillitis is amongst the most common diseases in childhood with detrimental effects that adversely affects their quality of life. Adeno-tonsillectomy is the commonest procedure performed by otorhinolaryngologists globally as well as in Kenya. Vitamin D levels vary based on several factors such as skin pigmentation and geographical location and very few local studies have studied Vitamin D deficiency in children. A correlation between Vitamin D deficiency and adenotonsillar disease has been shown to exist, but no study has been done locally. This data could assist in viewing the disease from a clinical and public health perspective.

2.2 Research question

How does the vitamin D level compare in children with and without obstructive adenotonsillar disease?

2.3 Primary Objective:

To compare the Vitamin D levels in children with obstructive adenotonsillar disease with those attending well-baby and pediatric surgical unit at the Kenyatta National Hospital

2.4 Secondary Objective:

To determine the association between age and Vitamin D levels in children with obstructive adenotonsillar disease and controls attending well-baby and pediatric surgical unit

CHAPTER THREE: METHODOLOGY

3.1Research Methodology

3.1.1 Study Design

This study was a case control study

3.1.2 Study Setting

The research was conducted at ENT clinic and well-baby and pediatric surgical unit at Kenyatta national hospital

3.1.3 Study Duration

The study took twelve months from March 2021 to March 2022

3.1.4 Study Population and sampling

Definition of cases

Children under 12 years with a diagnosis of obstructive adenotonsillar disease scheduled for surgery at the KNH

Definition of controls

Children under 12 years attending well-baby and pediatric surgical unit without symptoms of obstructive adenotonsillar disease. They were matched with cases based on age and sex.

3.1.5 Inclusion Criteria for cases

1. Patients who have a diagnosis of obstructive adenotonsillar disease and scheduled for surgery

2. Parents/guardians who are willing to give consent/assent

3.1.6 Exclusion Criteria for cases

1. Patients on systemic steroids/anticonvulsants/antiretrovirals/antifungals (ketoconazole)
2. Patients on Vitamin D supplementation

3.1.7 Inclusion Criteria for controls

1. Patients in well-baby and pediatric surgical unit (circumcision, orchidopexy, hypospadias and uncomplicated hernias)
2. Patients without symptoms of obstructive adenotonsillar disease (nasal obstruction, snoring, mouth breathing and/or tonsillar grade 3 or 4)
3. Parents/guardians who are willing to consent

3.1.8 Exclusion Criteria for controls

1. Patients who have had (adeno)tonsillectomy
2. Patients on Vitamin D supplementation or systemic steroids

3.1.9 Sample size calculation

The following formula was used to estimate the sample size for a quantitative variable in case-control studies (34):

$$n = \frac{r+1}{r} \times SD^2 \left(\frac{Z_\beta + Z_{\frac{\alpha}{2}}}{d} \right)^2$$

$$n = \frac{1+1}{1} \times 68.1^2 \left(\frac{1.28 + 1.96}{49.6} \right)^2$$

Where;

n=sample size

r=ratio of control to cases=1

SD= standard deviation=68.1nmol/l according to study by Yildiz et al (6)

Z_β= standard normal variate for power=1.28 for 90% power

$Z_{\frac{\alpha}{2}}$=expected prevalence or proportion=standard normal variate for level of significance=1.96

d=expected mean difference between cases and controls=49.6 nmol/l (6)

Substituting in the formula gave us 39.6, therefore 40 cases and 40 controls.

3.1.10 Sampling

Convenient sampling was used to recruit participants until the desired sample size was achieved

3.1.11 Study Procedure

Cases

Patient with a diagnosis of obstructive adenotonsillar disease scheduled for surgery at the KNH was given a detailed explanation of the purpose, voluntary nature of the study, benefits, confidentiality and costs of participation. Consequences of needle prick such as pain and possible injection site infection was also be explained by the principal investigator. After giving informed consent, each subject had demographic data including age, sex, weight, height and BMI captured in the data collection sheet by the principal investigator. Using sterile venipuncture technique, two milliliters of venous blood sample was obtained using a disposable syringe. Blood was put in a plain vacutainer tube (red topped) that was labelled, and taken to the Kenyatta National Hospital laboratory. Serum vitamin D was determined by LIASON R 25-OH vitamin D Assay technique, which adopts a “Flash” chemiluminescence technology (CLIA) with a pragmatic microparticle solid phase (MP). This method is rapid, accurate and precise. The, National committee for clinical laboratory standards (NCCLS) protocols has validated this method(35). Additionally, its comparable to the gold standard liquid chromatography isotope dilution tandem mass spectrometry (LC-IDM/MS) (36) and well correlated with radioimmunoassay technique (37). The normal reference ranges were derived from the equipment manufacturer (30-80 ng/ml)

Controls

Patients attending well-baby clinic and pediatric surgical unit were matched for age and gender and the study explained to the parents/guardians. Obstructive symptoms including snoring, nasal obstruction and mouth breathing were ruled out by the principal investigator. Patients were then examined by the principal investigator to assess their tonsil size and those with grade 3 or 4 were excluded from the study. Venous blood was drawn for measurement of $25(OH)D_3$ levels and the same procedure followed as for the cases.

3.1.12 Study Flow Diagram

Cases

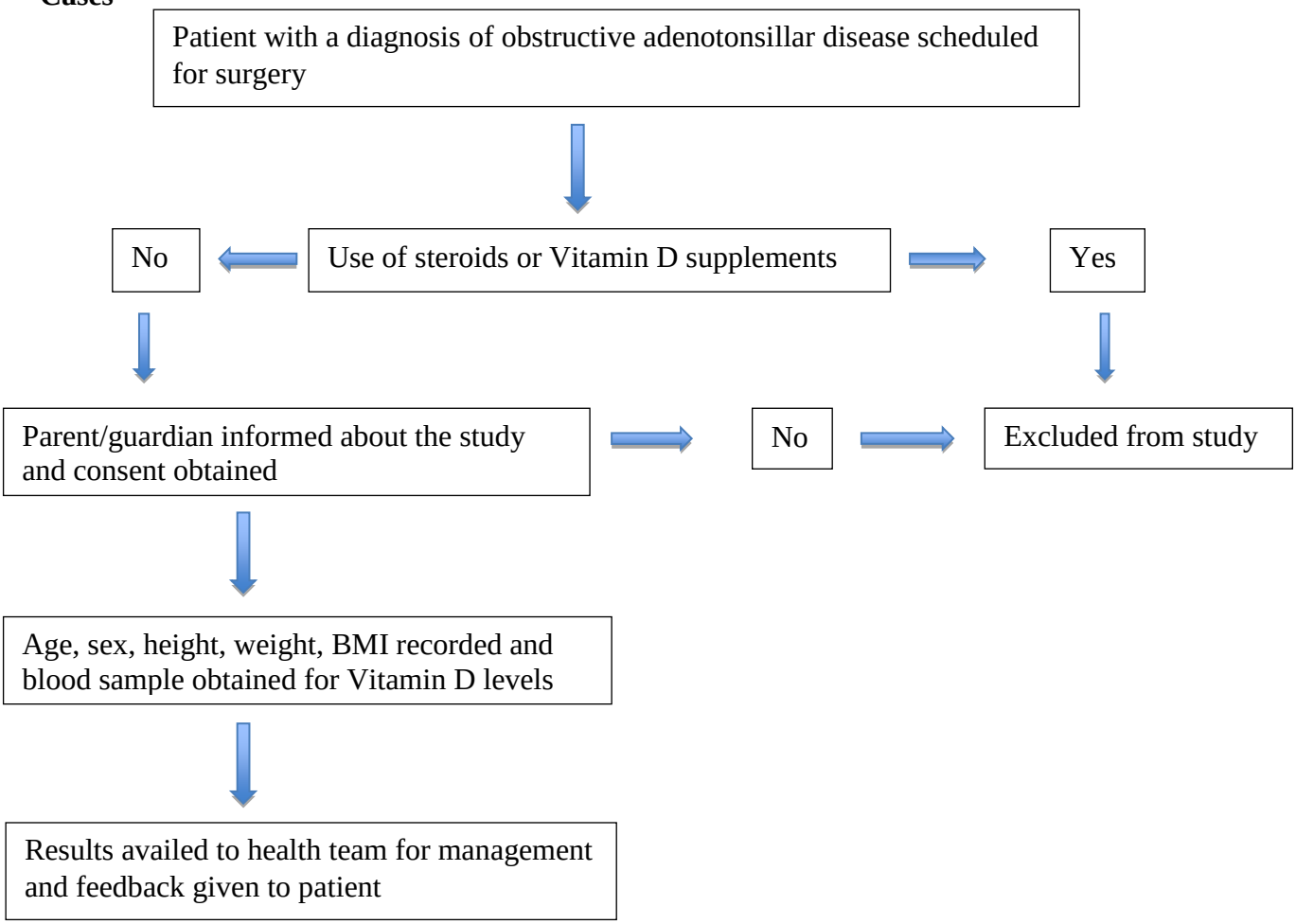


Figure 2

Controls

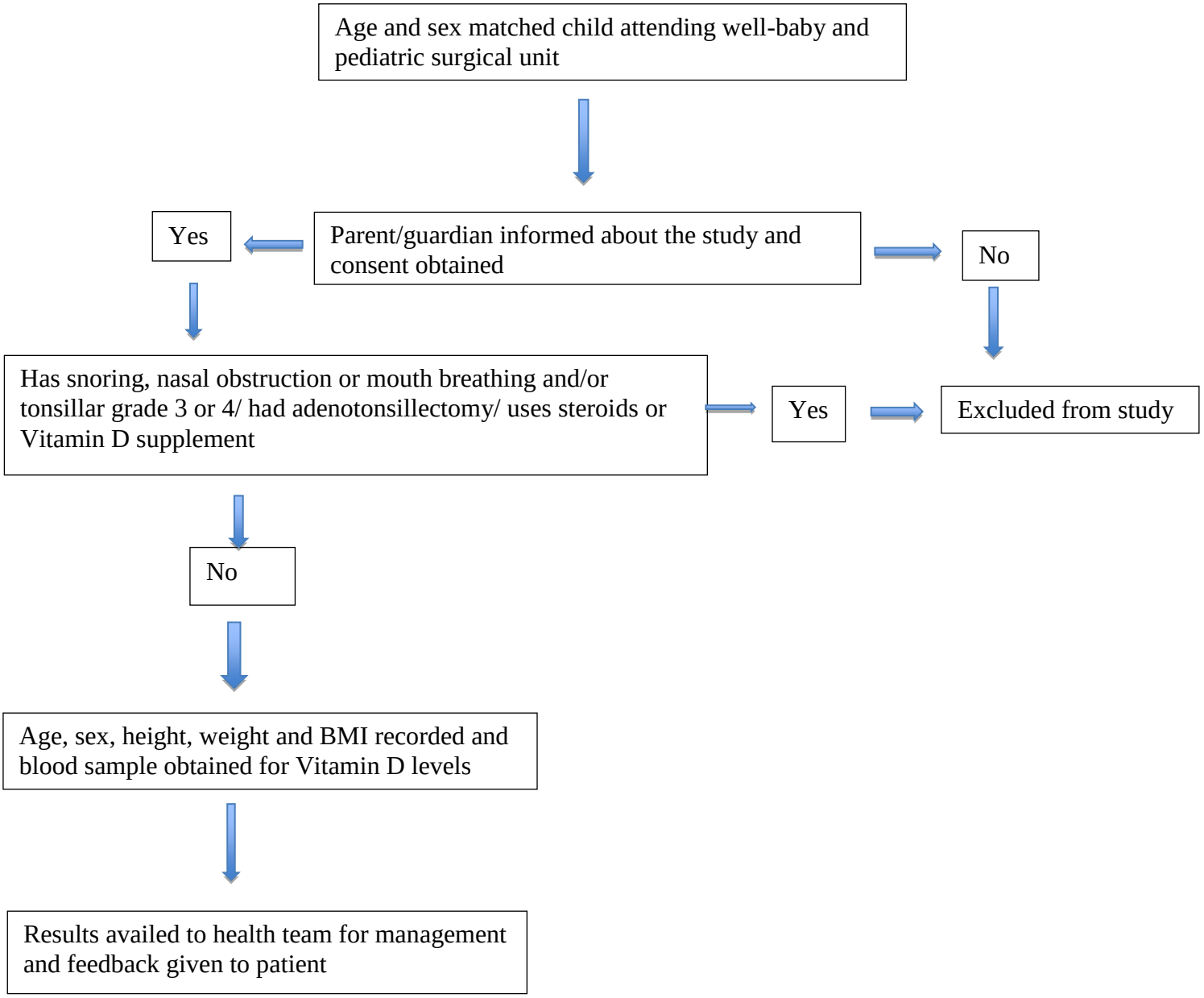


Figure 3

3.2 Data Management

Patients age, gender, history of prematurity, weight, height and Vitamin D levels were entered into a data collection sheet (Appendix II) for the cases. Similar data was entered for the controls indicating the exclusion of obstructive symptoms such as nasal obstruction, snoring, mouth breathing and/or tonsillar grade 3 or 4. Upon completion of the study, all required forms and consents were stored in a safe drawer in the Department of Surgery (ENT Section). A password-protected computer was used to store the electronic copies of the data. Data access was restricted to the principal investigator and supervisors only

3.3 Data Analysis

The collected data were sorted, cleaned, categorized, and entered into the statistical software package SPSS version 24 (Chicago). In order to prevent the loss of data, a daily backup was performed on the folder containing the data and uploaded to the cloud storage. Variables of interest include the outcome variable as Vitamin D3. Independent variables are sex, age height, weight and BMI. Univariate analysis was carried out. Continuous data was analyzed and summarized as means and standard deviations for normally distributed data and medians with inter-quartile range for skewed data. This was used to analyze age, proportions of gender, Vitamin D3 levels, while categorical data was analyzed and displayed in charts by use of frequencies and proportions. Chi-square test and fishers exact test was used for bivariate analysis, to establish the correlations between presence of vitamin D deficiency and clinic-demographic characteristics of the study population like age and gender. The Odds Ratios were compared between cases and controls to determine impact of vitamin D3 on obstructive adenotonsillar disease and impact of sex.

3.4 Quality control

Pre- analytical quality control

To ensure quality assurance samples were collected according to standard operating procedures of sample collection using proper vacutainers. Only one phlebotomist withdrew the samples and transportation of samples was in cool box. The refrigerators in which samples and reagents were kept, had their temperatures checked two times daily during the week of collection.

Analytical Errors

Tests were run using the manufactures laid down standard operating procedures. The tests were interpreted using the manufacture's insert. Samples were run with level one and two controls for each set of assays. When a new bottle of reagent was opened, before use sample controls were run. When reagent bottles were used or opened for use the first time before testing, controls were run. Test machines were calibrated according to the manufacturer's instructions.

Post- analytical errors

The researcher was careful to avoid causing post transcriptional errors while transferring lab results to the assigned laboratory numbers in the data entry form.

3.5 Ethical considerations

The study was carried out after approval by the KNH/UON Ethics and Research Committee. Informed consent was obtained from the caregiver once they understood the objective and methodology of the study. If, however, they were unwilling to participate in the study, they continued receiving care (surgery for the children with obstructive adenotonsillitis and vaccination for the children attending well-baby clinic and surgery for the pediatric surgical cases) without any discrimination.

Data collected was held in confidentiality and used for research purposes only. Anonymity was maintained by using patient codes which had no similarity to the patients' names, phone numbers or any such identification. The cost of the Vitamin D test was incurred by the principal investigator without any extra cost to the patient. Participants with abnormal laboratory values were referred to pediatrics for further evaluation and management.

Results of the study will be made available to the KNH, presented in medical conferences and published in medical journals for the benefit of the medical profession and the lay public. A soft copy of this dissertation will be available at the UON e-repository on the website.

CHAPTER FOUR: RESULTS

The study's major goal was to compare Vitamin D levels in children who had or didn't have obstructive adenotonsillar illness. Between the two groups, the relationship between age, gender, weight, height, BMI, and Vitamin D was also determined.

4.1 Social-demographics and anthropometric characteristics of study population

This study involved a total of 80 participants. Out of these, 40 had obstructive symptoms of adenotonsillar disease while 40 didn't have any obstructive symptoms. Those with obstructive symptoms, 28 were males and 12 were females and they were matched for age and sex with the control group giving a total sex distribution of 56(70%) for males and 24(30%) for females with a male: female ratio of 2.3:1.

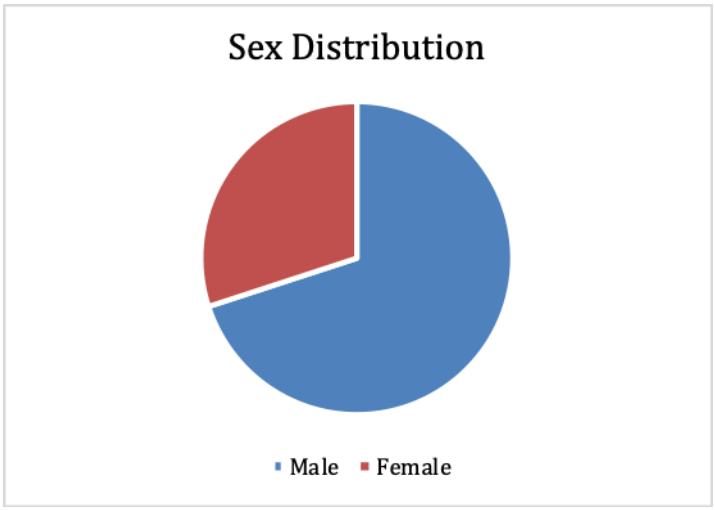


Figure 4- Sex distribution

The mean age for both groups was 4.4 +/- 1.6 years.

The average weight of the study group was slightly lower at 18.3 +/- 4.5kg and that of the control group was 19.4 +/- 4.6kg (p=0.26). Similarly, the average height of the study group was lower at 107.7cm +/- 9.6cm while that of the control group was 109.7cm +/- 10.2cm (p=0.37)

The average BMI of the study group was higher at 16.0 +/- 2.0 while that of the control group was 15.5 +/- 1.5 (p=0.35).

4.2. Vitamin D levels in cases and controls

Vitamin D deficiency was defined as a serum level of Vitamin D of <30ng/ml (75nmol/l). Both groups had normal average Vitamin D levels, however, the study group was lower at 32.8 +/- 13ng/ml and the control group at 35.6+/-8.6ng/ml (p=0.26) (Figure 4). The lowest Vitamin D level in the cases was 7.01ng/ml and 17.61ng/ml in the control group.

17 out of 40 cases (42.5%) had Vitamin D deficiency while 8 out of 40 controls (20%) were noted to be Vitamin D deficient (p=0.05, odd ratio=3.0 (95% CI:1.1-8.0)).

Vitamin D deficiency was found in 25 (31.3%) of the total population.

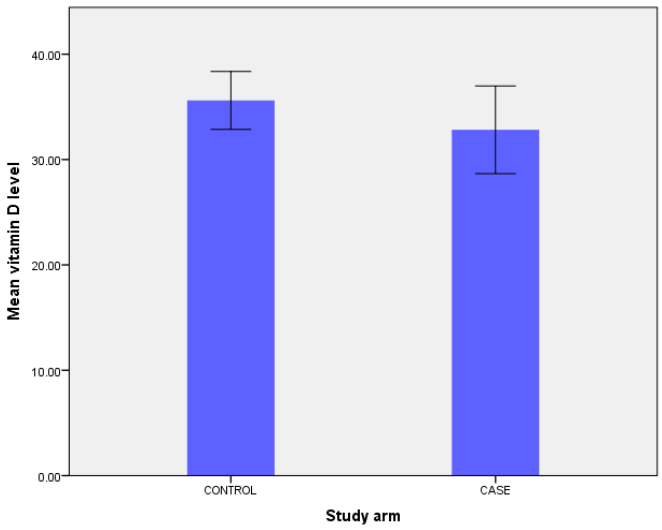


Figure 5- Mean Vitamin D levels

4.3 Correlation between age, gender, weight, height and BMI and Vitamin D for cases

Vitamin D deficiency was more prevalent amongst females then males (50% vs 39.3%, p=0.73), and more prevalent in children greater then 5 years (60% vs 36.7%, p=0.27) (table 2).

We used Spearmans rank correlation to explore correlations between the socio-demographic variables and Vitamin D. Age (r= -0.39, p=0.01), weight (r= -0.34, p=0.03)), height (r= -0.39, p=0.02) and BMI (r= -0.35, p=0.03) were inversely associated with Vitamin D levels (table 3)

Table 2: Correlation between age, gender and vitamin D for cases (Chi-squared test**)**

Variable		**Normal Vit D levels**	**Vitamin D deficiency**	**p-value**
Gender	Female	6(50%)	6(50%)	0.73
	Male	17(60.7%)	11(39.3%)	
Age	≤5	19(63.3%)	11(36.7%)	0.27
	>5	4(40%)	6(60%)	

Table 3: Correlation by spearmans rank correlation for cases

Variable	**Spearmans correlation coefficient**	**P-value**
Age	-0.39	**0.01**
Weight	-0.34	**0.03**
Height	-0.39	**0.02**
BMI	-0.35	**0.03**

4.4. Correlation between age, gender, weight, height and BMI and Vitamin D for controls

Vitamin D deficiency was more prevalent amongst the males then females (21.4% vs 16.7%, p=1.00), and more prevalent in children less then and equal to 5 years (26.2% vs 0, p=0.17). We used Spearmans rank correlation to explore correlations between socio-demographic variables and Vitamin D. Age (r= 0.52, p=0.001), weight (r=0.51, p=0.001), height (r=0.41, p=0.008) and BMI (r=0.25, p=0.10) were positively associated with Vitamin D levels.

4.5 Correlation between age, gender, weight, height and BMI and Vitamin D for the total population

Vitamin D deficiency was more prevalent amongst females then males (33.3% vs 30.4%, p=0.80), and more prevalent in children less then and equal to 5 years (31.7% vs 30%, p=1.00). We used Spearmans rank correlation to explore correlations between socio-demographic variables and Vitamin D. Age (r=0.003, p=0.88), weight (r=0.03, p=0.77), height (r=0.00, p=1.00) and BMI (r=0.046, p=0.68) exhibited positive relationships with Vitamin D.

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1: DISCUSSION

Although our country benefits from ample sunlight, higher levels of melanin in the dark-skinned population act as a natural sunscreen, necessitating more sunlight exposure for children (27). In their study, Kagotho et al found that black healthy people in Nairobi who were exposed to more than three hours of sunshine were less likely to be Vitamin D deficient than those who were exposed to less than one hour, although the difference was not statistically significant (27). Genetic polymorphisms lead to lower levels vitamin D binding protein in black populations. This being the principal vitamin D carrier protein consequently leads to lower Vitamin D levels (38).

This study involved 80 children, with 70 percent of them being male. Okerosi et al (unpublished data) reported a 65 percent male predominance in children with obstructive sleep disordered breathing undergoing adenoidectomy and or tonsillectomy at KNH.

The average age of the subjects was 4.4 years. Because the influence of adenoid/tonsil size decreases as a child develops, we discovered fewer older children with obstructive adenotonsillar disease.

Vitamin D deficiency was found in 31.3 percent of the participants in the study. Shin et al reported a higher prevalence of 52.3 percent, which could be attributed to Korea's higher latitude and thus colder temperatures than Kenya.

Although the means of Vitamin D did not differ significantly between the two groups in our study, the study group had a lower Vitamin D mean than the control group, and the number of participants with low blood Vitamin D was higher among the cases (42.5% vs 20%, p=0.05). Vitamin D plays a vital role in immune regulation and deficiency may lead to hypertrophy of the adenoids/tonsils. Reid et al (32) concluded that lower vitamin D levels were significantly associated with larger tonsil size. Shin et al showed that 91.3% of children with Vitamin D deficiency had adenoid and/or tonsillar hypertrophy. This high prevalence could be because of using tonsil grades 3 and 4 and adenoid nasopharyngeal ratio ≥ 0.8 to define hypertrophy while we only used obstructive symptoms. Our findings are in keeping with study done by Aydin et al in Turkey which also didn't show a statistically significant difference between the mean Vitamin D levels in the two groups but a higher prevalence of Vitamin D deficiency was seen in the study group (18% vs 0, $p<0.001$) (39). However, their study group comprised of children with recurrent tonsillitis. Another pilot study done in the US didn't show any difference in the levels of vitamin D in children undergoing adenotonsillectomies and controls. Again, the study group comprised of children with obstruction and recurrent infections and the season in which blood was collected was not specified. Differences in latitude, skin pigmentation, and seasons could be responsible for the conflicting findings.

Vitamin D is a fat-soluble vitamin which deposits in body fat, hence, children who are overweight or obese tend to have Vitamin D deficiency. We found that the study group had a higher BMI when compared to the control group though not statistically significant (16 vs 15.5,

p=0.35). However, BMI was inversely correlated with Vitamin D (spearmans rank= -0.35, p=0.03) in children with obstructive adenotonsillar disease but not in the controls group or the total population. Reid et al (32) and Shin et al (5) were also able to demonstrate an inverse relationship between BMI and serum Vitamin D levels. Similarly, even age, weight and height showed an inverse relationship with Vitamin D in the cases but not in the controls or the total population.

Deficiency of Vitamin D has been associated with the development of lower respiratory tract infections in children under the age of five (40) (41). In our study, there was no significant difference in Vitamin D deficiency in children who were less then 5 years when compared to children more than 5 years in cases, controls or the total population. This may be because of the study having fewer children who were older than 5 years. However, we found a statistically significant inverse relationship between age and Vitamin D levels in the cases which could be due to reduced levels of 7-dehydrocholesterol in the skin with older age.

Our study found no association between gender and Vitamin D deficiency in cases, controls, or the study population, however, Vitamin D deficiency was commoner in females with obstructive adenotonsillar illness. This is due to the fact that women have slightly increased body fat and, as a result, have lower Vitamin D levels. In addition, Aydin et al found no correlation between gender and vitamin D levels (38).

5.2: Conclusion

Almost half of the children with obstructive adenotonsillar disease were vitamin D deficient. This study showed that children with obstructive adenotonsillar disease were three times more likely to have vitamin D deficiency compared to those without. Age, weight, height and BMI were inversely associated with Vitamin D levels in the study group.

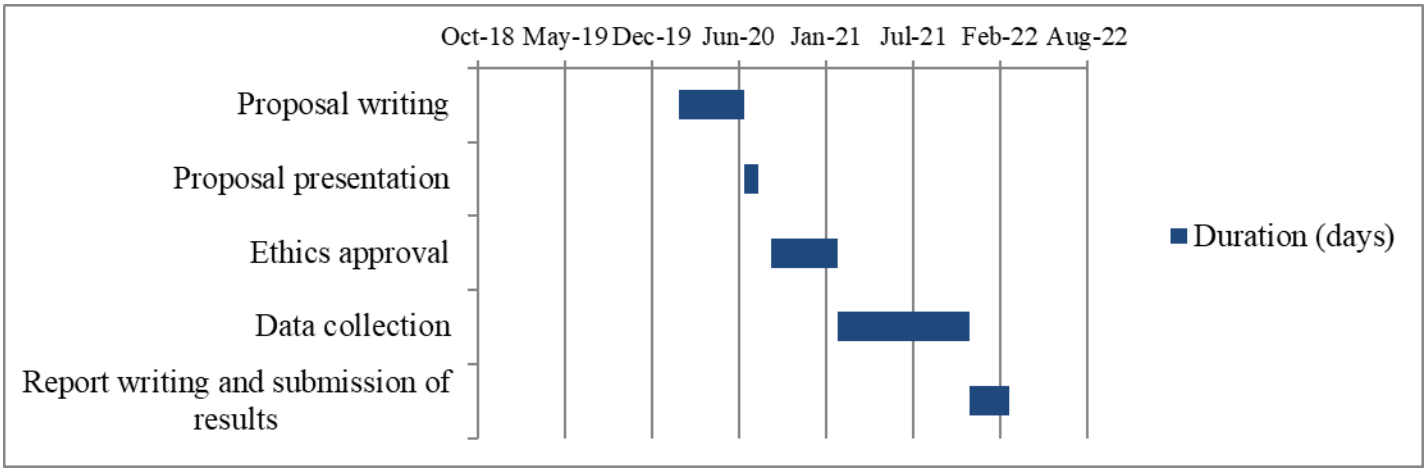
5.3: Recommendations

We recommend that children having obstructive adenotonsillar disease should be assessed for Vitamin D deficiency for prompt diagnosis and intervention which will also assist in protecting the child from further respiratory tract infections. In such patients, BMI should be monitored closely and corrected if abnormal. Further studies should be carried out in multiple centers with larger sample sizes to better define the prevalence of Vitamin D deficiency and associated risk factors. We also recommend that such children should be put on Vitamin D supplementation and observed for upper respiratory tract infections.

5.4: Limitations

- The study had a small sample size, to allow more detailed generalizations to be made
- Although we only used black children, the different skin types amongst them was not taken into consideration

TIMELINE



BUDGET

Item	No. of units	Cost per unit	Cost
Proposal copies	8	1000/=	8,000/=
Printing thesis	10	1500/=	15,000/=
Stationary	10	500/=	5,000/=
Statistician	1	35,000/=	35,000/=
Vitamin D test	80	2500/=	200,000/=
Contingency fund			20,000/=
ERC fee	1	2,000/=	2,000/=
Total cost			305,000/=

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APPENDICES

Appendix I: General information and consent

PARTICIPANT INFORMATION AND CONSENT FORM FOR ENROLMENT IN THE STUDY

Title of Study: Vitamin D levels in children booked for adenotonsillectomy at the Kenyatta National Hospital

Principal Investigator/and institutional affiliation: DR HUSSEIN HASSANALI, POSTGRADUATE STUDENT, UNIVERSITY OF NAIROBI

Introduction:

I would like to inform you about my study on Vitamin D levels in children booked for adenotonsillectomy at the KNH. This consent form is aimed at giving you the information you need to enable you to make an informed decision. You will be allowed to ask any questions regarding the study. Where you do not understand the benefits and risks of the study, kindly seek clarification. Upon answering your questions and concerns to your satisfaction, you may decide to be included in the study or not. You shall be required to sign a consent form that you have agreed to voluntarily participate in this study. Note that your decision to participate in this study should be voluntary and you may withdraw from the study if you wish to do so without having to give reasons for your withdrawal.

Refusal to participate in the research will not affect the services your child is entitled to in this health facility or other facilities. We will give you a copy of this form for your records.

May I continue? YES / NO

This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee protocol No……..

WHAT IS THIS STUDY ABOUT?

The researcher listed above is examining your child to see if there is any relationship between adenotonsillar disease and Vitamin D levels. Parents/ guardians in this research study will be asked basic demographic questions after which participant will undergo assessment of their Vitamin D levels by obtaining a venous blood sample. There will be approximately 80 participants divided into 40 children with adenotonsillar disease and 40 children from well-baby clinic. We are asking for your consent to consider participating in this study.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen: You will be interviewed by the investigator in a private area where you feel comfortable answering questions. The interview will last approximately 15 minutes. The interview will cover use of certain drugs.

Your child will proceed to have a venous blood sample taken for measurement of Vitamin D levels, results of which will be availed to the clinicians managing the patient. We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reasons why we may need to contact you is to provide feedback on the Vitamin D results.

ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY?

Medical research has the potential to introduce psychological, social, emotional and physical risks. Effort should always be put in place to minimize the risks. One potential risk of being in the study is loss of privacy. We will keep everything you tell us as confidential as possible. We will use a code number to identify your child in a password-protected computer database and will keep all child's paper records in a locked file cabinet.

However, no system of protecting your child's confidentiality can be secure, so it is still possible that someone could find out your child was in this study and could find out information about you. In addition, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

The study will not expose your child to any risks except for the slight pain felt while blood is being taken from your vein.

ARE THERE ANY BENEFITS BEING IN THIS STUDY?

The information you provide will help us better understand the relationship between adenotonsillar disease and Vitamin D. This might help us to develop other treatment strategies for children like yours.

WILL BEING IN THIS STUDY COST YOU ANYTHING?

There are no costs to be incurred.

WILL YOU GET REFUND FOR ANY MONEY SPENT AS PART OF THIS STUDY?

There are no refunds as there are no costs.

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your rights as a research participant, you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. (254-020) 2726300-9. Ext. 44355 email uonknh_erc@uonbi.ac.ke.

WHAT ARE YOUR OTHER CHOICES?

Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time without it affecting your child's treatment.

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement: I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw any time. I freely agree to participate in this research study. I understand that all efforts will be made to keep information regarding my child's personal identity confidential.

By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I agree to participate in this research study:	**Yes**	**No**

I agree to provide contact information for follow-up:	Yes	No

Participant printed name: _____

Participant signature / Thumb stamp _____Date _____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Researcher's Name: Dr Hussein Hassanali **Date**: _____

Signature_____

Role in the study: _____

For more information contact,

Dr. Peter Mugwe, Consultant ENT, University of Nairobi, 0722-513778.

Dr Catherine Irungu, Consultant ENT, University of Nairobi, 0722-385710

Dr. Tim Jumbi, Consultant Pediatric Surgeon, University of Nairobi, 0720-780303

Dr. Paul Laigong, Consultant pediatric endocrinologist, University of Nairobi, 0720-386861

Ethics and Research Committee Telephone No. (254-020) 2726300-9. Ext. 44355 email uonknh_erc@uonbi.ac.ke.

Kiambatisho; Fomu ya Maelezo Kuhusu Idhini ya Wateja

Kitangulizi

Mimi ni daktari Hussein Hassanali anayesomea masoma ya juu ya kitengo cha upasuaji wa masikio, mapua, koo, kichwa na shingo katika chuo kikuu cha Nairobi. Ningependa kuomba idhini yako ya kushiriki katika utafiti wenye lengo la kujua kuwepo kwa mchanganyiko wa koo la kati katika mtoto wako.

Jinsi ya kushiriki

Baada ya wewe kupeana idhini ya kushiriki katika utafiti huu, nitachunguza koo ya mtoto wako, kisha utajibu maswali kadhaa nitakayokuuliza. Mtoto wako atafanyiwa uchunguzi zaidi wa kupimwa damu kuamua Vitamin D . Hakuna gharama yoyote zaidi utakayolipishwa na hakuna madhara yoyote ya kushiriki katika utafiti huu.

Una haki ya kuondoa mtoto wako kutoka kwa utafiti huu wakati wowote bila adhabu yoyote. Utapewa habari kuhusu uchunguzi utakaofanywa na umuhimu wa matokea yatakayopatikana.

Jinsi gani kushiriki kwako kunaweza kuleta madhara?

Utafiti huu hautakudhuru kwa njia yoyote; taarifa yote kuhusu mgonjwa wako itakuwa ni siri, utambulizo wa mtoto wako pia utakuwa kwa siri.

Je tutafanyia nini matokeo ya utafiti huu?

Maarifa tutakayopata yatasaidia kuboresha huduma inayopewa watoto walio na ugonjwa wa adenoids and tonsils.

Kuna uwezekano wa kuchapishwa kwa matokeo ya utafiti huu katika majarida ya kisayansi au kuwakilishwa katika mikutano ya kisayansi na umma kwa jumla bila kuwataja wahusika.

Je umeridhika?

Iwapo umeridhishwa na maelezo uliyoyasoma na kufafanuliwa, tafadhali weka sahihi yako kwenye fomu ya idhini inayofuata:

(ii) Sehemu ya pili – Idhini ya mgonjwa

Mimi (Jina)…….…… kwa hiari yangu, nimekubali mtoto wangu kushiriki katika utafiti huu ambao unafanywa na Daktari Hussein Hassanali. Nimeelezewa manufaa na madhara ya utafiti huu kwa undani na nimeridhika.

Jina la Mzazi/Mlezi……

Sahihi……...

Tarehe……..

Nambari ya utafiti……..

Appendix II: Data collection tool

DATA COLLECTION TOOL FOR CASES

PART A- DEMOGRAPHIC INFORMATION

Study number …… DATE……...

BIODATA

AGE……

GENDER…….

Weight .....

Height .....

BMI (kg/m^2).....

PART B- LAB RESULT

25(OH)D_3 LEVELS ……

DATA COLLECTION TOOL FOR CONTROLS

PART A- DEMOGRAPHIC INFORMATION

Study number……. DATE…….

BIODATA

AGE…….

GENDER…….

Weight (kg) .....

Height (cm).....

BMI (kg/m^2).....

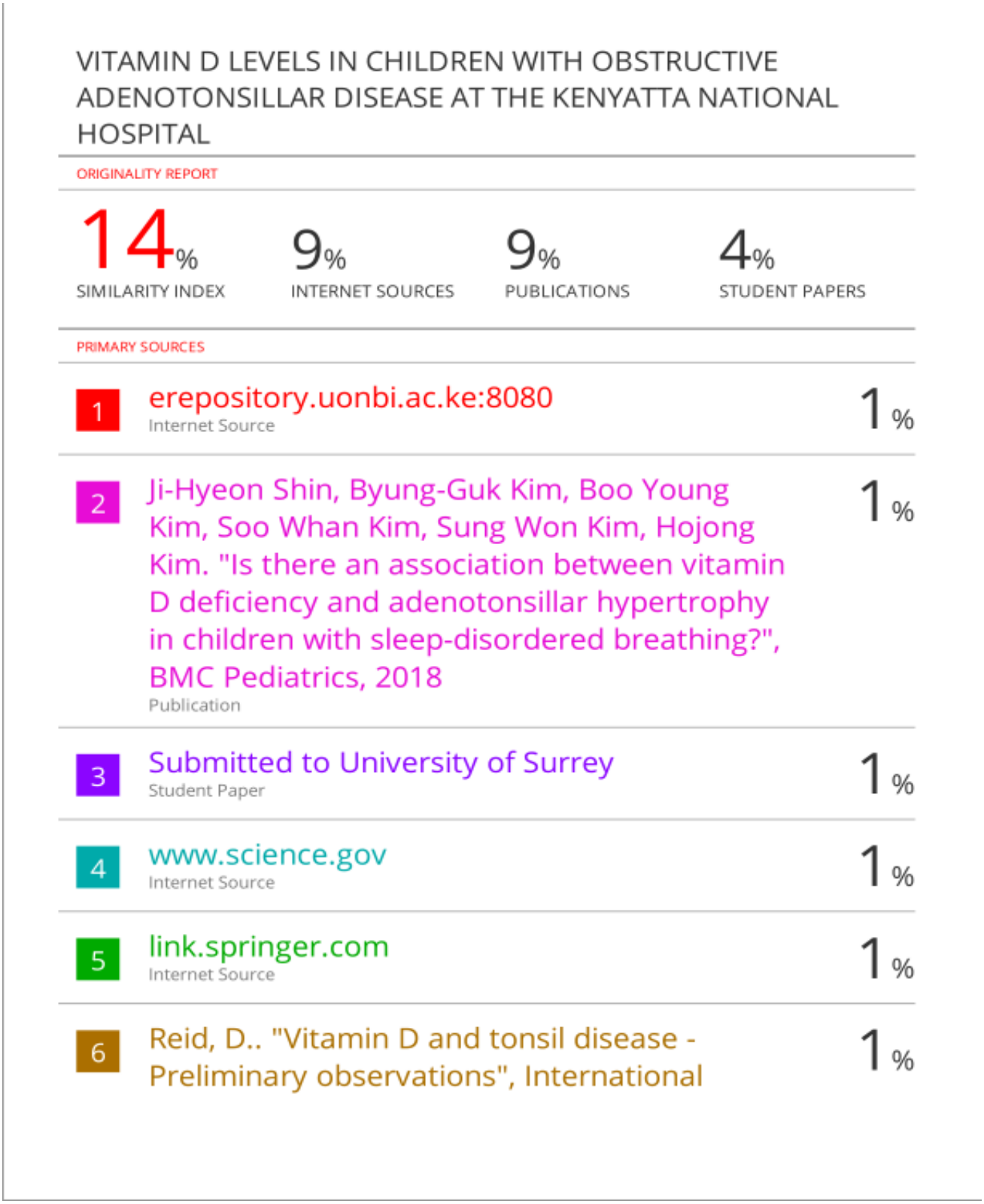
Snoring, nasal obstruction or mouth breathing Yes/No

Tonsillar grade 3 or 4 Yes/No

PART B- LAB RESULT

$25(OH)D_3$ LEVELS……...

Appendix III: Plagiarism report.



VITAMIN D LEVELS IN CHILDREN WITH OBSTRUCTIVE ADENOTONSILLAR DISEASE AT THE KENYATTA NATIONAL HOSPITAL

ORIGINALITY REPORT

14%	9%	9%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	erepository.uonbi.ac.ke:8080 Internet Source	1%
2	Ji-Hyeon Shin, Byung-Guk Kim, Boo Young Kim, Soo Whan Kim, Sung Won Kim, Hojong Kim. "Is there an association between vitamin D deficiency and adenotonsillar hypertrophy in children with sleep-disordered breathing?", BMC Pediatrics, 2018 Publication	1%
3	Submitted to University of Surrey Student Paper	1%
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