

**COMPARISON OF TESTOSTERONE: DIHYDROTESTOSTERONE
RATIOS IN CHILDREN WITH HYPOSPADIAS TO A CONTROL
GROUP AT KENYATTA NATIONAL HOSPITAL**

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DECLARATION

This dissertation is my original work and to the best of my knowledge has not been submitted anywhere else for consideration for publication or for the award of another degree.

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LIST OF ABBREVIATIONS

T – Testosterone

DHT – Dihydrotestosterone

ERC – Ethics Review Committee

KNH – Kenyatta National Hospital

SPSS – Statistical Package for Social Sciences version 22.0

UON – University Of Nairobi

AR – Androgen Receptor

5 α R – 5 alpha reductase

ICBDMS - International Clearing House for Birth Defects Monitoring Systems

ABSTRACT

BACKGROUND: Hypospadias is a common birth defect occurring in male children. Its worldwide incidence is approximately 1 in 125 live male new-borns. It occurs due to an arrest in the normal development of the urethra, foreskin and ventral part of the penis. The external genitalia of male embryos undergoes masculinization under the influence of testosterone and dihydrotestosterone. Dihydrotestosterone (DHT) is responsible for the complete differentiation of the penis with a male urethra and glans. It is converted from testosterone by the enzyme type 2 5 α -reductase. Research done shows that a deficiency of 5 α -reductase could be one of the causes of hypospadias.

OBJECTIVE: The aim of this study was to compare the Testosterone: Dihydrotestosterone ratio in children with hypospadias to a control group without hypospadias.

STUDY SETTINGS AND DESIGN: This was an observational case-control study carried out in the KNH paediatric surgical ward and paediatric surgical outpatient clinic.

PATIENTS AND METHODS: The study involved patients with hypospadias and children with inguinal hernias with no other congenital anomalies as the control group. The patients were reviewed, a questionnaire administered and blood drawn. Blood levels of testosterone and dihydrotestosterone were measured at the KNH Biochemistry laboratory and a T: DHT ratio calculated. The control group was paediatric patients with inguinal hernias and no other congenital anomaly. Data collected was analysed using SPSS version 22.0.

RESULTS: On the testosterone levels in children with hypospadias and the control group, the study found that there were no statistical differences ($p = 0.290$).

On the dihydrotestosterone levels in children with hypospadias and the control group, the study found that there were no statistical differences (0.304)

On the Testosterone: Dihydrotestosterone ratio in children with hypospadias and the control group, the study found that there were no statistical differences (0.130).

CONCLUSION: Since there was no statistical difference in the testosterone: dihydrotestosterone ratio between the case and control groups, the study concludes that children with isolated hypospadias may not have a deficiency or defect in the enzyme 5 alpha reductase.

1.0 CHAPTER ONE: INTRODUCTION

Hypospadias is a common birth defect occurring in male children. Its worldwide incidence is approximately 1 in 125 live male new-borns [1]. Despite several studies, the aetiology of hypospadias is not well understood and could be dependent on a number of factors, including genetic, endocrine and environmental factors [2].

Hypospadias as a birth defect has significant socio-economic impact on the parent/parents of the child. Such children may need several surgeries to correct the defect thus affecting the economic status of the parents both as a direct cost of surgeries and lost working hours. These children are also stigmatised by the community and may suffer psychological trauma.

Hypospadias occur due to an arrest in the normal development of the urethra, foreskin and ventral part of the penis. In hypospadias the urethral opening can be located anywhere along the ventral part of the penis, within the scrotum or in the perineum. It is usually also associated with a chordee, which is a ventral curvature of the penis [3].

Hypospadias can be classified as mild, moderate or severe (posterior), depending on the location of the urethral meatus.

Mild hypospadias are further sub classified as glanular, coronal or distal (where the meatus is located in the distal third of the shaft of the penis).

In moderate hypospadias the meatus is located along the middle third of the shaft of the penis. Severe hypospadias are further sub classified as posterior penile, penoscrotal, scrotal or perineal [4].

Development of the penis occurs between 8-16 weeks of intrauterine life and is influenced by testosterone and its metabolite; dihydrotestosterone. A surge in the levels of luteinizing hormone which is produced from the anterior pituitary gland leads to masculinization of the external genitalia of the developing foetus. This causes an increase in the anogenital distance, penile elongation, male-type urethra formation from the urethral groove and preputial development [5,6].

The urethral groove appears on the ventral surface of the shaft of the penis between the two urethral folds. The urethral folds, which are of endodermal origin, fuse to form the penile urethra while fusion of the edges of the urethral groove, which are of ectodermal origin, lead to formation of the median raphe.

The glanular urethra develops during the 16th week. There are two theories which have been stipulated to explain the mechanism of formation of the glanular urethra. These include the:

- (i) Endodermal cellular differentiation. This is a newer theory and has replaced the older one amongst researchers as the more acceptable one.
- (ii) Ectodermal tissue ingrowth from the glans. This was initially widely accepted but is now being replaced.

The future prepuce forms during the same time as the urethra is developing and is therefore reliant on development of a normal urethra. In abnormal urethral development, where the genital folds do not fuse, the tissues responsible for development of the prepuce do not form ventrally and are excessive dorsally [7].

Dihydrotestosterone (DHT) is the male hormone which is responsible for the full differentiation of the penis with a male urethra and glans. It must be converted from testosterone by the enzyme 5α -reductase type 2 [8]. It is also reported that dihydrotestosterone is 50 times more effective than testosterone [9]. It is therefore absolutely necessary for development of the male external genitalia and urethra during foetal development [10].

5α -reductase deficiency is a disease that causes 46XY genetic male patients to have partial virilisation of their external genitalia and testes [11]. The disease spectrum of 5α -reductase deficiency can vary from a female phenotype to a fully developed male with hypospadias or only micro phallus [12,13]. These patients usually have normal spermatogenesis if they have normally descended testes [14]. Their testosterone levels are either normal or elevated, with decreased DHT levels in relation to testosterone [15]. Patients with 5α -reductase type 2 deficiency have an increased serum testosterone-to-dihydrotestosterone ratio (T/DHT). An increased T/DHT ratio is therefore diagnostic of the disease. Prepubertal males with 5α -reductase-2 deficiency have a T/DHT ratio of more than 30 while in normal prepubertal males the mean T/DHT ratio is 10.7 (3.5-14) [16].

Androgen receptor antagonists and inhibitors of 5α -reductase have been used to induce hypospadias experimentally [17].

In the human genome the steroid 5α -reductase type 2 gene is located on chromosome 2, p-arm, band 2 and sub-band 3 [18].

1.1 Literature Review

Paulozzi looked at the International Clearing House for Birth Defects Monitoring Systems data and noted that there was an increased incidence of hypospadias in the United States, Norway, and Denmark while in England, Canada and Holland the increasing incidences appear to have levelled off since 1985. The data also showed that the incidence was not increasing in the less developed countries [19].

In the United States of America, data from Metropolitan Atlanta Congenital Defects Program (MACDP) and Birth Defects Monitoring Program (BDMP) show an unexplained doubling in the incidence of hypospadias in the country [20].

Wu et al studied the prevalences of various congenital anomalies in Kenya and found the prevalence of hypospadias to be at 0.9 per 1000 children (95% CI of 0.3-2.1) [21].

In one series most patients (72%) presented with anterior hypospadias, while middle and posterior hypospadias occurred in 16% and 12% of cases, respectively. The most common associated malformations were undescended testis (12%), inguinal hernias (8%), and intersex disorders (5%) [22].

McArdle and Lebowitz examined 200 patients with hypospadias and found only 6 patients with genitourinary anomalies (3%) [23].

Albers et al evaluated thirty three patients with posterior hypospadias to classify and establish its cause. Various techniques were used such as clinical evaluation, ultrasound assessment, karyotyping, endocrine assessment, molecular analysis of the androgen receptor (AR) and genetic evaluation of 5 α -reductase genes. In only 12 patients (36%) were they able to determine the aetiology. The remaining 21 patients (64%) were labelled as having hypospadias of unknown aetiology [24].

McPhaul et al. and Hiort et al. examined the androgen receptors (AR) in children with hypospadias and concluded that androgen receptor defects or mutations in the genes for androgen receptor were rare in these children [25,26].

Bentvelsen et al. measured androgen receptor levels in the prepuces of male children with hypospadias and compared them to controls without hypospadias. They didn't find any significant difference in mean androgen receptor content [27].

Gearhart and colleagues studied the preputial skins of male children with hypospadias and evaluated the androgen receptor levels and enzyme 5 α -reductase activity. They didn't find any deficiencies in the levels of androgen receptor nor in the activity of the enzyme 5 α -reductase [28].

Allera and colleagues also analysed the androgen receptor genes in patients with hypospadias and found a defect in the open-reading frame in only one patient out of the nine patients they analysed with severe hypospadias [29].

Bloch et al inhibited the 3β -hydroxysteroid dehydrogenase activity in pregnant rats by injecting them with 2-cyano-4,4,17-trimethyl-17-hydroxyandrost-5-en-3-one(WIN). The anogenital distances of the male foetuses were markedly reduced. These male foetuses had also developed hypospadias. The testes of these foetuses were found to synthesize a reduced amount of testosterone and androstenedione but had an increased concentration of 17α -hydroxypregnenolone and dehydroepiandrosterone (substrates for the enzyme 3β -hydroxysteroid dehydrogenase in the testosterone synthesis pathway) [30].

Clark et al administered finasteride (5α reductase inhibitor) orally once a day to pregnant rats during gestation. First generation offspring were assessed on the 20th day of gestation as well as after birth. The male offspring had dosage-related incidences of hypospadias with a threshold dosage level of about 0.1 mg/kg/day. At the dose of 100 mg/kg/day (with dosing through the 20th day of gestation) there was a 100% effect. Finasteride also caused decreased anogenital distance in the male offspring of these rats [31].

Studies have also been done in order to attribute all hypospadias to some sort of genetic defect in the testosterone synthesis pathway. However these studies have not been completely successful. Aaronson et al. studied the three major enzymes in the testosterone synthetic pathway; 3β -hydroxy-steroid dehydrogenase, 17α -hydroxylase, and $17,20$ -lyase in thirty boys with completely descended testes and with proximal shaft or penoscrotal hypospadias. Fifteen boys (50%) were found to have an intrinsic defect in the synthesis of testosterone. However the cause in the remaining fifteen boys (50% of the cases) was not established [32].

Silver et al performed a genomic analysis on the 5α -reductase type 2 genes in penile skin tissues of boys with hypospadias with no other congenital anomalies. Eighty one specimens were studied and seven (8.6%) were found to involve a mutation in at least one for 5α -reductase type 2 gene, while two other specimens were noted to have a mutation in both alleles. It was also concluded that a partial deficiency of the enzyme 5α -reductase with reduced levels of dihydrotestosterone in the foetal urethra could be enough to cause isolated hypospadias without causing the other clinical spectrum of 5α -reductase deficiency [33].

Thai HT et al found that the leucine version of the enzyme 5α -reductase to be 30% less effective than the valine variant with insufficient dihydrotestosterone levels in the leucine version. It was therefore determined that the leucine version of 5α -reductase may contribute to the aetiology of hypospadias [34].

K. Zhang et al conducted a meta-analysis on several studies on V89L functional polymorphism of the 5 α -reductase type 2 gene caused by a transversion of Guanine to Cytosine (valine to leucine) at codon 89. They concluded that that the V89L polymorphism of the SRD5A2 gene leads to an increase in the risk of developing hypospadias and the Cytosine allele is a genetic risk factor for the development of hypospadias [35].

Pang et al in 1979 compared the Testosterone: Dihydrotestosterone ratio in prepubertal males with varying conditions such as undescended testes, delayed puberty and treated congenital adrenal hyperplasia to that in normal male infants. They found that the ratio was higher in the prepubertal males with the varying conditions (11 ± 4.4) than in the normal male infants (4.9 ± 2.8) [36].

Antje et al in 2004 studied the T: DHT ratios in 10 unrelated children with severe hypospadias and found it to be elevated at >11 . He compared it to 49 adult fertile males as controls. He then analysed the steroid 5 α -reductase type1 (SRD5A1) gene in the children with hypospadias and an increased T:DHT ratio and failed to detect any mutation in this gene. Therefore he could not support the theory that a mutation in the SRD5A1 gene could cause an increased T: DHT ratio and thus be the main cause of virilisation defects [37].

The use of testosterone injections and dihydrotestosterone creams have been successfully used for penile lengthening in children with 5 α -reductase 2 deficiency prior to surgical correction of hypospadias. Mendonca et al in 1996 evaluated sixteen subjects with hypospadias with male pseudo-hermaphroditism due to steroid 5 α -reductase 2 deficiency. The diagnoses were made on the basis of normal plasma testosterone values, normal or low plasma dihydrotestosterone levels and high testosterone/dihydrotestosterone ratios. Ten of 13 subjects of postpubertal age had surgical correction of the hypospadias, and were treated with high-dose testosterone esters by parenteral injection and subsequently with dihydrotestosterone cream. These regimens brought serum dihydrotestosterone levels to the normal male range (or above). Treatment of the prepubertal boys with testosterone and/or dihydrotestosterone resulted in a doubling of penis size allowing for successful hypospadias repair [38].

1.2 Conceptual Framework

Narrative

The enzyme 5 α reductase is responsible for conversion of testosterone to dihydrotestosterone which is the active component. DHT is responsible for the development of the penis and male urethra. It is postulated that deficiency in the enzyme 5 α reductase is responsible for reduced testosterone activity as very little would then be converted to its active component; DHT. A reduced DHT level would impair development of penis and male urethra and lead to development of hypospadias. The activity of 5 α reductase can be determined by calculating the Testosterone: DHT ratio. Due to reduced synthesis of DHT (due to decreased 5 α reductase activity) the T:DHT ratio in children with hypospadias will be increased when compared to the control group.

Schematic

Testosterone $\xrightarrow{5\alpha \text{ reductase}}$ DHT

Testosterone $\xrightarrow{5\alpha \text{ reductase deficiency}}$ ↓ DHT Therefore increased T: DHT ratio

Children with hypospadias \longrightarrow ? 5 α reductase deficiency (Increased T: DHT ratio)

2.0 CHAPTER TWO: PROBLEM STATEMENT AND JUSTIFICATION OF THE STUDY

Hypospadias is a common birth defect which occurs in approximately 1 in 125 live male newborns.

Treatment of hypospadias is very expensive and sometimes involves multiple surgeries making it an economic burden and psychologically stressful especially to the parents of the child.

The aetiology of hypospadias is thought to be either genetic or due to exposure to environmental endocrine factors with anti-androgenic activity.

In this study we want to find out whether children with hypospadias have a genetic defect leading to defective or deficient 5α reductase activity with decreased DHT production. DHT is the active metabolite of testosterone and is responsible for development of the penis and male urethra.

If not, then the hypospadias could be due to environmental endocrine factors, which could be a basis of other studies.

Therefore this study will go a long way in helping us to establish the possible cause and could be a basis of future studies on methods to prevent hypospadias.

It could also be a basis for administration of testosterone or dihydrotestosterone supplements before surgery thus reducing the need for multiple surgeries and therefore reduce the cost of treatment.

No similar study has been done on children in Kenya.

2.1 Hypotheses

Null Hypothesis: There is no difference in T: DHT ratio in children with hypospadias when compared to a control group

Alternate Hypothesis: There is an increase in T: DHT ratio in children with hypospadias when compared to a control group

2.2 Research Question

Is there an increase in T: DHT ratio in children with hypospadias when compared to a control group?

2.3 Objectives

2.3.1 Broad Objective

To compare the Testosterone: Dihydrotestosterone ratio in children with hypospadias with a control group

2.3.2 Specific Objectives

1. To measure testosterone levels in children with hypospadias and the control group.
2. To measure dihydrotestosterone levels in children with hypospadias and the control group.
3. To calculate the Testosterone: Dihydrotestosterone ratio in children with hypospadias and the control group.

3.0 CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

Kenyatta National hospital paediatric surgical ward and paediatric surgical outpatient clinic

3.2 Study design

This is an observational case control study designed to compare testosterone: dihydrotestosterone ratios in children with hypospadias to children without hypospadias.

3.3 Study population

The study population comprised male children born with hypospadias and the control being male children in surgical paediatric wards and paediatric surgical outpatient clinic with inguinal hernia with **no congenital anomalies**. Development of inguinal hernias in male children is not associated with any hormone deficiency, therefore such children with inguinal hernias and no other congenital anomalies can be used as a control group.

Frequency matching of the control group to the study population was done i.e

- there were an equal number of children in the control group as in the study population
- the children were below 12 years of age

3.4 Inclusion criteria

- All children with hypospadias who are under 12 years of age

3.5 Exclusion criteria

- Children more than 12 years of age
- Children with disorders of sex development
- Children on medication that will affect the testosterone level.

3.6 Sampling

Convenient sampling procedure was used to recruit patients into the study. All patients who fulfilled the eligibility criteria were enrolled until the full sample size was achieved.

3.7 Sample size calculation

The basis for the calculation of the sample size was derived from data from the following.

For continuous exposure use different in means formula

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

n = Sample size

r = ratio of controls to cases

S.D² = Pooled variance (S_p)² of the outcome variable

Z_β = Desired power (typically 0.84 for 80% power)

Z_{α/2} = level of statistical significance

d = difference in means

Calculating sample size assuming the tabulated parameters from a similar study (Antje et al (2004) [36]) below;

Parameter	Cases (1)	Controls (2)
Means	17.7	11.9
Difference in means=d	17.7-11.9=5.8	
Standard Error (S _x)	1.77	0.97
Standard deviation(S) =S.E*Square root(sample size)	=1.77*√ 10 =5.6	=0.97*√ 49 =6.8
Variance=S ²	(S ₁) ² =(5.6) ² =31.4	(S ₂) ² = (6.8) ² =46.2
Pooled Variance(S _p) ²	(S _p) ² =[(31.4*9)+(46.2*48)]/57=43.86	
Z _β	0.84	
Z _{α/2}	1.96	
r is ratio of controls to cases (1:1)	1	1

Substituting the above parameters in the sample size estimation formula;

$$n = \frac{1 + 1}{1} 43.86 \frac{(0.84 + 1.96)^2}{5.8 \times 5.8} = 20.44$$

Therefore, cases= 21 and controls=21

3.8 Data Collection

Data collection was done after consent was sought from the guardians of the patients. Data was collected through interviewing subjects, physical examination and collection of blood samples. Trained interviewers used pre-tested structured questionnaires to collect the data; the questionnaires were available in both English and Kiswahili.

A separate data collection tool was used for the control group.

The blood samples were collected by trained phlebotomist provided by the Biochemistry laboratory in KNH. 4mls of whole blood was drawn and placed into red top vacutainers.

Before the actual data collection, the standardized study questionnaire was pre-tested through administration to nurses in the paediatric surgical ward at the KNH. This feedback was analysed to enable ascertainment of the clarity and specificity of the questionnaire, as well as the ability to analyse the data to be obtained.

Data collection was done at the KNH paediatric surgical ward and paediatric surgical outpatient clinic. The nurses in charge of the respective units were informed and the logistics of data collection was discussed. The eligibility of inclusion was ascertained by verification from the recorded data and decisions made in the files of the patients, in addition to the information provided by the patient. The data was collected by trained research assistants with minimum qualification of Kenya Registered Community Health Nurse (KRCHN), with experience in handling paediatric patients.

The patients who fulfilled the study inclusion criteria were invited into a separate room, accompanied by their legal guardians, within the facility. The purpose and objectives of the study was introduced and discussed. Both verbal and written consent was sought. A baseline physical examination was performed at the time of recruitment, the study questionnaire was administered in private and confidentiality assured. 4mls of whole blood were then drawn and placed into two red top vacutainers (2mls in each).

Once a participant was recruited, a sticker was inserted onto the inner cover of the file of the patient to prevent duplication of data.

3.9 Data Collection Instrument

1. A structured mainly pre-coded questionnaire with a few open-ended questions that shall be focused on the following areas based on the objectives of the study:
 - a. Biodata
 - b. Pathology of the hypospadias
2. Laboratory results on the level of testosterone and dihydrotestosterone and a calculated T:DHT ratio
3. A separate data collection sheet was used for the control group. It focused on
 - a. Biodata
 - b. Laboratory results on the level of testosterone and dihydrotestosterone and a calculated T:DHT ratio

3.10 Quality Assurance

All aspects of this study were subjected to strict quality control. The study instrument was pre-tested to ensure clarity of the questions. There was strict adherence to the inclusion criteria in order to avoid collecting irrelevant data. Regular meetings were scheduled to review any emerging issues that would be relevant to quality control among the principal investigator and the research assistants. The legibility of the handwriting of the research assistants was assured through appropriate criteria and surveillance of recording, in addition to providing adequate space in the questionnaire. The benefit of this strategy was that it would ensure that recording biases due to illegibility are minimized. Stringent training of the research assistants was undertaken, including in observation of the ethical considerations while handling the study participants. There was strict surveillance of the data collection and entry procedures in order to minimize the risk of omission-generated biases and transcriptional errors. The primary investigator verified each questionnaire to confirm that responses are filled correctly, with no skipped questions.

All blood samples were taken to the biochemistry laboratory at KNH and the same kit and machine were used to run the tests. Excess participant blood was discarded using the standard protocol for discarding harmful body fluids.

3.11 Quality Assurance Protocol For The Assay Determinations

3.11.1 Preanalytical phase

1. Blood samples was only be drawn from patients who meet the inclusion criteria. Patients on medications affecting testosterone or dihydrotestosterone levels were not included.
2. A phlebotomist who is experienced in drawing blood from the paediatric age group was used.
3. Aseptic technique was used to draw blood.
4. Depending on age and physical stature of the child the right gauge of needle was used.
5. 4mls of blood were drawn and placed in two red top vacutainers and gently shaken. 2mls in each.
6. The vacutainers were clearly labelled with identity codes which corresponded to the identity codes on the data collection sheet.
7. The vacutainers were taken to the KNH Biochemistry laboratory within 30 minutes.
8. The blood samples were stored in a refrigerator at temperatures between 2° C – 8° C in a space dedicated to the study until the entire sample size was reached.
9. One laboratory technician was responsible for handling and storage of these blood samples.

3.11.2 Analytical Phase

1. The analysis of Testosterone and Dihydrotestosterone was done at the KNH Biochemistry laboratory.
2. The laboratory is ISO 15189: 2012 certified. This is a quality management certification for medical laboratories.
3. All the tests were performed by one laboratory technician.
4. The tests were done using COBAS® 6000 Biochemical analyser machine.
5. The test kit reagents were procured from the same supplier of the machine.
6. The test kit reagents were checked to ensure they are not expired.
7. The machine was calibrated using a calibration set provided by the manufacturer of the kits.
8. Since one reagent kit can perform up to 100 tests, all tests were done in a single run. i.e. all analysis of testosterone levels were done in a single run and that of dihydrotestosterone in a single run.

9. Controls, provided by the same manufacturer, were used to ensure quality control.

3.11.2 Postanalytical phase

1. Correct interpretation of results was done.
2. Results were recorded using the correct identifier code.
3. Results were reported correctly.
4. The data was entered into a computer and a hard copy printed.
5. Records were maintained and secured.
6. The entire quality control chain was be re-evaluated.

NB: Upon completion of study all the laboratory results were destroyed.

3.12 Data Management

Once data collection was completed, the completeness of the filling of the questionnaire was ascertained. The database was password protected for security and to prevent tampering or alterations. Regular file back-up was done to an external hard disk to avoid any loss. Data cleaning and validation was performed in order to achieve a clean dataset ready for analysis.

3.13 Data Analysis

Statistical analysis of the collected was performed using the Statistical Package for Social Sciences (SPSS) version 22.0. Patients' socio-demographic and clinical information were summarized into percentages and means/medians for categorical and continuous variables respectively. The laboratory values of testosterone of each patient were divided by the laboratory values of dihydrotestosterone of the same patient to derive the T: DHT ratio. A mean ratio of patients with hypospadias was compared to the mean ratio of patients without hypospadias. Student's t test was used to compare means. All statistical tests were interpreted at 5% level of significance (p value less or equal to 0.05). Study findings were presented in tables and graphs.

3.14 Ethical Considerations

This study was subjected to review by the Kenyatta National Hospital/ University of Nairobi Ethical Review Committee (KNH/UON ERC) and ethical approval granted.

Informed consent was sought from the respondents (parents/guardians of the children) before administration of the data collection tool and drawing of blood sample. The participants were assured that their participation is voluntary and they were free to refuse to participate in the

study without giving any reason; and this would not affect the quality of care that they receive. There was also a separate consent form for children in the control group.

Informed assent was sought from children between the ages of 6 – 12 years who are able to read and write. They were assured of their right to participate or refuse to participate without affecting their treatment for the condition.

The findings were treated with the greatest amount of confidentiality and used only for the purpose of this research. The objectives of the study were explained to the parents/guardians of the children. The entire interview was done in private, and the identities of the children and personal details were kept strictly confidential. Identity codes were used for the data collection tool and the sample bottle in order to keep the data anonymous.

3.15 Materials

Supplies and equipment: Stationery such as printing paper, manila paper, envelopes, printing ink, staplers, stapling pins, paper punch, files, pens, storage space, photocopying, a computer, access to the Internet and canvases for preparing poster presentations.

Personnel: The principal investigator, two research assistants, one phlebotomist and one laboratory technician.

Training procedure: In order to control for inter-examiner variability the research assistants were trained to perform the clinical examination and on how to administer the questionnaire to both the study and control groups by the principal investigator. An interview guide was also used. The expected responses were explained to them. Successful training was confirmed by occasional supervision of administration of the questionnaire by the Principal investigator during the pre-testing phase.

The role of the principal investigator was to train and supervise the research assistants, coordinate with the laboratory technician and ensure quality and reproducibility is maintained throughout the study.

3.16 Expected Application of Results

The findings and recommendations of this study will be disseminated to scientific fora, stakeholders in the health sectors and published in reputable medical journals. It will help inform and improve knowledge in the possible causes of hypospadias in African children. The study is also expected to serve as a baseline for those who may wish to make further research on the area.

3.17 Limitations of the Study

The study was done on patients who have been exposed to the environment, some for up to 12 years. These factors may affect hormone levels and were not being considered. Since these children will be seen at Kenyatta National Hospital which is a national referral facility, then these children will be coming from different environments. It was therefore difficult to control for the environment

4.0 CHAPTER FOUR: RESEARCH FINDINGS

4.1 Introduction

The findings of the study are presented in this chapter. The main objective of the study was to compare the Testosterone: Dihydrotestosterone ratio in children with hypospadias with a control group at the Kenyatta National Hospital. A total of 42 patients (21 cases and 21 controls) were examined between the months of January 2019 to February 2019.

4.2 Patient characteristics

This section describes the patient characteristics who received treatment at the Kenyatta National Hospital. Means and standard deviations are presented as Mean (SD) where applicable.

Table 1: Patient characteristics

The characteristics of the patients is as shown by the table below.

	Frequency n (%)	
	Cases	Controls
Residency		
Urban	13 (61.9)	
Rural	8 (38.1)	
Age		
<1 month	1 (4.8)	0 (0.0)
1-5 months	1 (4.8)	3 (14.3)
6-24 months	9 (42.9)	7 (33.3)
2-5 years	8 (38.1)	8 (38.1)
6-9 years	2 (9.5)	2 (9.5)
10-11 years	0 (0.0)	1 (4.8)
Family history		
Yes	3 (14.3)	
No	18 (85.7)	
Meatal location		
Glanular	3 (14.3)	
Midshaft	3 (14.3)	
Penoscrotal	7 (33.3)	
Perineal	1 (4.8)	
Subcoronal	7 (33.3)	
Chordee		
Absent	5 (23.8)	
Mild	7 (33.3)	
Moderate	2 (9.5)	
Severe	7 (33.3)	
Scrotal Anatomy		
Normal	15 (71.4)	

Bifid	6 (28.6)	
Preoperative/Postoperative		
Preoperative	16 (76.2)	
Postoperative	5 (23.8)	

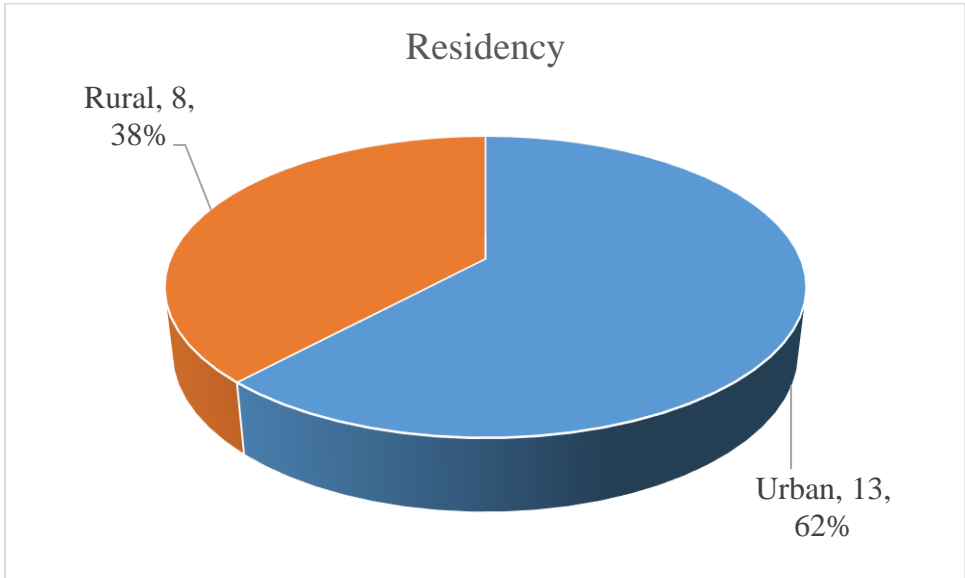


Figure 1: Residency

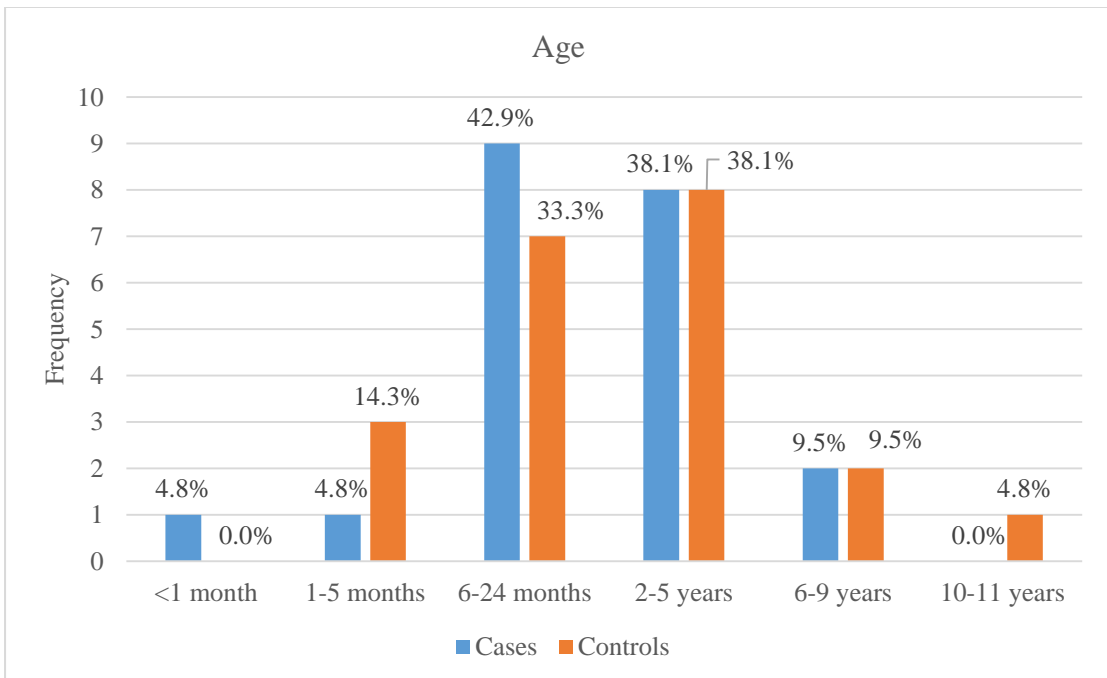


Figure 2: Age

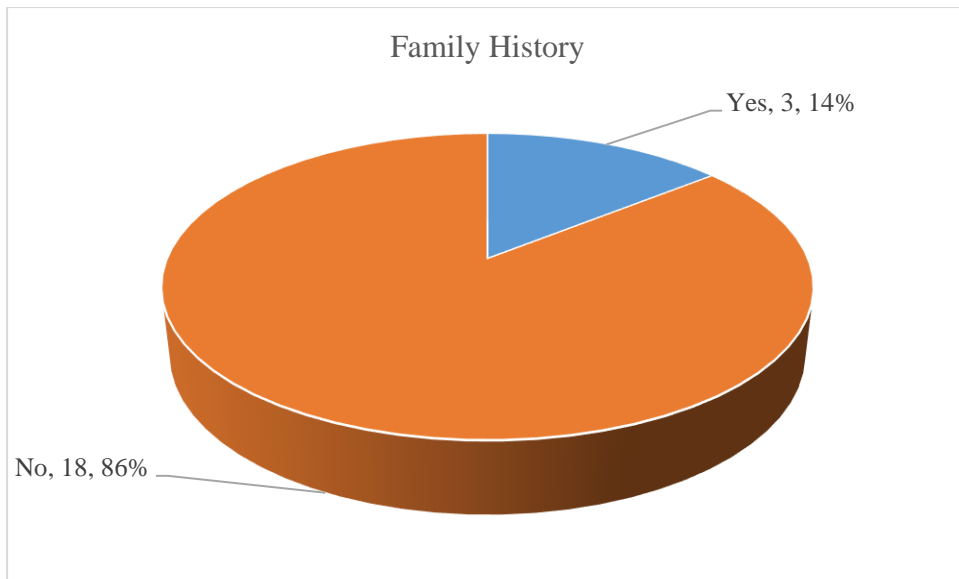


Figure 3: Family History

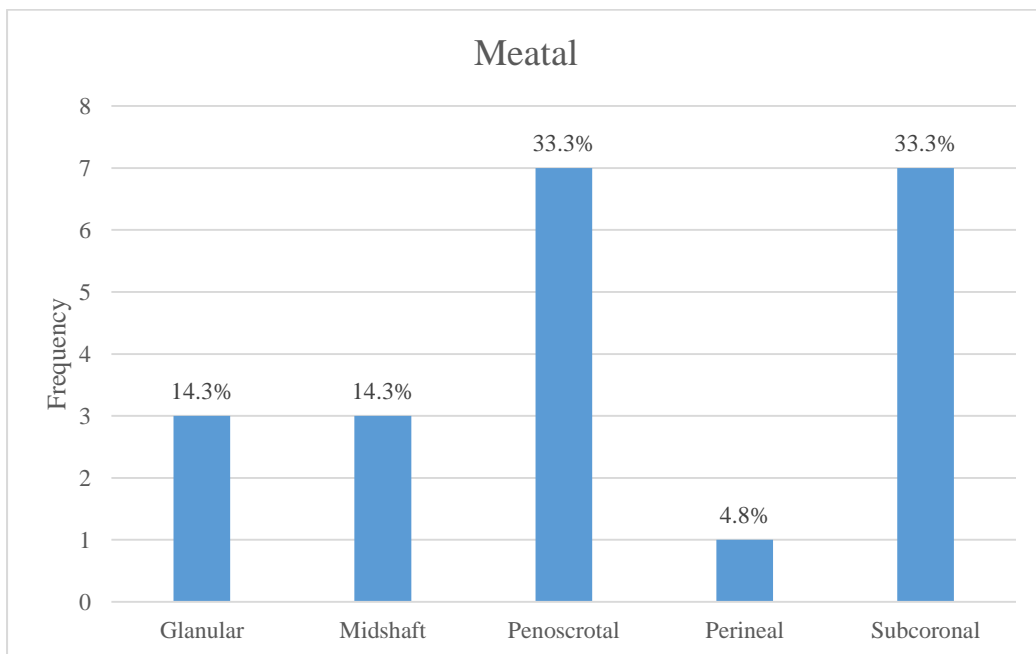


Figure 4: Meatal Location

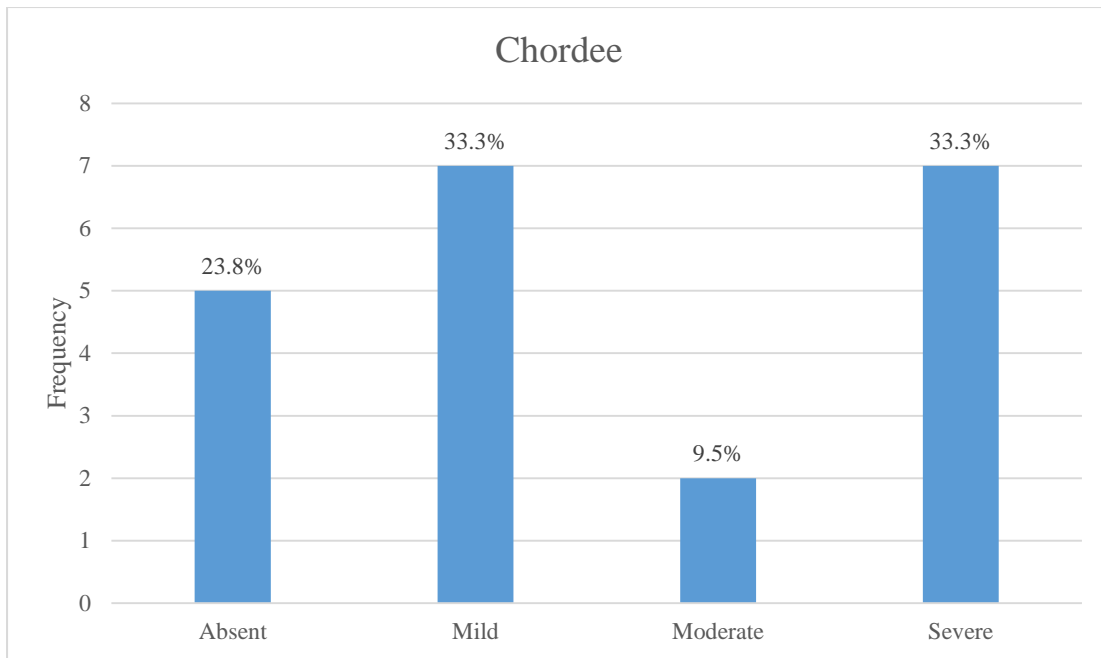


Figure 5: Chordee

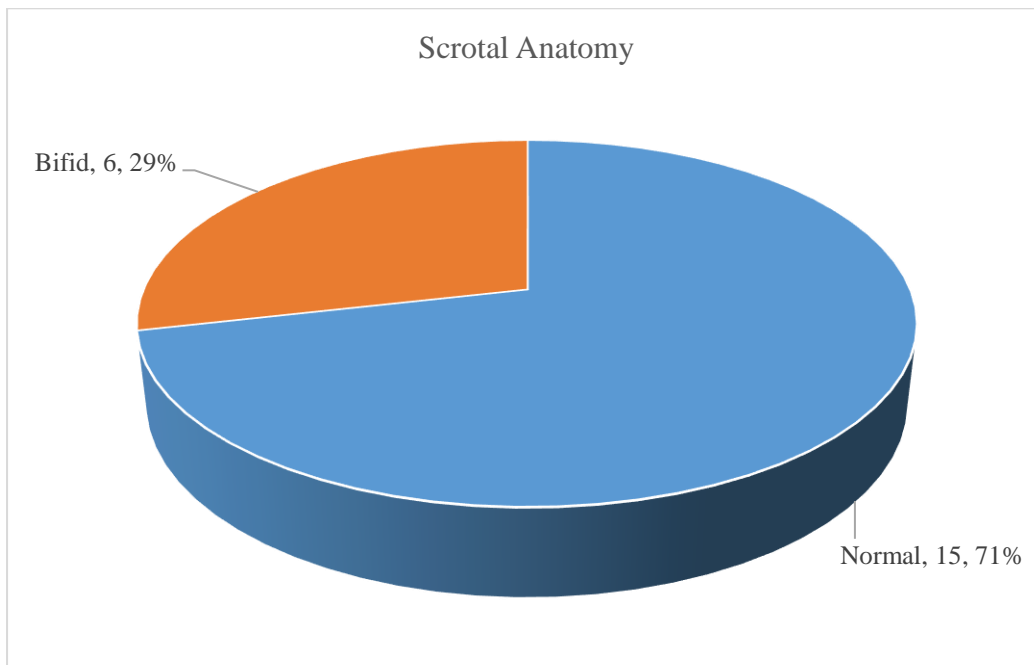


Figure 6: Scrotal Anatomy

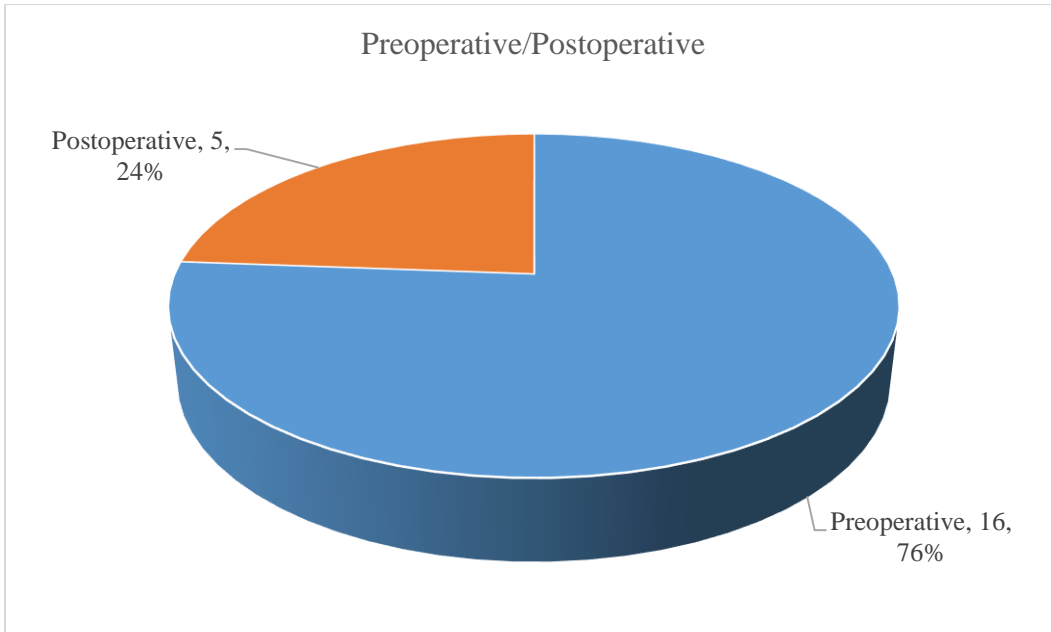


Figure 7: Preoperative/Postoperative

4.3 Testosterone and Dihydrotestosterone levels

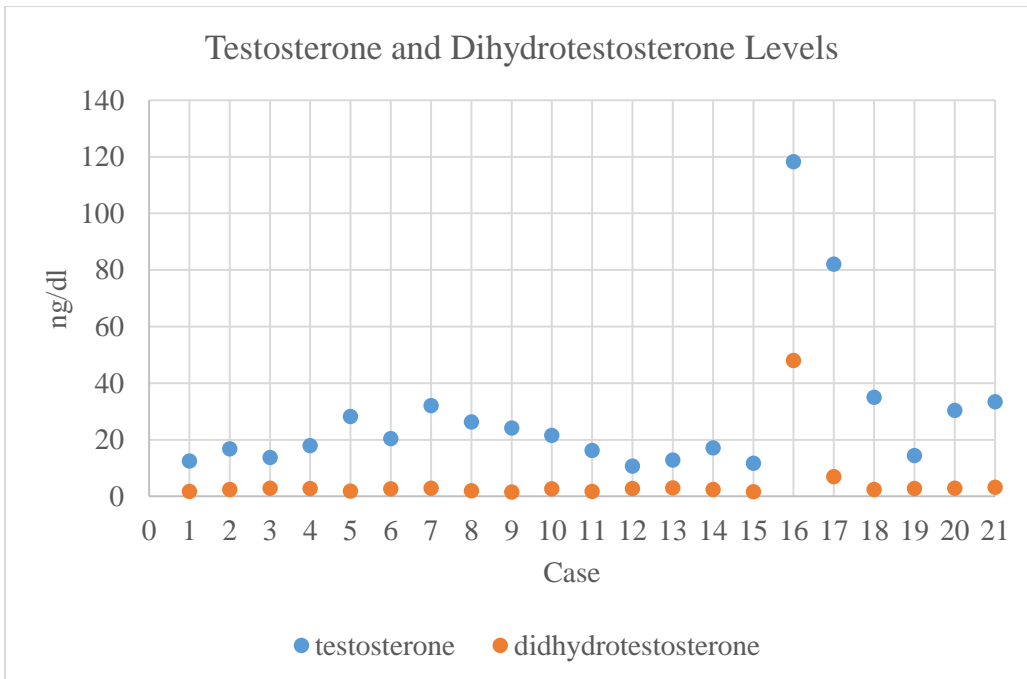


Figure 8: Levels of Testosterone and Dihydrotestosterone in children with hypospadias

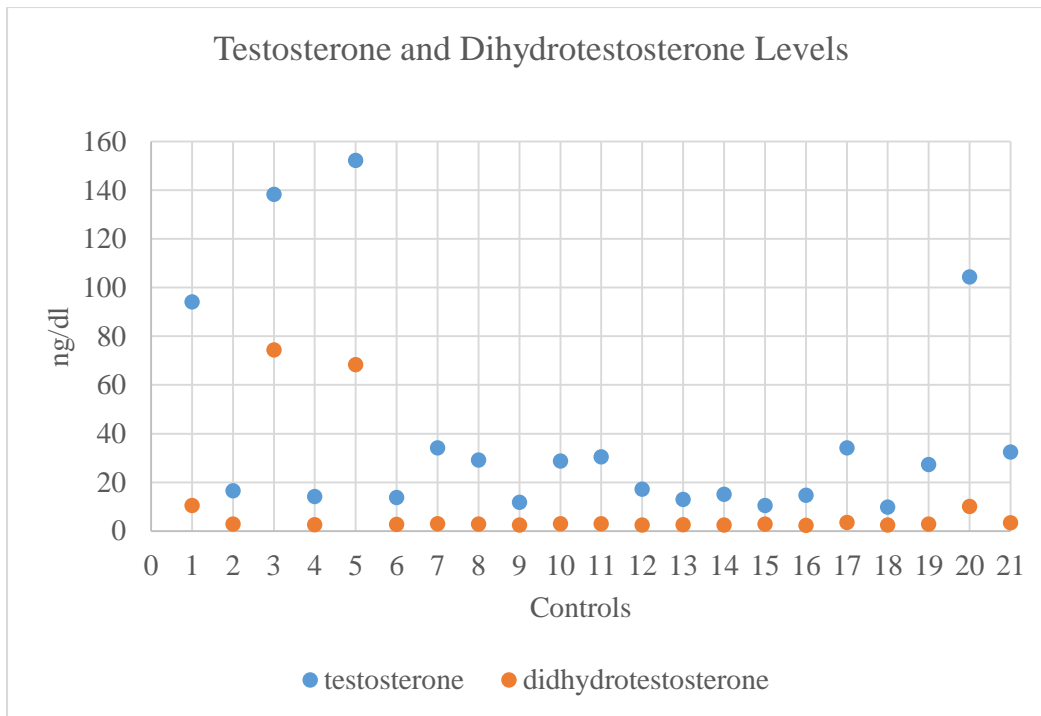


Figure 9: Levels of Testosterone and Dihydrotestosterone in the control group.

4.3.1 Testosterone levels

This section presents the results of the measures of testosterone levels in children with hypospadias and the control group.

Table 2: Testosterone Levels Results

The results of the testosterone levels in children with hypospadias and the control group is as shown by the table below.

	N	Mean	SD	p-value
Cases	21	28.4	25.7	0.290
Controls	21	40.1	43.0	

An independent-samples t-test was run to determine if there were differences in the testosterone levels in children with hypospadias and the control group. There were no statistical differences in the means of testosterone levels ($p = 0.290$)

4.3.2 Dihydrotestosterone levels

This section presents the results of the measures of dihydrotestosterone levels in children with hypospadias and the control group.

Table 3: Dihydrotestosterone Level Results

The results of the measures of dihydrotestosterone levels in children with hypospadias and the control group is as shown by the table below.

	N	Mean	SD	p-value
Cases	21	4.8	9.9	0.304
Controls	21	10.0	20.6	

An independent-samples t-test was run to determine if there were differences in the dihydrotestosterone levels in children with hypospadias and the control group. There were no statistical differences in the means of dihydrotestosterone levels ($p = 0.304$)

4.3.3 Testosterone: Dihydrotestosterone Ratio

This section presents the results of the measures of testosterone:dihydrotestosterone ratio in children with hypospadias and the control group.

Table 4: T:DHT Ratios

The results of the measures of testosterone:dihydrotestosterone ratio in children with hypospadias and the control group is as shown by the table below.

	N	Mean	SD	p-value
Cases	21	8.7	3.8	0.130
Controls	21	7.1	3.0	

An independent-samples t-test was run to determine if there were differences in the dihydrotestosterone ratio in children with hypospadias and the control group. There were no statistical differences in the means of testosterone:dihydrotestosterone ratio ($p = 0.130$).

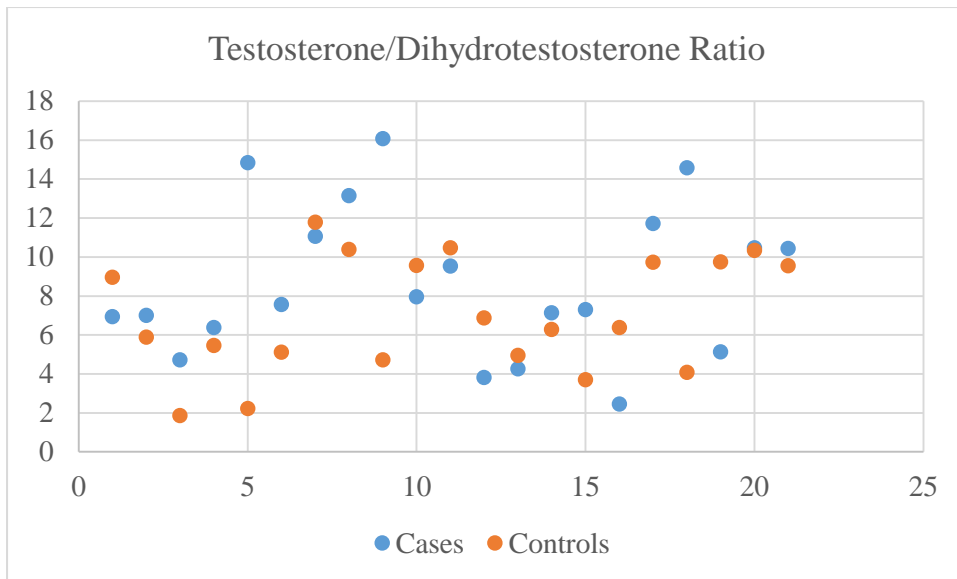


Figure 10: A scatter plot showing the T:DHT ratios in both the cases and controls

4.3.4 T:DHT ratios comparison between the proximal and distal hypospadias

Although subgroup analysis was not part of the objectives, this section was added to compare the means of proximal and distal hypospadias

Table 5: Comparing the means of the T:DHT ratios between proximal and distal hypospadias

	N	Mean	SD
Proximal Hypospadias	8	11.2	3.9
Distal Hypospadias	13	7.2	3.0

The sample size however was noted to be too small to infer any statistical significance.

5.0 CHAPTER FIVE: DISCUSSIONS, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The purpose of this study was to compare the Testosterone: Dihydrotestosterone ratio in children with hypospadias with a control group at the Kenyatta National Hospital. The study was led by the following objectives: to measure testosterone levels in children with hypospadias and the control group, to measure dihydrotestosterone levels in children with hypospadias and the control group., and to calculate the Testosterone: Dihydrotestosterone ratio in children with hypospadias and the control group.

A total of 42 patients were recruited for the study between January 2019 and February 2019. Out of these 21 were male children with hypospadias with no other congenital anomalies and 21 were male children with inguinal hernias and no other congenital anomalies. The children with inguinal hernias were used as a control group because development of inguinal hernias is not associated with any hormone deficiency.

In the case group i.e. children with hypospadias 61.9% came from urban areas while 38.1% came from rural areas. This would be expected as Kenyatta National Hospital (KNH) is located in Nairobi, so most patients would be from Nairobi and its environs. However, there was a significant number from rural areas as KNH is also a national referral hospital.

Most children with hypospadias were between 6-24 months (42.9%) and 2-5 years (38.1%). This is in keeping with the ideal time for surgery for hypospadias as reported by Manzoni et al [39].

The number of cases with a family history of hypospadias was at 14.3% compared to those without family history at 85.7%. This was slightly lower than noted in a study by Olivier M. et al where the family history was noted to be at 22.3% [40].

Of the hypospadias subtypes the commonest were subcoronal (33.3%) and penoscrotal (33.3%) followed by glanular (14.3%), midshaft (14.3%) and perineal (4.8%). This was in contrast to a study by Anikwe et al which showed anterior hypospadias to be at 72% [22].

76.2% of children with hypospadias had chordee while in 23.8% it was absent. Those with mild chordee were mainly associated with distal hypospadias while those with severe chordee were mainly associated with proximal hypospadias.

28.6% of the children with hypospadias had a bifid scrotum. These were only present in those with proximal hypospadias.

On the testosterone levels in children with hypospadias and the control group, the study found that there were no statistical differences ($p = 0.290$). However when comparing the mean testosterone levels in the two groups; 28.4ng/dl (S.D = 25.7) in the cases group and 40.1ng/dl (S.D = 43.0) in the controls group, it could be noted that the children with hypospadias tend to have a lower testosterone level when compared to the controls. Testosterone levels however vary markedly in different age groups and since the cases and controls were not matched by age it would be difficult to draw such an inference.

On the dihydrotestosterone levels in children with hypospadias and the control group, the study found that there were no statistical differences ($p = 0.304$). However when comparing the mean dihydrotestosterone levels in the two groups; 4.8ng/dl (S.D = 9.9) in the cases group and 10.0ng/dl (S.D = 20.6) in the controls group, it could be noted that the children with hypospadias tend to have a lower dihydrotestosterone level when compared to the controls. Dihydrotestosterone levels however vary markedly in different age groups and since the cases and controls were not matched by age it would be difficult to draw such an inference.

On the calculated mean of the testosterone:dihydrotestosterone (T:DHT) ratio the mean T:DHT ratio in children with hypospadias was at 8.7 (S.D = 3.8) while the T:DHT ratio in the control group was at 7.1 (S.D = 3.0). However there were no statistical differences in the means of testosterone:dihydrotestosterone ratio ($p = 0.130$). This shows that in this particular cohort there was no deficiency or defect in the enzyme type 2 alpha reductase which is responsible for conversion of testosterone to dihydrotestosterone. This was in keeping with a study done by Gearhart et al which didn't show any deficiency in activity of the enzyme 5 alpha reductase [28]. The pathology could then be at the level of DHT receptors responsible for its actions [8]. There could also be chemical modifiers from the environment which act as competitive inhibitors at the DHT receptors level [1,2].

One coincidental finding was that the mean T:DHT ratios in patients with proximal hypospadias (11.2 (S.D = 3.9) (N = 8)) was slightly higher than that in distal hypospadias (7.2 (S.D = 3.0) (N = 13)). However due to the small sample size a subgroup analysis would be beyond the scope of this study so a further similar study is recommended concentrating on proximal hypospadias.

5.2 Conclusions

Since there was no statistical significance in the testosterone: dihydrotestosterone ratio between the case and control groups, the study concludes that children with isolated hypospadias in our environment may not have a deficiency or defect in the enzyme type 2 5-alpha reductase. The hypospadias could be due to a defect in DHT receptors at the point of activity i.e. in the urethra or there could be other environmental chemical modifiers which act as competitive inhibitors at the DHT receptors.

5.3 Recommendations for Further Research

Due to the small sample size, the study is not conclusive. So another study with larger numbers with subgroup analysis is recommended.

Since this study didn't establish a deficiency in the enzyme type 2 5-alpha reductase other causes such as in-utero exposure to environmental chemical modifiers could also be looked at to establish other possible causes of hypospadias.

The functional activity of DHT receptors in male urethras/ penises of children with hypospadias could also be analysed to assess for abnormal physiology of the DHT receptors.

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APPENDICES

APPENDIX A: BUDGET AND BUDGETARY CONSIDERATIONS:

Budget Item	Amount (Kshs)
Research fee for KNH-ERC	2,000/=
Statistician consultation fee	30,000/=
Purchase of reagents	102,530/=
Laboratory fees	50,000/=
Stationery; (a) Printing (b) Photocopying (c) Binding (d) Pens	15,000/= 6,000/= 32,000/= 500/=
Research assistants fee @20,000 each (three assistants)	60,000/=
Contingency fund	20,000/=
Total	318,030/=

APPENDIX B: TIME LINES

Activity	July 2018	Aug 2018	Sept 2018	Oct 2018	Nov 2018	Dec 2018	Jan 2019	Feb 2019	Mar 2019
Proposal development									
Ethical approval									
Data collection									
Data analysis									
Dissertation submission									

APPENDIX C

INFORMED CONSENT FORM:

This Informed Consent form is for parents/guardians of male African children with hypospadias and will be administered to the eligible children's parents or guardians. We are requesting these patients to participate in this research project whose title is "**COMPARISON OF TESTOSTERONE: DIHYDROTESTOSTERONE RATIO IN CHILDREN WITH HYPOSPADIAS TO A CONTROL GROUP AT KENYATTA NATIONAL HOSPITAL**".

Principal Investigator: Dr. Bagha Mohamed Suhayl

Institution: Department of Surgery, School of Medicine, University of Nairobi.

This Informed Consent Form has three parts:

- 1) Information Sheet (to share information about the research with you).
- 2) Certificate of Consent (for signatures if you agree to take part).
- 3) Statement by the researcher/person taking consent.

You will be given a copy of the full informed consent form.

PART I: Information Sheet

Introduction

My name is Dr. Bagha Mohamed Suhayl, a post graduate student in General Surgery at the University of Nairobi. I am carrying out a research to determine the ratio of testosterone to dihydrotestosterone in African children with hypospadias.

Purpose of the research

Hypospadias is a major problem in male children occurring in 1 in 125 male newborns worldwide. Various theories have been postulated as the cause but none established. No study has been done on African children to establish the cause. I am doing this study to find out if levels of male hormones i.e testosterone and its metabolite Dihydrotestosterone have an impact on development of hypospadias.

I am going to give you information and invite you to be a participant in this research. There may be some words that you may not understand. Please ask me to clarify as we go through

the information and I will explain. After receiving the information concerning the study, you are encouraged to seek clarification in case of any doubt.

Type of Research Intervention

This research will involve asking relevant questions, examination of your child and medical records with your doctor's permission [or their representative] to establish type of hypospadias and other congenital anomalies. Four (4)mls of blood will then be drawn by a well-trained health practitioner and sent to the laboratory to assess the levels of testosterone and dihydrotestosterone.

Voluntary participation/right to refuse or withdraw

Your participation is entirely voluntary. Whether you decide to participate in this research or not, all the services that you receive at this hospital will continue and there will be no change. If you choose not to participate, you will still be offered the treatment that is routinely offered in this hospital for your condition. You have a right to refuse or withdraw your participation in this study at any point.

Confidentiality

The information obtained will be treated with confidentiality and only be available to the principal investigator and the study team. The child's name will not be used. Any information about him will have a number on it instead of his name. We will not share the identity of those participating in this research with anyone else.

Sharing the results

The knowledge that we get from this study will be shared with other research institutions and doctors through publications and conferences. Confidential information will not be shared.

Benefits

You may get no direct benefit from the information you provide for this study. However, the results will greatly contribute towards the advancement of health science by providing knowledge on possible cause of hypospadias in our society and further research on how to prevent it.

Risks

There are no direct risks anticipated in this study as it only seeks to measure levels of hormones in blood.

Cost and compensation

There will be no extra cost incurred for participating in this study nor will there be any compensation offered. This proposal has been reviewed and approved by UoN/KNH Ethics Committee, which is a Committee whose task is to make sure that research participants are protected from harm.

Who to contact

If you wish to ask any questions later, you may contact:

1. Principal Researcher:

Dr. Bagha Mohamed Suhayl,

Department of Surgery, School of Medicine, University of Nairobi

P.O. Box 19676-00202 KNH, Nairobi..

Mobile no. 0721349759

2. University of Nairobi Supervisors:

1. PROF. PETER .W. NDAGUATHA

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P.O. Box 480-00202 KNH, Nairobi, Kenya

3. Kenyatta National Hospital Supervisors

1. DR. HAMDUN SAID HAMDUN

MB.CHB, M.MED (Surg.UON),

Consultant Paediatric Surgeon: Kenyatta National Hospital,

P.O Box 2063-00202 KNH, Nairobi, Kenya

If you have any ethical concerns, you may contact:

Secretary, UON/KNH-ERC,

P.O. Box 20723- 00202,

KNH, Nairobi.

Tel: 020-726300-9

Email: KNHplan@Ken.Healthnet.org

PART II: Certificate of Consent

I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate as a participant in this research.

Name of child _____

Name of Parent/Guardian _____

Signature of Parent/Guardian _____

Date _____

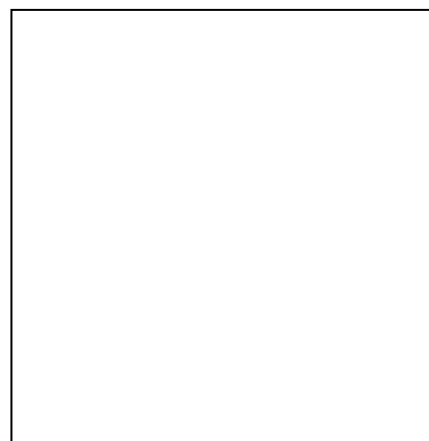
If Non -literate:

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Thumb print of Parent/Guardian.

Signature of witness _____

Date _____



PART III: Statement by the researcher

I have accurately read out the information sheet to the participant, and to the best of my ability made sure that the participant understands that the following will be done:

- Refusal to participate or withdrawal from the study will not in any way compromise the care of treatment.
- All information given will be treated with confidentiality.
- The results of this study might be published to facilitate better understanding of hypospadias in children.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Name of researcher/person taking consent _____

Signature of researcher/person taking consent _____

Date _____

FOMU YA MAKUBALIANO.

Fomu hii ya makubaliano ni kwa ajili ya wazazi au walezi wa watoto wa kiume wenye ugonjwa wa hypospadias watakaopewa ili wakubali watoto wao wahusike katika utafiti huu.

Tunakusishi ukubali mtoto wako ashiriki katika utafiti huu wa maarifa ambao anwani yake ni:

“KULINGANISHA UWIANO WA TESTOSTERONE NA DIHYDROTESTOSTERONE KATIKA WATOTO WALIO NA UGONJWA WA HYOSPADIAS NA WALE WASIOKUWA NAYO KATIKA HOSPITALI YA TAIFA YA KENYATTA”

Mtafiti mkuu: Dkt. Bagha Mohamed Suhayl

Chuo Kikuu cha Nairobi,

Kitivo cha utabibu.

Fomu hii ina sehemu tatu:

- 1) Habari itakayo kusaidia kukata kauli
- 2) Fomu ya makubaliano (utakapo weka sahihi)
- 3) Ujumbe kutoka kwa mtafiti

Utapewa nakala ya fomu hii.

SEHEMU YA KWANZA: Ukurasa wa habari

Kitambulizi

Mimi ni daktari Bagha Mohamed Suhayl, ninayesomea uzamili katika idara ya upasuaji Chuo Kikuu cha Nairobi. Ninafanya utafiti kwa anwani ya: **“Comparison of testosterone: dihydrotestosterone ratio in children with hypospadias to acontrol group at Kenyatta National Hospital”**. Inayo tafsiriwa kuwa **‘Kulinganisha uwiano wa Testosterone na Dihydrotestosterone katika watoto wa walio na ugonjwa wa hypospadias na wasiokuwa nayo katika Hospitali ya Taifa ya Kenyatta.’**

Lengo kuu la utafiti.

Ugonjwa wa hypospadias ni shida kuu inayopatikana katika vijana wa kiume na hudhuru 1 kati ya vijana 125 wanaozaliwa duniani. Kuna maelezo mengi yaliyozinduliwa lakini hakuna lililothibitishwa kueleza linalosababisha hypospadias. Hakuna utafiti uliofanywa kwa watoto wa kiafrika.

Ninafanya utafiti huu kujaribu kutambua kama viwango vya homoni za kiume za testosterone na dihydrotestosterone zinaweza kuathiri kutokea kwa hypospadias.

Nitakupa ujumbe kuhusu utafiti huu kasha nikupe fomu utakayojaza kama kibali cha kujiunga kwa utafiti. Iwapo kuna baadhi ya mambo hutaelewa, una uhuru wa kuuliza kwa maelezo zaidi.

Aina ya utafiti.

Utafiti huu utahusu kujibu maswali kupitia kwa dodoso, kupima mtoto wako na pia kudurusu hifadhi ya jumbe za afya yake kulingana na hiari ya daktari wake ili kutambua aina ya hypospadias na kuweko na ugonjwa maumbile mwingine. Millilita tano za damu zitatolewa na kupelekwa kwa mahabara ambapo viwango vya testosterone na dihydrotestosterone vitapimwa.

Haki ya kukataa utafiti

Kushiriki kwako kwa utafiti huu ni kwa hiari yako. Una uhuru wa kukataa kushiriki, na kukataa kwako hakutatumiwa kukunyima tiba. Unayo haki ya kujitoa katika utafiti wakati wowote unapoamua.

Taadhima ya siri

Ujumbe kuhusu majibu yako yatahifadhiwa. Ujumbe kuhusu ushiriki wako katika utafiti huu waweza kupatikana na wewe na wanaoandaa utafiti na wala si yeyote mwingine. Jina lako halitatumika bali ujumbe wowote kukuhusu utapewa nambari badili ya jina lako.

Hatari unayoweza kupata

Hakuna hatari yoyote ambayo yaweza kutokea kwa sababu ya kuhusishwa kwa utafiti huu. Mtoto wako atatolewa tu damu kidogo ili kupima viwango vya homoni.

Hifadhi ya matokeo.

Matokeo ya utafiti huu yatachapishwa kwa nukuu mbali mbali za sayansi kupitia kwa idhini ya mtafiti mkuu. Nakala za chapisho zitahifadhiwa katika idara ya upasuaji, chuo kikuu cha Nairobi na katika maktaba ya sayansi za Afya, kitivo cha utabibu. Hivyo basi, matokeo ya utafiti huu hayatasambazwa kwa umma au jukwaa lisiloidhinishwa kihalali. Ujumbe ulio kwa dodoso hautahifadhiwa baada ya uchanganuzi wa matokeo.

Gharama au fidia.

Utafiti huu hautakugharimu zaidi ya matibabu yako ya kawaida. Vilevile, hakuna malipo yoyote au fidia utakayopokea kutokana na kujiunga kwako katika utafiti huu. Muda wako ndio utakaotumiwa wakati wa mahojiano

SEHEMU YA PILI: Fomu ya makubaliano

Nimeelezwa utafiti huu kwa kina. Nakubali kushiriki katika utafiti huu kwa hiari yangu. Nimepata wakati wa kuuliza maswali na nimeelewa kuwa iwapo nina maswali zaidi, ninaweza kumwuliza mtafiti mkuu au watafiti waliotajwa hapa juu.

Jina la Mshiriki _____

Jina la Mzazi/Mlezi _____

Sahihi ya Mzazi/Mlezi _____

Tarehe _____

Kwa wasioweza kusoma na kuandika:

Nimeshuhudia usomaji na maelezo ya utafiti huu kwa mshiriki. Mshiriki amepewa nafasi ya kuuliza maswali. Nathibitisha kuwa mshiriki alipeana ruhusa ya kushiriki bila ya kulazimishwa.

Jina la shahidi _____

Alama ya kidole cha gumba
cha Mzazi/Mlezi

Sahihi la shahidi _____

Tarehe _____



Anwani za Wahusika

Ikiwa uko na maswali ungependa kuuliza baadaye, unaweza kuwasiliana na:

1. Mtafiti mkuu:

Dkt. Bagha Mohamed Suhayl,
Idara ya upasuaji, Shule ya Afya, Chuo Kikuu cha Nairobi,
SLP 19676 KNH, Nairobi 00202.
Simu: 0721 349 759

2. Wahadhiri husika:

1. PROFESSA P.L.W NDAGUATHA

(MB.Ch.B, MMED (Gen Surg.) UON, Fellow (Urology)U.K, FCS(ECSA)
S.L.P 19676-00202 KNH, KNH, Nairobi 00202

2. DKT. FRANCIS OSAWA

MB.CHB, M.MED (Gen. Surg. UON),
S.L.P 480-00202 KNH, Nairobi.

3. DKT. HAMDUN SAID HAMDUN

MB.CHB, M.MED (Gen. Surg. UON),
S.L.P 2063-00202 KNH, KNH, Nairobi 00202

Wahusika wa maslahi yako katika Utafiti:

- Karani,
KNH/UoN-ERC
SLP 20723-00202 KNH, Nairobi.

SEHEMU YA TATU: Ujumbe kutoka kwa mtafiti

Nimemsomea mshiriki ujumbe kiwango ninavyoweza na kuhakikisha kuwa mshiriki amefahamu yafuatayo:

- Kutoshiriki au kujitoa kwenye utafiti huu hakutadhuru kupata kwake kwa matibabu.
- Ujumbe kuhusu majibu yake yatahifadhiwa kwa siri.
- Matokeo ya utafiti huu yanaweza chapishwa kusaidia utambuzi wa shida ya hypospadias katika watoto.

Ninathibitisha kuwa mshiriki alipewa nafasi ya kuuliza maswali na yote yakajibiwa vilivyo. Ninahakikisha kuwa mshiriki alitoa ruhusa bila ya kulazimishwa.

Mshiriki amepewa nakala ya hii fomu ya makubaliano.

Jina la mtafiti

Sahihi ya Mtafiti

Tarehe

INFORMED CONSENT FORM FOR THE CONTROL GROUP:

This Informed Consent form is for parents/guardians of male children with inguinal hernias who will be used as a control group and will be administered to the eligible children's parents or guardians. We are requesting these patients to participate in this research project whose title is **“COMPARISON OF TESTOSTERONE: DIHYDROTESTOSTERONE RATIO IN CHILDREN WITH HYPOSPADIAS TO A CONTROL GROUP AT KENYATTA NATIONAL HOSPITAL”**.

Principal Investigator: Dr. Bagha Mohamed Suhayl

Institution: Department of Surgery, School of Medicine, University of Nairobi.

This Informed Consent Form has three parts:

- 4) Information Sheet (to share information about the research with you).
- 5) Certificate of Consent (for signatures if you agree to take part).
- 6) Statement by the researcher/person taking consent.

You will be given a copy of the full informed consent form.

PART I: Information Sheet

Introduction

My name is Dr. Bagha Mohamed Suhayl, a post graduate student in General Surgery at the University of Nairobi. I am carrying out a research to determine the ratio of testosterone to dihydrotestosterone in male children with hypospadias.

Purpose of the research

Hypospadias is a major problem in male children occurring in 1 in 125 male newborns worldwide. Various theories have been postulated as the cause but none established. No study has been done on African children to establish the cause. I am doing this study to find out if levels of male hormones i.e testosterone and its metabolite Dihydrotestosterone have an impact on development of hypospadias.

Your child will be part of the control group whose ratio of Testosterone: Dihydrotestosterone will be compared to that in children with hypospadias.

I am going to give you information and invite you to be a participant in this research. There may be some words that you may not understand. Please ask me to clarify as we go through the information and I will explain. After receiving the information concerning the study, you are encouraged to seek clarification in case of any doubt.

Type of Research Intervention

This research will involve asking relevant questions, drawing of 4mls of blood by a well-trained health practitioner, sending the blood to the laboratory to assess the levels of testosterone and dihydrotestosterone and comparing the ratio of Testosterone: Dihydrotestosterone to that in children with hypospadias.

Voluntary participation/right to refuse or withdraw

Your participation is entirely voluntary. Whether you decide to participate in this research or not, all the services that you receive at this hospital will continue and there will be no change. If you choose not to participate, you will still be offered the treatment that is routinely offered in this hospital for your condition. You have a right to refuse or withdraw your participation in this study at any point.

Confidentiality

The information obtained will be treated with confidentiality and only be available to the principal investigator and the study team. The child's name will not be used. Any information about him will have a number on it instead of his name. We will not share the identity of those participating in this research with anyone else.

Sharing the results

The knowledge that we get from this study will be shared with other research institutions and doctors through publications and conferences. Confidential information will not be shared.

Benefits

You may get no direct benefit from the information you provide for this study. However, the results will greatly contribute towards the advancement of health science by providing knowledge on possible cause of hypospadias in our society and further research on how to prevent it.

Risks

There are no direct risks anticipated in this study as it only seeks to measure levels of hormones in blood.

Cost and compensation

There will be no extra cost incurred for participating in this study nor will there be any compensation offered. This proposal has been reviewed and approved by UoN/KNH Ethics Committee, which is a Committee whose task is to make sure that research participants are protected from harm.

Who to contact

If you wish to ask any questions later, you may contact:

3. Principal Researcher:

Dr. Bagha Mohamed Suhayl,

Department of Surgery, School of Medicine, University of Nairobi

P.O. Box 19676-00202 KNH, Nairobi..

Mobile no. 0721349759

4. University of Nairobi Supervisors:

3. PROF. PETER .W. NDAGUATHA

MB.CHB, M.MED (Surg.UON), FELLOW UROLOGY. (UK), FCS (ECSA),

Associate Professor, Chairman: Department of Surgery: University Of Nairobi.

P.O. Box 19676-00202 KNH, Nairobi, Kenya

4. DR. FRANCIS OSAWA

MB.CHB, M.MED (Surg.UON),

Lecturer: Department of Surgery: University Of Nairobi.

P.O. Box 480-00202 KNH, Nairobi, Kenya

3. Kenyatta National Hospital Supervisors

1. DR. HAMDUN SAID HAMDUN

MB.CHB, M.MED (Surg.UON),

Consultant Paediatric Surgeon: Kenyatta National Hospital,

P.O Box 2063-00202 KNH, Nairobi, Kenya

If you have any ethical concerns, you may contact:

Secretary, UON/KNH-ERC,

P.O. Box 20723- 00202,

KNH, Nairobi.

Tel: 020-726300-9

Email: KNHplan@Ken.Healthnet.org

PART II: Certificate of Consent

I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate as a participant in this research.

Name of child _____

Name of Parent/Guardian _____

Signature of Parent/Guardian _____

Date _____

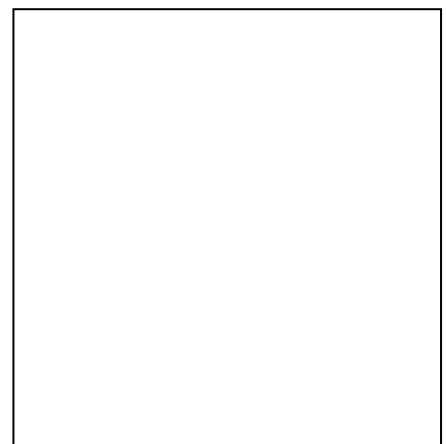
If Non -literate:

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Thumb print of Parent/Guardian.

Signature of witness _____

Date _____



PART III: Statement by the researcher

I have accurately read out the information sheet to the participant, and to the best of my ability made sure that the participant understands that the following will be done:

- Refusal to participate or withdrawal from the study will not in any way compromise the care of treatment.
- All information given will be treated with confidentiality.
- The results of this study might be published to facilitate better understanding of hypospadias in children.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Name of researcher/person taking consent _____

Signature of researcher/person taking consent _____

Date _____

FOMU YA MAKUBALIANO KWA KUNDI LA KUDHIBITI:

Fomu hii ya makubaliano ni kwa ajili ya wazazi au walezi wa watoto wa kiume wenye ugonjwa wa mshipa wa ngiri, watakaokuwa katika kundi la kudhibiti, watakaopewa ili wakubali watoto wao wahusike katika utafiti huu.

Tunakusihii ukubali mtoto wako ashiriki katika utafiti huu wa maarifa ambao anwani yake ni:
“KULINGANISHA UWIANO WA TESTOSTERONE NA DIHYDROTESTOSTERONE KATIKA WATOTO WALIO NA UGONJWA WA HYPOSPADIAS NA WALE WASIOKUWA NAYO KATIKA HOSPITALI YA TAIFA YA KENYATTA”

Mtafiti mkuu: Dkt. Bagha Mohamed Suhayl

Chuo Kikuu cha Nairobi,

Kitivo cha utabibu.

Fomu hii ina sehemu tatu:

- 4) Habari itakayo kusaidia kukata kauli
- 5) Fomu ya makubaliano (utakapo weka sahihi)
- 6) Ujumbe kutoka kwa mtafiti

Utapewa nakala ya fomu hii.

SEHEMU YA KWANZA: Ukurasa wa habari

Kitambulizi

Mimi ni daktari Bagha Mohamed Suhayl, ninayesomea uzamili katika idara ya upasuaji Chuo Kikuu cha Nairobi. Ninafanya utafiti kwa anwani ya: **“Comparison of testosterone: dihydrotestosterone ratio in children with hypospadias to a control group at Kenyatta National Hospital”**. Inayo tafsiriwa kuwa **‘Kulinganisha uwiano wa Testosterone na Dihydrotestosterone katika watoto wa walio na ugonjwa wa hypospadias na wasiokuwa nayo katika Hospitali ya Taifa ya Kenyatta.’**

Lengo kuu la utafiti.

Ugonjwa wa hypospadias ni shida kuu inayopatikana katika vijana wa kiume na hudhuru 1 kati ya vijana 125 wanaozaliwa duniani. Kuna maelezo mengi yaliyozinduliwa lakini hakuna lililothibitishwa kueleza linalosababisha hypospadias. Hakuna utafiti uliofanywa kwa watoto wa kiafrika.

Ninafanya utafiti huu kujaribu kutambua kama viwango vya homoni za kiume za testosterone na dihydrotestosterone zinaweza kuathiri kutokea kwa hypospadias.

Mtoto wako atukuwa katika kundi la kudhibiti ambao viwango vyao vya homoni za testosterone na dihydrotestosterone zitalainganishwa na viwango vya watoto wenye ugonjwa wa hypospadias.

Nitakupa ujumbe kuhusu utafiti huu kasha nikupe fomu utakayojaza kama kibali cha kujiunga kwa utafiti. Iwapo kuna baadhi ya mambo hutaelewa, una uhuru wa kuuliza kwa maelezo zaidi.

Aina ya utafiti.

Utafiti huu utahusu kuulizwa maaswali kwa njia ya dodoso, kutolewa millilita nne (4) za damu na kupelekwa kwa mahabara ambapo viwango vya testosterone na dihydrotestosterone vitapimwa.

Haki ya kukataa utafiti

Kushiriki kwako kwa utafiti huu ni kwa hiari yako. Una uhuru wa kukataa kushiriki, na kukataa kwako hakutatumiwa kukunyima tiba. Unayo haki ya kujitoa katika utafiti wakati wowote unapoamua.

Taadhima ya siri

Ujumbe kuhusu majibu yako yatahifadhiwa. Ujumbe kuhusu ushiriki wako katika utafiti huu waweza kupatikana na wewe na wanaoandaa utafiti na wala si yeyote mwingine. Jina lako halitatumika bali ujumbe wowote kukuhusu utapewa nambari badili ya jina lako.

Hatari unayoweza kupata

Hakuna hatari yoyote ambayo yaweza kutokea kwa sababu ya kuhusishwa kwa utafiti huu. Mtoto wako atatolewa tu damu kidogo ili kupima viwango vya homoni.

Hifadhi ya matokeo.

Matokeo ya utafiti huu yatachapishwa kwa nukuu mbali mbali za sayansi kupitia kwa idhini ya mtafiti mkuu. Nakala za chapisho zitahifadhiwa katika idara ya upasuaji, chuo kikuu cha Nairobi na katika maktaba ya sayansi za Afya, kitivo cha utabibu. Hivyo basi, matokeo ya utafiti huu hayatasambazwa kwa umma au jukwaa lisiloidhinishwa kihalali. Ujumbe ulio kwa dodoso hautahifadhiwa baada ya uchanganuzi wa matokeo.

Gharama au fidia.

Utafiti huu hautakugharimu zaidi ya matibabu yako ya kawaida. Vilevile, hakuna malipo yoyote au fidia utakayopokea kutokana na kujiunga kwako katika utafiti huu. Muda wako ndio utakaotumiwa wakati wa mahojiano

SEHEMU YA PILI: Fomu ya makubaliano

Nimeelezwa utafiti huu kwa kina. Nakubali kushiriki katika utafiti huu kwa hiari yangu. Nimepata wakati wa kuuliza maswali na nimeelewa kuwa iwapo nina maswali zaidi, ninaweza kumwuliza mtafiti mkuu au watafiti waliotajwa hapa juu.

Jina la Mshiriki _____

Jina la Mzazi/Mlezi _____

Sahihi ya Mzazi/Mlezi _____

Tarehe _____

Kwa wasioweza kusoma na kuandika:

Nimeshuhudia usomaji na maelezo ya utafiti huu kwa mshiriki. Mshiriki amepewa nafasi ya kuuliza maswali. Nathibitisha kuwa mshiriki alipeana ruhusa ya kushiriki bila ya kulazimishwa.

Jina la shahidi _____

Alama ya kidole cha gumba
cha Mzazi/Mlezi

Sahihi la shahidi _____

Tarehe _____



Anwani za Wahusika

Ikiwa uko na maswali ungependa kuuliza baadaye, unaweza kuwasiliana na:

3. Mtafiti mkuu:

Dkt. Bagha Mohamed Suhayl,
Idara ya upasuaji, Shule ya Afya, Chuo Kikuu cha Nairobi,
SLP 19676 KNH, Nairobi 00202.
Simu: 0721 349 759

4. Wahadhiri husika:

1. PROFESSA P.L.W NDAGUATHA

(MB.Ch.B, MMED (Gen Surg.) UON, Fellow (Urology)U.K, FCS(ECSA)
S.L.P 19676-00202 KNH, KNH, Nairobi 00202

2. DKT. FRANCIS OSAWA

MB.CHB, M.MED (Gen. Surg. UON),
S.L.P 480-00202 KNH, Nairobi.

3. DKT. HAMDUN SAID HAMDUN

MB.CHB, M.MED (Gen. Surg. UON),
S.L.P 2063-00202 KNH, KNH, Nairobi 00202

Wahusika wa maslahi yako katika Utafiti:

- Karani,
KNH/UoN-ERC
SLP 20723-00202 KNH, Nairobi.

SEHEMU YA TATU: Ujumbe kutoka kwa mtafiti

Nimemsomea mshiriki ujumbe kiwango ninavyoweza na kuhakikisha kuwa mshiriki amefahamu yafuatayo:

- Kutoshiriki au kujitoa kwenye utafiti huu hakutadhuru kupata kwake kwa matibabu.
- Ujumbe kuhusu majibu yake yatahifadhiwa kwa siri.
- Matokeo ya utafiti huu yanaweza chapishwa kusaidia utambuzi wa shida ya hypospadias katika watoto.

Ninathibitisha kuwa mshiriki alipewa nafasi ya kuuliza maswali na yote yakajibiwa vilivyo. Ninahakikisha kuwa mshiriki alitoa ruhusa bila ya kulazimishwa.

Mshiriki amepewa nakala ya hii fomu ya makubaliano.

Jina la mtafiti

Sahihi ya Mtafiti

Tarehe

APPENDIX D

MINOR ASSENT FORM

Study title: “COMPARISON OF TESTOSTERONE: DIHYDROTESTOSTERONE RATIO IN CHILDREN WITH HYPOSPADIAS TO A CONTROL GROUP AT KENYATTA NATIONAL HOSPITAL”.

Principal Investigator: Dr. Bagha Mohamed Suhayl

Institution: Department of Surgery, School of Medicine, University of Nairobi.

I am doing a study where we will be measuring testosterone and dihydrotestosterone levels in your blood. The reason I am doing this study is to find out the cause of your condition (hypospadias).

We will draw 4mls of blood from your veins and take it to the laboratory. In the laboratory we will measure the levels of testosterone and dihydrotestosterone and calculate a ratio between the two.

You will feel a little pain when we will remove the blood but there will be no other harmful effects.

You can choose to participate in the study or not. If you choose not to participate then you will continue to receive the treatment you are currently receiving and there will be no change in your treatment.

When we are finished with this study we will write a report about what was learned. This report will not include your name or that you were in the study.

Your parents know about the study and have agreed for you to participate.

If you decide you want to be in this study, please sign your name.

I, _____, want to be in this research study.

(Sign your name here)

(Date)

ASSENT FORM FOR THE CONTROL GROUP

Study title: “COMPARISON OF TESTOSTERONE: DIHYDROTESTOSTERONE RATIO IN CHILDREN WITH HYPOSPADIAS TO A CONTROL GROUP AT KENYATTA NATIONAL HOSPITAL”.

Principal Investigator: Dr. Bagha Mohamed Suhayl

Institution: Department of Surgery, School of Medicine, University of Nairobi.

I am doing a study where we will be measuring testosterone and dihydrotestosterone levels in your blood. The reason I am doing this study is to find out the cause of your hypospadias.

The levels of these hormones in your blood will be compared to those in the blood of children with hypospadias.

We will draw 4mls of blood from your veins and take it to the laboratory. In the laboratory we will measure the levels of testosterone and dihydrotestosterone and calculate a ratio between the two.

You will feel a little pain when we will remove the blood but there will be no other harmful effects.

You can choose to participate in the study or not. If you choose not to participate then you will continue to receive the treatment you are currently receiving and there will be no change in your treatment.

When we are finished with this study we will write a report about what was learned. This report will not include your name or that you were in the study.

Your parents know about the study and have agreed for you to participate.

If you decide you want to be in this study, please sign your name.

I, _____, want to be in this research study.

(Sign your name here)

(Date)

APPENDIX E: DATA COLLECTION TOOL

Biodata

Date _____

Name _____

Age _____

Area of residency

Urban

Rural

Family history of hypospadias

Yes

No

Pathology

1. Meatal location

- a. Glanular
- b. Subcoronal
- c. Distal penis
- d. Midshaft
- e. Proximal penile
- f. Penoscrotal
- g. Perineal

2. Testicular descent

- a. Undescended
- b. Descended

3. Chordee + degree

- a. Present
 - i. Severe
 - ii. Moderate
 - iii. Mild
- b. Absent

- 4. Scrotal anatomy
- a. Normal
- b. Bifid

5. Other associated anomalies

INTERVENTION

- 1. Is the patient
- a. preoperative
- b. postoperative
- 2. Is the patient on oral or topical testosterone supplementation?
- a. Yes
- b. No

LAB VALUES

Testosterone levels _____ ng/dl

DHT levels _____ ng/dl

T: DHT ratio _____

DATA COLLECTION TOOL FOR THE CONTROL GROUP

Biodata

Date_____

Name_____

Age_____

Area of residency

Urban

Rural

LAB VALUES

Testosterone levels_____ng/dl

DHT levels_____ng/dl

T: DHT ratio _____

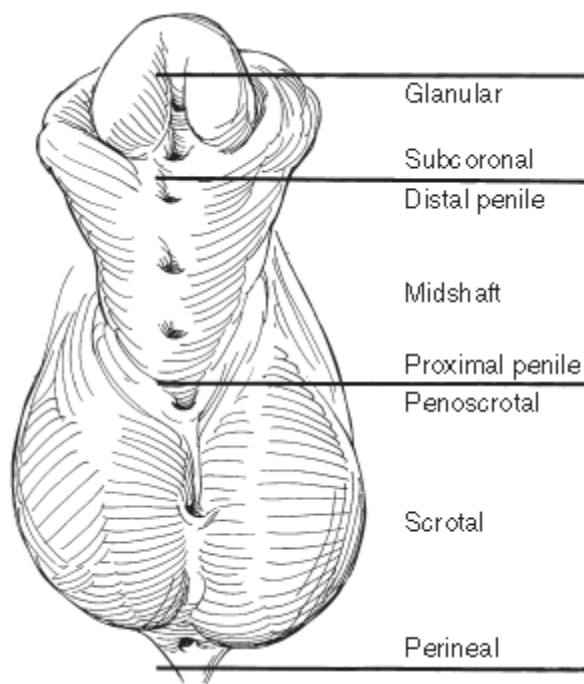
APPENDIX F

INTERVIEW GUIDE

The interviewer will use the following questions as a guide in filling the data collection tool.

1. What is the name of the child?
2. How old is he?
3. Is there anyone in your family who has a similar condition as the child i.e. hypospadias?
4. Has the child undergone any procedure/ surgery to correct the hypospadias?
5. Is the child on any testosterone supplement?

The following diagram will be used by the interviewer to determine the type of hypospadias and other associated pathologies.

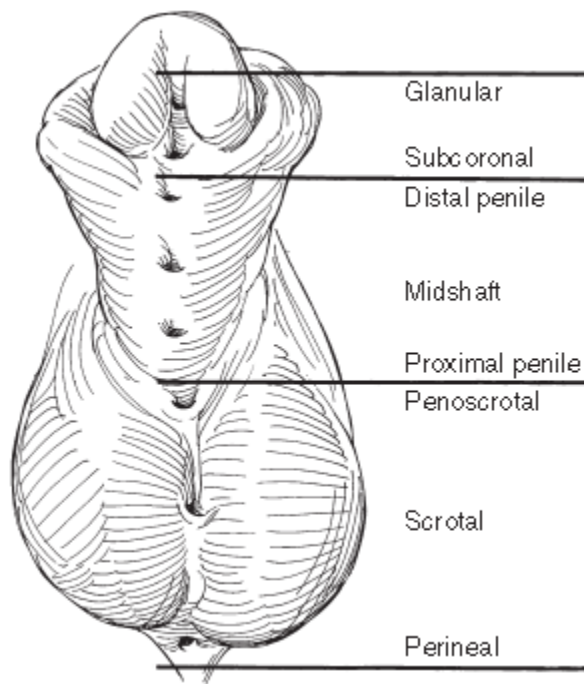


MWONGOZO WA MAHOJIANO

Mhoji atatumia maswali yafuatayo ili kumwongoza kujaza dodoso.

1. Jina la mtoto ni nini?
2. Mtoto ana miaka mingapi?
3. Je, kuna mtu yeyote katika jamii yenu mwenye shida ya hypospadias?
4. Je, mtoto amefanyiwa upasuaji wowote ili kurekebisha hiyo ugonjwa wa hypospadias?
5. Je, motto anatumia dawa zozote zilizo na uwiano wa testosterone?

Mhoji atatumia picha ifuatayo kumwongoza kutambua aina ya hypospadias.



APPENDIX G

DUMMY TABLES

Table 1

For Socio-demographic data

	Cases
Residence	Mean(S.D)
Urban	n(%)
Rural	n(%)

Table 2

For cases i.e patients with hypospadias

Patient code	Testosterone	DHT	T:DHT RATIO	Meatal location
AVERAGE T:DHT				

Table 3

For Controls

Patient code	Testosterone	DHT	T:DHT RATIO
AVERAGE T:DHT			

Table 4

	Case	Control
AVERAGE T:DHT		