HUMAN PAPILLOMA VIRUS GENOTYPES IN RECURRENT RESPIRATORY PAPILLOMATOSIS PATIENTS AT KENYATTA NATIONAL HOSPITAL

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Medicine in Otorhinolaryngology-Head & Neck Surgery, University of Nairobi

MARCH, 2022

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I declare that this dissertation is my own original work and has not been presented for a Degree in any other University.

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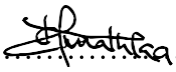
Signature……  …… Date: …29/08/2022……

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ABBREVIATIONS

AORRP- Adult onset recurrent respiratory papillomatosis

DNA - Deoxyribonucleic acid

ENT - Ear, Nose and Throat

HPV - Human papilloma virus

IFN - Interferon

JORRP- Juvenile onset recurrent respiratory papillomatosis

KNH - Kenyatta National Hospital

PCR - Polymerase chain reaction

RPM - Revolutions per minute

RRP - Recurrent respiratory papillomatosis

ABSTRACT

Background: Recurrent Respiratory Papillomatosis is an aggressive, recurrent, difficult to manage disease, caused by Human Papillomavirus. It affects both children and adults. Determination of the human papilloma virus genotype is of prognostic value in management of recurrent respiratory papillomatosis.

Objective: To determine human papilloma virus genotypes and correlate with severity of disease in patients with recurrent respiratory papillomatosis at Kenyatta National Hospital.

Study design: Hospital based cross-sectional study

Study site: The study was conducted at Kenyatta National Hospital Ear, Nose and Throat department, operating theatre and University of Nairobi Kenya Aids Vaccine Initiative-institute of clinical research laboratory.

Study Population: Patients diagnosed with recurrent respiratory papillomatosis.

Methodology: Forty patients diagnosed with recurrent respiratory papillomatosis were recruited. Patients history was taken. Two biopsy samples were taken, one for Human papillomavirus genotyping and the other for histopathology. Disease severity was classified as aggressive or non-aggressive using Doyle criteria.

Data analysis: SPSS version 23.0 statistical software was used to analyze data. Descriptive analysis was performed on all variables and summarized into frequency, tables and charts. Pearson chi square was used to test association between independent variables.

Results: There were 25(62.5%) male and 15(37.5%) female patients. HPV 6 was isolated in 67.5% patients and HPV 11 in 32.5% with no co-infection. The patients had an age range between 3 to 66 years where majority (92.5%) had JORRP and 7.5 % had AORRP. 40% had aggressive disease while 60% had non-aggressive disease. HPV 11 was found in 81.3% of patients with aggressive disease and HPV 6 in 18.8%. There was a positive correlation between HPV 11 and disease aggression ($p<0.001$).

Conclusion: HPV 6 is the most common (67.5%) HPV genotype in patients with RRP at KNH and HPV 11 is associated with aggressive disease.

1.0 CHAPTER ONE: INTRODUCTION

1.1 Background

Recurrent respiratory papillomatosis (RRP) is a disease of the airway mucosa. It affects both children and adults. Incidence in USA is estimated as 4.3/100000 in children and 1.8/100000 in adults [1]. Due to its involvement of the airway and a prolonged disease history, RRP poses fatal consequences. The disease burden is significant, necessitating frequent hospitalizations and surgical operations. This causes high financial and psychological burden to the patients. Financial burden in USA is estimated to be 150 million dollars annually [1]

The disease results from a persistent infection of the respiratory epithelium with human papillomavirus (HPV). The most commonly associated genotypes in disease pathogenesis are HPV genotypes 6 and 11. HPV 11 is the most virulent of the two genotypes and often lead to severe disease course while HPV 6 is the most common but less virulent [2]. The presence of any of the following criteria indicates aggressive disease: At least ten procedures in total, at least three procedures per year at any time, distal spread (involvement of the trachea or lung), and tracheostomy, Doyle *et al* [3]. Disease onset at young age in juveniles is associated with severe disease and subsequent clinical course is more aggressive in JORRP than in AORRP[2,3,4]

Exophytic lesions of connective tissue covered by epithelium, are the hallmarks of the disease. Although papillomas are most commonly found in the larynx, they can occur anywhere along the respiratory tract [1,5,6]. Depending on age, there are two types of RRP: juvenile onset recurrent respiratory papillomatosis (JORRP) and adult onset recurrent respiratory papillomatosis (AORRP). It is more prevalent in males than females [6,7,8]. RRP is frequently misdiagnosed as croup, bronchitis or asthma, resulting in late diagnosis and acute airway complications [6,9]

As a result, some patients require a tracheotomy for immediate relieve of obstruction [9].

A first born baby delivered vaginally by a mother less than 20 years with genital warts in pregnancy has been described as an important clinical triad and risk factors for vertical transmission of JORRP[10]. Vaginal delivery has the highest risk while caesarian section offers a degree of protection. This protection is not absolute as studies show there is a possibility of intrauterine transmission[18]. High number of sexual partners and oral sex are known risk factors for AORRP[10,11].

There is no cure for RRP at the moment. The goal of treatment is to keep the airways open and to improve the quality of the patient's voice. Surgical excision is the standard of care. Adjuvant therapies including interferons, cidofovir, HPV vaccine and immunomodulators are used in addition to surgical excision especially when surgery alone is inadequate in controlling the disease. Use of cidofovir in aggressive disease requiring frequent surgical excision every 3-4 months has shown good results by increasing interval between surgical procedures and remission in other cases [12]

1.2 Aetiology of RRP

The human papillomavirus (HPV), which belongs to the papoviridae family, causes RRP. It is a non-enveloped icosahedral virus with double stranded DNA. There are almost 100 known HPV genotypes. Based on the risk of malignant transformation of the accompanying lesions, these genotypes are divided into two categories: high risk and low risk.

Genotypes 16,18,31,33,35,45,51,52,56 are high risk, while 6,11,42,43,44 are low risk.

RRP is primarily caused by HPV 6 and 11. Genital condylomas(warts) are caused by the same genotypes (HPV 6,11). The clinical course and severity of disease have been linked to the viral genotypes involved. Young patients infected with HPV 11 frequently present with aggressive papilloma growth, resulting in airway obstruction and need a tracheostomy to maintain airway. The high-risk genotypes have been linked to genital and aero digestive tract cancers [13].

HPV attacks mucosa stem cells [14]. Although the virus is present in the mucosa as a latent infection, the mucosa is clinically normal. HPV causes disease by an unknown mechanism that causes cellular growth. Activation of epidermal growth factor (EGF) receptor have been postulated as this pathway is known to cause epithelial cells proliferation. Virus gene products, on the other hand, bind to and inactivate tumor suppressor proteins, resulting in tumor formation [14, 15]. It is thought that in children with RRP, both the humoral and cellular immune responses are impaired, leading to severe disease course. [16,17].

1.3 Transmission of HPV in RRP

The most common form of transmission in children is vertical transmission following vaginal delivery through an HPV-infected birth canal [18]. More than half of mothers who give birth to infants with RRP have visible maternal condylomas (warts) [19]. A caesarian section as a mode of delivery, has been shown to reduce transmission [19]

Trans placental transmission of HPV, direct contact and sexual abuse play a minor role in transmission to children[20]. Primigravida mothers frequently have a prolonged second stage labor, which exposes the baby to the virus for an extended period of time. Additionally, newly acquired genital condylomas shed more HPV viruses than long-standing lesions, resulting in an elevated risk in the offspring of low-income young women. Despite the close relationship between maternal condylomas and RRP, only a small number of children exposed at birth develop clinical RRP [21]. As a result, factors such as viral exposure length and timing, patient immunity as well as local trauma have been postulated to determine disease causation in children.

1.4 Clinical Presentation of RRP

Hoarseness of voice is the main presenting symptom in RRP. The voice may be hoarse or weak (presenting as a weak cry) from birth. This is followed by inspiratory stridor which then progresses to a biphasic stridor. Other presenting symptoms include chronic cough, dyspnea, recurrent respiratory tract infection, as well as poor weight gain and respiratory distress at the time of diagnosis. RRP has a variable natural history. It may spontaneously remit or persist and reoccur several times, necessitating frequent surgical treatment.

The initial presentation of papillomas is usually in the larynx. Extra laryngeal spread of RRP occurs in 30% and 16% of affected child and adults respectively [22] Outside the larynx, the trachea, bronchi, lungs and oral cavity are the most commonly involved sites. After the larynx, the trachea is the next most frequently affected site.

1.5 Diagnosis of RRP

Diagnosis of RRP involves a high index of suspicion based on history and presenting symptoms. Preoperative diagnosis is best done with flexible laryngoscope. This entails inspecting the airway from nasopharynx to subglottic region for papilloma in a sequential manner. It also enables evaluation of the laryngeal lumen, the mobility of the vocal cords, and the urgency of surgical excision.

Definitive diagnosis is made through histopathology of laryngeal biopsy and HPV DNA genotyping. Macroscopic papillomata appear as pedunculated, grape-like lesions on laryngeal mucosa. These papillomas are then grasped with micro laryngeal instruments and excised for

histopathology examination. Microscopically papillomata appear as show keratinized squamous epithelium over a fibro- vascular core.

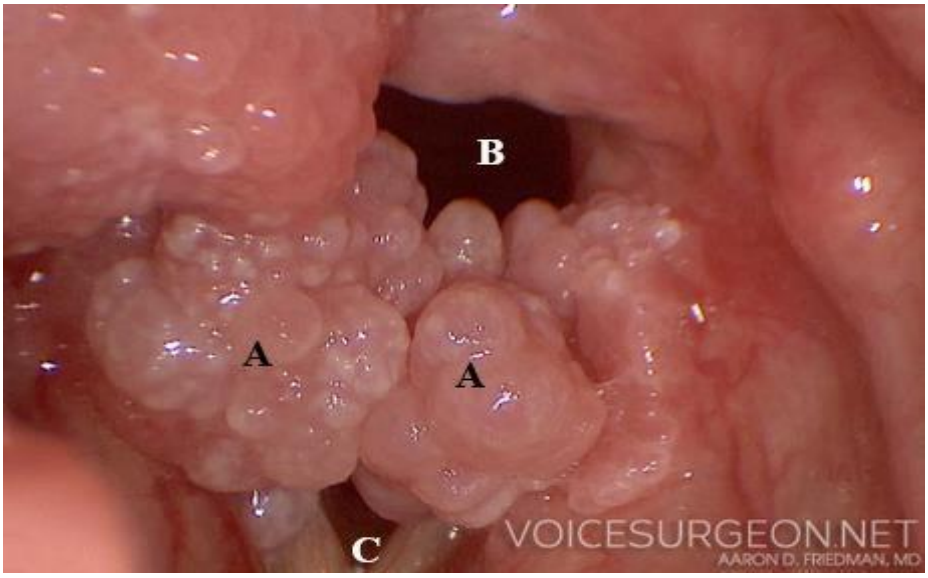

Figure 1: Endoscopic view of laryngeal papillomas(A**), the posterior Rima glottidis (**B**) and anterior glottis with vocal cords (**C**) .*Courtesy of voicesugeon.net*(23)**

1.6 Staging of RRP

Several RRP staging systems have been proposed but failed universal acceptance. The Derkey staging system is the most recent and dependable staging system [24]. It is the most commonly used and accepted staging system in research papers, and it has been adopted by the American Society of Pediatric Otolaryngologists [25]. It's based on the anatomical location and extent of lesions, as well as their clinical impact on the voice and airway integrity. This staging approach is used to determine the extent of the disease in terms of anatomical location, treatment response over time, and the requirement for adjuvant medicinal therapy.

A score of 0 to 3 (0=absent,1=surface lesion, 2=raised lesion,3=bulky lesion) is assigned to each site of the aero-digestive tract. A total score is calculated by adding the scores from each involved site. The maximum total score is 75. A score of less than 20 is categorized as low risk for need of adjuvant medical therapy while a score of 20 or more as high risk and indicate need for early initiation of adjuvant therapy. The Doyle et al criteria, which has been widely used in studies, is used to classify RRP as either aggressive or non-aggressive disease [26].

The presence of one or more of the following criteria is defined as aggressive disease.

i) A total of ten surgical excision procedures or more
ii) Three or more surgical excision procedures within one year.

iii) Distal papilloma extension into subglottic and tracheal areas
iv) Need for tracheostomy.

1.7 Treatment of RRP

The mainstay of RRP management is surgical excision. Medical management is largely adjunct to the surgical excision.

1.7.1 Surgical Excision

Surgical excision is the standard of care for RRP. The main aim of surgery is to maintain airway patency and preserve voice quality [27]. Aggressive resection is not recommended in disease involving anterior commissure. This sub site requires staged or subtotal excision of papillomas. The staged surgical approach is aimed at reducing scar formation at the anterior commissure and hence minimizing dysphonia and airway obstruction. In severe disease, surgery limits spread of papilloma to lower respiratory tract [19,28]. Tracheostomy is indicated in patients with aggressive disease causing upper airway obstruction. Studies have shown that tracheostomy in these patients causes rapid spread of the papillomata through the tracheal stoma as well as distal spread. As a result, tracheostomy should be performed only in patients in whom it is extremely necessary, and patients should be decannulated when adequate airway patency has been established [28,29]. Different types of instruments are used for surgical excision of papilloma. These include cold instruments, microdebriders and lasers. These are used independently or in combination [30-33]

Cold steel excision is widely available and a popular method especially where other modalities of excision are unavailable.

Laser treatment with Carbon dioxide (CO_2) and potassium-titanyl- phosphate (KTP) is widely employed where available. CO_2 laser is more commonly used as compared to KTP laser [34,35]. Use of microdebrider has gained popularity in RRP treatment because it is quick in excising bulk lesions and don't cause thermal injury [36]. It enables the surgeon to simultaneously excise lesions and suction the bleeding sites [37]. Microdebrider can be used in conjunction with lasers. The microdebrider excises the gross papilloma first, and then the laser provides hemostasis and further precise excision of the remaining papilloma.

1.7.2 Medical Therapy

Adjuvant therapy is required in close to 20% of RRP patients because surgery alone in these patients cannot adequately control the disease [30]. Adjuvant therapy is considered in patients who require surgical excisions of papilloma more than three to four times per year or who

have aggressive disease [1]. Adjuvant therapy currently used include interferon, cidofivir, bevacizumab, PD-1 inhibitor and HPV vaccine.

One of the first adjuvants used in the treatment of RRP was interferon. Interferons are produced by leukocytes in response to infection and are known to activate other immune cells and upregulate antigen presentation. Its use in RRP has produced mixed results. thrombocytopenia, leukopenia, fever, neurologic deficits and hair loss, are common side effects of IFN therapy. As a result, it is rarely utilized in RRP treatment because to its low efficacy and side effect profile.

Cidofovir is also used in RRP treatment. It acts by blocking viral DNA replication by inhibiting viral DNA polymerase. It is administered as an intralesional injection and has been well tolerated with minimal side effects [38,39]. In both JORRP and AORRP patients, cidofivir treatment resulted in papilloma reduction and complete illness remission [12,40,41]. HPV vaccine has in recent times been used for both prevention and treatment of RRP. Vaccines such as Cervarix, Gardasil, and Gardasil 9 are used. Cervarix is used to prevent high-risk HPV types 16 and 18, whereas Gardasil protects against both low-risk HPV types 6 and 11 and high-risk HPV types 16 and 18. The new Gardasil 9 vaccine is recommended by the Centers for Disease Control and Prevention (CDC) for both prevention and treatment and has Sprotects against HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58.

It is hypothesized that routine vaccination of pre-teenage girls will reduce incidence of genital warts and thus the incidence of RRP by reducing vertical transmission from mother to child.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Literature Review

Human Papillomavirus types 6 and 11 cause Recurrent Respiratory Papillomatosis, which is an aggressive, recurring, and difficult-to-manage disease. HPV 11 is the more virulent of the two genotypes, and it frequently causes severe disease, whereas HPV 6 is the most common but less virulent [2]. Disease severity is classified as aggressive or non-aggressive using the Doyle criteria [3]. Several studies have done to correlate HPV genotype and disease severity. Young age at onset among juveniles and presence of HPV 11 is associated with aggressive disease [3-4].

Seedat R.Y et al [42] in study on HPV types causing JORRP in south Africa isolated HPV in 94.7 % of the patients with JORRP. In this study HPV 6 and 11 were isolated without co-infection of the two. The mean age at diagnosis was between 1.4 years and 9.5yrs. It was noted that HPV 11 caused aggressive disease where affected patients required more surgical excisions procedures. In similar study in Bulgaria Draganov et al [43] isolated HPV DNA in all samples (100 %). HPV 11 was isolated in 61.9 % of patients, HPV 6 in 23.8 % and 14.3 % of patients where positive for both HPV 6 and 11. They found out that patients infected with HPV 11 had an aggressive disease course ($p=0.0265$), and none of the samples had HPV 16,18,31,33.

A study from 1974 -2012 in Netherlands involving 55 patients, Tjion P et al [44], found out that 67% of RRP patients had HPV 6 whereas 24 % had HPV 11. The patients with HPV 11 had anatomically more wide spread disease unlike patients with HPV 6. In this study, patients younger than 5 years positive for HPV 11 and adult with HPV 6 experienced aggressive disease. None of the patients had co-infection with high risk HPV genotypes.

Wiatrak et al [5] found out that patients with HPV 11 had higher severity scores and required frequent surgical intervention and adjuvant medical treatment to control the disease progression in his study in the United States comparing the severity of RRP, HPV 6 and 11 genotypes and other risk factors in children. HPV 6 was isolated in 53.5% and HPV 11 in 39.7% of the patients while 6.9% had mixed infection. It was also found out in this study, that patients less than 3 years at onset of disease developed an aggressive disease.

In Thailand in a two-year prospective study, Intakorn P et al [45] comparing disease staging and severity with the HPV type, found out that 40% of cases of RRP where had HPV 6, while 60% of the patients were infected with HPV11. There was no co-infection between HPV 6

and 11 in patients in this study. They noted that patients infected with HPV 11 had high severity score and experience an aggressive disease(p=0.013).

Eftekhaar et al [46] in a 2-year prospective cross sectional study in Iran, isolated HPV DNA in all 12 patients with RRP. It was noted that 75% of patients had HPV 6 while 16.7 % had HPV 11. One patient had co –infection with both HPV 6 and 11. The patients' average age was 9.8 years, with a male to female ratio of 1:1. It is evident in this study that HPV 6 is more prevalent than HPV 11 which is in keeping with a study done by Sanchez et al [47] in Colombia in 2013 involving 129 patients. Sanchez et al found HPV 6 in 69% of patients, HPV 11 in 27.1% and HPV 16 in 7.8%. There were 9.3% of patients co infected with multiple HPV types. In this study 36.7% of patients were children less than 12 year and majority of adults with RRP were males.

2.2 Study Justification

RRP is a devastating illness that often poses challenges in its management and has huge financial and psychological impact on patients due to repeated hospitalization for surgical excision. There is lack of data on the HPV genotypes in RRP patients in Kenya, and how these genotypes impact on the disease severity. Studies have shown that HPV genotyping is of prognostic value since the genotype present determines the course and severity of disease. Knowledge of this at diagnosis can be utilized in patient counselling on expected disease course and outcome and also provide valuable data that will guide in formulation of guidelines on adjuvant therapy in patients with aggressive disease.

2.3 Research Question

What are the HPV genotypes and the associated disease severity in RRP patients presenting at KNH?

2.4 Main Objective

To determine the HPV genotypes and to correlate the genotypes to the disease severity in RRP patients at KNH.

2.5 Specific Objectives

1. To determine the HPV genotypes in patients with RRP at KNH
2. To assess the disease severity in patients with RRP at KNH
3. To determine the correlation between HPV genotypes and disease severity in patients with RRP at KNH.

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study Design

A hospital based cross –sectional descriptive study design was used.

3.2 Study Site

The study was conducted at the Kenyatta National Hospital ENT department, operating theatre, and the University of Nairobi KAVI-ICR laboratories.

3.3 Study Population

The participants were patients diagnosed with RRP.

3.4 Participant Recruitment

3.4.1 Inclusion Criteria

a) Patients with a diagnosis of RRP and scheduled for excision of papillomas.
b) Patients with histologic diagnosis of RRP
c) Patients who gave consent

3.4.2 Exclusion Criteria

a) Patients with histologic diagnosis other than RRP.

3.5 Sample Size Calculation

A total of 58 patients diagnosed with RRP were seen in KNH in last one year, according to data from KNH hospital records. Therefore, a representative sample size was calculated out of this population using the finite population formula.

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

Where:

n' = sample size with finite population correction,

N = size of the target population = 58,

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

P = Prevalence of RRP =3.88% derived from a study by Seedat *et al* in South Africa.

d = margin of error = 5% (0.05)

Therefore:

$$n' = \frac{58\ x1.96^2x\ 0.0388(1-0.0388)}{0.05^2(58-1)+1.96^20.0388(1-0.0388)}$$

n'= 31.077 rounded off to a minimum of **35** patients.

3.6 Sampling Technique

A convenience sampling technique was used to recruit patients.

3.7 Study Tools

a) QI amp DNA Mini Spin kit
b) HPV Real-Tm kit (TV11-100FRT)
c) Real time thermal cycler (Rotor GeneQ, QIAGEN)
d) Sample collection bottles.

3.8 Study Procedure

In this study, patients diagnosed with RRP and scheduled for surgical excision of laryngeal papillomas in KNH were the target population. Patients who meet the inclusion criteria had the study explained to them by the principle researcher and written consent/ assent sought (Appendix I). Those who gave written consent/ assent to participate in the study were recruited. The patient's history was taken using a structured data collection sheet (Appendix II) which included current age, gender, age at diagnosis, number of surgical procedures done, history of tracheostomy, age of the mother at time of delivery, order of birth, history genital warts in mother, mode of delivery and history of oral sex where applicable.

Hospital records were reviewed to confirm diagnosis, histopathology and assess the course of disease during follow up for the patients with prior diagnosis of RRP. During surgical excision of laryngeal papillomas in theatre, the site and extent of papillomas were assessed. Two biopsy samples were taken from the sites with laryngeal papillomas. The biopsy samples were put in two labelled specimen bottles, one for histology and the other one for HPV genotyping. The biopsy sample for HPV genotyping was collected in a specimen bottle with normal saline while the sample for histology was put in 10% buffered formalin. The sample for histology was taken to KNH histopathology laboratory while the sample for HPV genotyping was taken to the University of Nairobi KAVI-ICR laboratories immediately.

At the KAVI-ICR laboratory, the sample was frozen at -80^0C until DNA extraction. HPV DNA was extracted from approximately 25mg of each frozen tissue sample. The sample was placed in 1.5 ml micro-centrifuge tube. 100 μl of buffer ATL was added to each sample followed by 20 μl of proteinase K. The contents were then mixed by vortexing and incubated at 56^oc in a shaking water bath until the tissues were completely lysed. 200 μl of buffer AL was added to each sample and mixed by pulse-vortexing for 15 seconds then incubated at 70^0c for 10 minutes. The mixture was then briefly centrifuged. 200 μl of 100% ethanol was added to the sample and mixed for 15 seconds and then centrifuged. The mixture was

pipetted into the QIAamp mini spin column in a 2ml tube, which was then closed and centrifuged at 8000 revolutions per minute(rpm) for 1 minute. The QIAamp mini spin column was placed in a clean 2ml tube and 500μl of buffer AW1 was added. The mixture was then centrifuged at 8000 rpm for 1 minute. The tube with filtrate was discarded. 500μl of buffer AW2 was added to the QIAamp mini spin column and the mixture centrifuged at 14,000 rpm for 3 minutes. The QIAamp column was placed in a new tube and centrifuged at full speed for 1 minute to eliminate the buffer AW2 carryover. The QIAamp mini spin column was put in a new centrifuge tube and 200 μl of buffer AE was added, incubated at room temperature for 1 minute and then centrifuged at 8000 rpm for 1 minute. This last step was repeated to increase DNA yields. The extracted DNA was stored at -20^{o}c for HPV DNA genotyping.

Real time PCR was done on 10 μl of the extracted DNA using HPV Genotype Real-TM kit (TV11-100FRT) from SACACE and in adherence to manufacturers instruction. This kit detects HPV genotypes 6 and 11. The samples were tested after preparation of a reaction mix of hot stat DNA polymerase, PCR- buffer FRT and PCR-mix-1. Test tubes with test sample as well as negative control were incorporated in the set up. The test tubes were then transferred to Real time thermal cycler (Rotor Gene Q, QIAGEN). This machine was set as per the kit PCR cycling conditions. The Rotor Gene Q software was used to interpret the results of the PCR. If the corresponding fluorescence accumulation curve crossed the thresh hold line, the signal was considered positive.

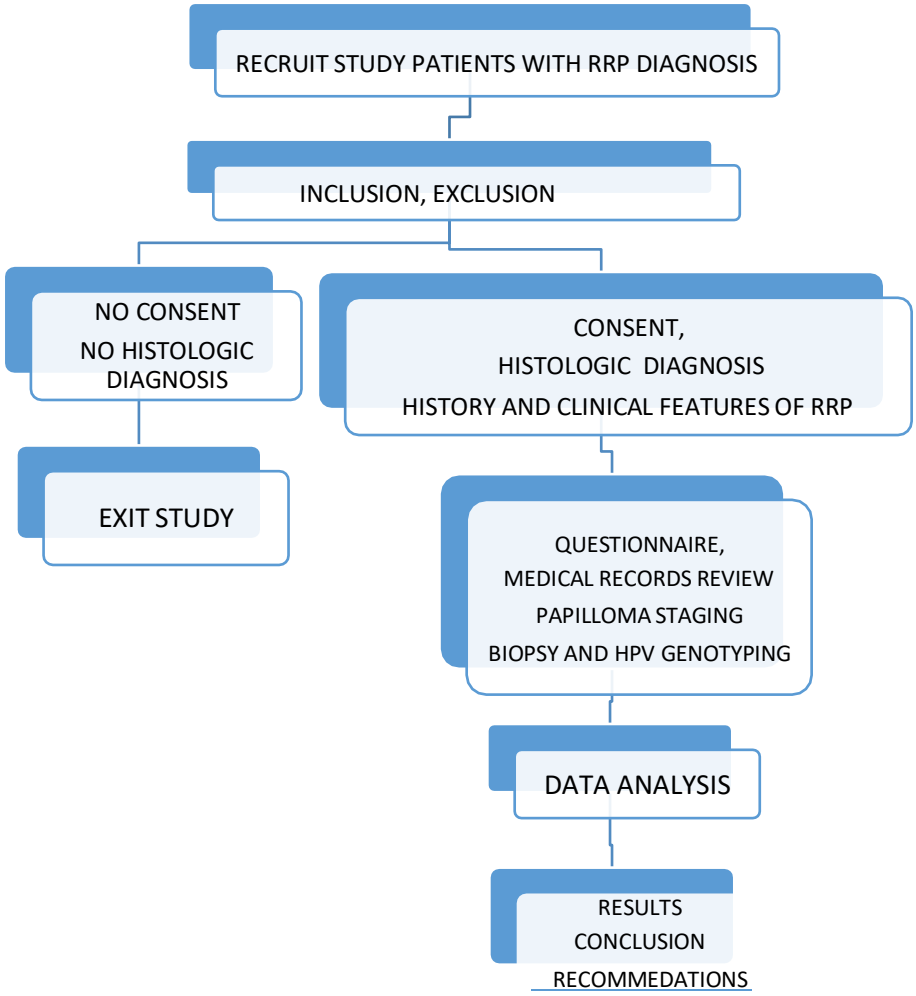

Figure 2: Study procedure flow chart

3.9 Quality Control

Quality control was a continuous process throughout the study to maximize validity and reliability of the study findings.

i) All collected data was cross checked to make sure it was complete.

ii) The principal researcher did patient interview, examine patient records, collected other relevant data and filled the data collection sheet.

iii) The same laboratory was utilized for genotyping.

iv) Specimen were collected, transported and stored as per recommended standards.

v) DNA extraction and genotyping were done using the same test kits and with strict adherence to manufacturer's instructions.

vi) The same PCR machine was used for all samples and the machine was programmed as per the kits PCR cycling conditions.

vii) A negative and a positive control was prepared and run with each test sample panel.

3.10 Data Analysis

Data was collected using a data collection sheet and entered into an excel spread sheet. The data was then cleaned for any errors and inconsistencies. SPSS version 23.0 statistical software was used to analyse data. Descriptive analysis was performed on all variables and summarised into frequency, tables and charts. Pearson chi square was used to test strength of association between variables.

3.11 Ethical Considerations

The study began after approval by UON-KNH Ethics and Research Committee (ERC), study No P642/07/2021.

Each patient received a detailed explanation of the study prior to giving a written informed consent. Patients did not incur any additional fees as a result of their participation in this study. Patients' confidentiality was maintained at all times as study numbers were used to identify them instead of their names. All data collection sheets and soft copy data was kept safely by the principal investigator and will not be shared with unauthorized personnel.

3.12 Study Dissemination

The study findings shall be disseminated in academic meetings, scientific conferences and in journals or newspapers where necessary. Hard copies of the study will be availed at the surgery department, the College of health sciences library and the ENT department library. For reference and dissemination, a soft copy will be made available on the University of Nairobi's online portal. For partial fulfilment of the Masters of Medicine in Ear, Nose, and Throat Surgery degree, a manuscript will be prepared and submitted for publication in a journal.

4.0 CHAPTER FOUR: RESULTS

The main objective of the study was to determine the HPV genotypes in patients with recurrent respiratory papillomatosis at KNH and correlate the genotypes with disease severity. A total of 40 patients were included in this study.

4.1 Demographic characteristics of study patients

Table 1:Age and sex distribution

		Frequency *(n=40)*	**Percentage**
Sex	Male	25	62.5
	Female	15	37.5
Age	≤ 5	4	10.0
	6 - 10	25	62.5
	11 - 15	5	12.5
	16-20	1	2.5
	>20	5	12.5

Majority of the patients were males at 62.5% while female patients accounted for 37.5%. The median age was 8.5 (IQR 6.0 – 11.0) years. The mean age of the patients was 12.6 years (SD 13.1) years, where the youngest was 3.0 years and the oldest 66.0 years.

Table 2: Patients characteristics on clinical history

		Frequency *(n=40)*	**Percent**
Age at first diagnosis	Juvenile onset	37	92.5
	Adult onset	3	7.5
History of Tracheostomy	Yes	7	17.5
	No	33	82.5
Age of mother at delivery	Unknown	4	10.0
	<20 yrs.	11	27.5
	≥20 yrs.	25	62.5
History of maternal genital warts	Yes	16	40.0
	No	19	47.5
	Unknown	5	12.5
Mode of delivery	Unknown	4	10.0
	Vaginal	36	90.0
Order of birth	1	30	75.0
	2	6	15.0
	3	2	5.0
	4	1	2.5
	10	1	2.5
History of oral sex	Yes	1	2.5
	No	39	97.5

Majority of the patients had JOORP at 92.5 % and only three patients (7.5%) where found to have AORRP. Then mean age at diagnosis for the patients with JORRP was 4.2 (SD 1.9) years, where the youngest was 2.0 years and the oldest age was 9.0 years. The median age was 3.5 (IQR 3.0 – 6.0) years. The patients with AORRP had a mean age of 29 years at time of diagnosis.

The mean age of the mother at time of delivery was 20.9 (SD 2.6) years, where the youngest mother was 16.0 years and oldest was 26.0 years. The median age was 21.0 (IQR 19.0 – 22.5) years. Twenty-seven percent of the patient's mothers were less than 20yrs while 62.5% were older than 20 years at time of delivery while four (4) patients didn't know their mothers age at time they were born.

Forty percent of patients were born of mother with history of genital warts while 47% had no history of the same. Majority (90%) of patients were born via vaginal delivery while in 10%, the mode of delivery was unknown. Majority (75%) of the patients where first born in the family whereas 25% had other order of birth. Only one patient had history of oral sex prior to onset of disease.

4.2 Extra laryngeal spread and HPV genotypes

Table 3: Extra laryngeal spread of papillomas.

	Extra laryngeal spread	No extra laryngeal spread
Frequency(*n=40*)	3 (7.5 %)	37 (92.5%)
Trachea	3(100%)	-
Other sites	0(0%)	-

Majority (92.5%) of the patients had no extra laryngeal spread of papillomas whereas three patients (7.5%) had spread to trachea.

4.2.1 HPV Genotypes distribution

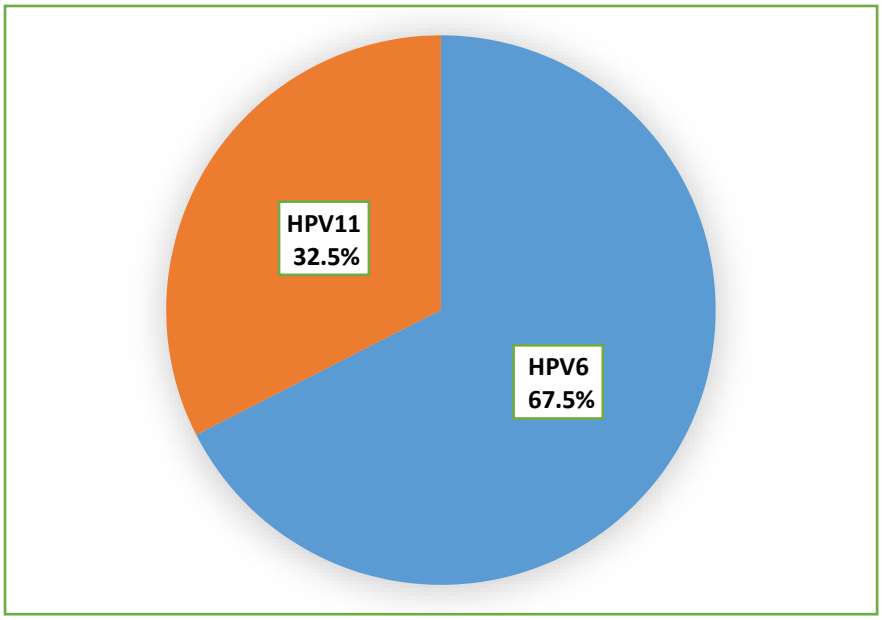

Figure 3: HPV genotype distribution percentage

HPV 6 was the most common genotype. None of the patients had co-infection with both HPV genotypes.

4.3 Disease severity

Table 4: Disease severity distribution.

	Frequency *(n=40)*	**Percentage**
Aggressive	16	40.0
Non-Aggressive	24	60.0

Majority (60%) of patients had non- aggressive disease. Disease severity was determined using Doyle criteria.

Table 5: Correlation between HPV genotypes and disease severity.

	Aggressive	**Non-Aggressive**	**p-value**
HPV6	3 (18.8%)	24 (100.0%)	**<0.001**
HPV11	13 (81.3%)	0 (0.0%)	

Majority (81.3%) of patients with aggressive disease had HPV 11. All the patients with non-aggressive disease had HPV 6. The correlation between the HPV genotypes and disease severity, indicate a statistically significant association between them ($p < 0.001$).

5.0 CHAPTER FIVE: DISCUSSION, CONCLUSION & RECOMMENDATIONS

5.1 DISCUSSION

Recurrent respiratory papillomatosis is a disease which results from persistent infection of respiratory epithelium with HPV. It is commonly associated with HPV 6 and HPV 11. HPV 6 is the most common genotype as compared to HPV 11 [2]. In this study HPV 6 was found to be the most prevalent at 67.5% while 32.5% of the patients had HPV 11. This finding compares with results of study done by Tjion etal [44] which reported that 67% of the patients had HPV 6 whereas 22% had HPV 11. RRP has a predilection to male gender [2,6,8]. In this study 62.5% of the patients with RRP were male while 37.5% were female.

Young maternal age (less than 20 years), vaginal delivery and first born child is triad of risk factors for vertical transmission of HPV in patients with juvenile onset recurrent respiratory papillomatosis (JORRP) [10]. Young prim gravid mothers often have prolonged second stage labour which result into exposure of the baby to HPV for prolonged periods of time. In this study, the mean age of the mother at time of delivery was found to be 20.9 years. It was also found that majority (75%) of the patients were first born children. Vaginal delivery has the highest risk of transmission of HPV in JORRP while caesarean section as mode of delivery offers a degree of protection [19]. In our study 90% of the patients were born via vaginal delivery, 10% had unknown mode of delivery while none of the patients were born via a caesarean section.

Maternal genital warts is also a risk factor for HPV transmission in children [18]. More than half of mothers with children with RRP have visible genital warts at time of delivery. In this study it was found that 40% of the mothers reported history of genital warts, 47.5% had no history genital warts while in 12.5% the history of genital warts was unknown.

Oral sex has been implicated as potential mode of transmission of HPV in adults. In this study, one of the 3 patients with adult AORRP reported history of oral sex. Our sample size

for the patients with AORRP was limited to allow proper assessment of the significance of oral sex in these patients.

The average age at diagnosis for children with JOORP is between 3 and 5 years whereas diagnosis in AOORP can be at any age. In this study the mean age at diagnosis for patients with JOORP was found to be 4.2yrs and range between 2 to 9 years. This finding compares with a study by Seedat etal [42] which reported a mean age of 5.4 years and ranges between 1.4 to 9.5 years.

Human papilloma virus 11 often causes an aggressive disease while HPV 6 is less virulent but more common. Disease severity is this study was assessed using the Doyle criteria [26], whereby presence of one or more of the criteria was described as aggressive disease i.e. Ten or more total surgical papilloma excision procedures, three or more surgical papilloma excision procedures in one year, distal extension of papilloma into subglottic area or trachea and need for tracheostomy.

In this study, it was found that 40% of patients had an aggressive disease and 60% had non-aggressive disease. Majority (81.3%) of the patients with aggressive disease had HPV 11 while only 3 (18.8%) patients had HPV 6. This shows a statistically significant ($p<0.001$) correlation between HPV 11 and aggressive disease. This finding is in keeping with other studies e.g. Seedat et al, Dragnov et al, and Tjion et al [42,43,44] which reported aggressive disease in patients infected with HPV 11.

5.2 : CONCLUSION

Human papilloma virus 6 was the most common (67.5%) genotype in patients with RRP. Majority of the patients had juvenile onset disease as compared to adult onset disease. Most of the patients had non aggressive disease. HPV 11 was found to cause an aggressive disease as compared to HPV 6($p<0.001$)

5.3 : RECOMMENTATIONS

We recommend HPV genotyping to be done at time of diagnosis for patients with recurrent respiratory papillomatosis as patients who are found to have HPV 11 could benefit from adjuvant medical treatment to slow down the disease and increase interval between surgical excisions. Further studies with bigger sample size should be done especially in patients with adult onset RRP to determine their socio-demographic characteristics and HPV genotypes.

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APPENDICES

Appendix I (a): Consent Form (English)

APPENDIX I (a): GENERAL INFORMATION SHEET

STUDY TITLE: HUMAN PAPILLOMA VIRUS GENOTYPES AND ASSOCIATED DISEASE SEVERITY IN RECURRENT RESPIRATORY PAPILLOMATOSIS PATIENTS IN KENYATTA NATIONAL HOSPITAL

Principal Investigator:

Dr. Charles Matheka

(M.Med student in Ear, Nose and Throat surgery, University of Nairobi)

Supervisors:

Dr. Joyce Aswani, consultant E.N.T surgeon

Dr. John Ayugi, Consultant E.N.T surgeon

Dr. Moses Masika, microbiologist

Introduction

This is an explanation of the study being conducted by the above listed researcher. Recurrent respiratory papillomatosis is a condition that occurs in both children and adults. It is characterised by growths in the airway passages that block or reduce the size of the airway. As a result, patients get difficulties in breathing and voice production. The growths are caused by a virus called Human Papilloma Virus. There many subtypes of this virus. The common subtypes which are known to cause this disease are subtype 6 and 11. The subtype involved influences how the disease will progress and how severe the disease will be. This virus is transmitted to the child during child birth, if the mother is infected with the virus in her birth canal. In the birth canal, the human papilloma virus causes growths called genital warts. Adults acquire the infection in the airways through oral sex.

The researcher is investigating the virus subtypes that caused the recurrent respiratory papillomatosis and how these subtypes have impacted on severity of the disease.

In this form, we have provided information that is required for the research. We request you to go through it and ask any questions that you may have before agreeing to participate in this study.

As some information may be sensitive, you may request more privacy and limit the interaction to only two people, that is you and the principal supervisor.

Purpose of the study

The results of the study will provide local data on HPV genotypes in our patients and how they influence disease severity. This data will also be useful formulating further treatment modalities. it can also be used as a forum for implementation of vaccination programmes against HPV, with the intention of reducing the number of cases of respiratory papillomatosis in the long run.

Description of study

Before taking part in this study, you will be allowed to ask questions about this study. All your concerns will be addressed and once satisfied you will be given a consent form that you will sign to accept to participate in this study. The principal researcher with or without an assistant researcher will take your details or your child's details (if the child is the patient) which will include sex, age, age diagnosis, surgical procedures that have been done to treat the disease and how the disease has been progressing. During surgery to reduce the growths (papilloma's) a biopsy (tissue sample) will be taken and will be taken to the laboratory in order to determine the human papilloma virus type.

Risks Involved

This study will be conducted in regards to ethics and research guidelines. You will not be subjected to any additional risks by participating in this study. No treatment shall be withdrawn, if you do not participate in this study, or if you drop out of the study.

Benefits

This study will provide information on human papilloma virus types and associated disease severity. This study will provide this valuable data which is lacking in our hospital/country. The results of this study will help you, as our patient in knowing the virus type involved and how this will impact on severity and disease progression going forward. The results of this study will also be valuable in formulating guidelines for use of additional treatment in patients with severe disease.

The principal investigator will avail and explain to you results of this study and you will continue to receive the appropriate care for your disease.

Confidentiality

A code will be assigned to you, to identify you as a study participant. Your name will not be used, in order to ensure confidentiality.

Payments

You will not incur any extra costs above your normal treatment nor will you receive any monetary benefits by participating in this study. The cost of testing the virus subtypes will be met by the principle investigator.

Use of Data Collected

The information obtained from the study will only be shared, after it has been authorized by KNH – UON Ethics committee. The information will be shared in scientific forums like journals, conferences and specialities meetings.

Rights as a participant

You will be allowed to voluntarily participate in this study and you have the right withdraw from the study at any time without any penalty

Investigator's declaration

I, as the principal investigator, declare that I have not received any financial payments, nor the supervisors, from any company or institution, to finance this study. Such action will and may compromise the study.

If you have any questions, please feel free to seek information through the contacts given below;

Principal Investigator:

Dr. Charles Matheka David
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Kenyatta National hospital

P.O BOX 20723 code 00202

Tel. 726300-9 ext 44355, 44102.

Appendix I (b): Consent Form - English

I .....freely give consent to take part in the study conducted by Dr. Charles Matheka David, the nature of which has been explained to me by him. I have been informed and I have understood that my participation is entirely at my own will. I comprehend that if I so wish, I can freely withdraw from the study and this will not in any way alter the care being given to me. The results of the study may not directly be of benefit to me, my kin and other patients.

Signature/ left thumb print(self)……

Date……

Witness: Name: …….

Signature: …….

Appendix II (a): Fomu ya Idhini(Kiswahili)

Karatasi ya Habari ya Jumla

Utangulizi

Haya ni maelezo kuhusu utafiti unaofanya na mtafiti aliyeorodheshwa. Recurrent respiratory papillomatosis(RRP) ni ugonjwa ambao upata watoto na watu wazima. Katika huu ugonjwa growth umea katika njia na kupumua ambazo ufunga kabisa ama kupugua kwa kima. Kutoka na hizi growth mgonjwa hupata matatizo ya kupumua na sauti yake ubadilika. Huu ugonjwa husababishwa na virus vya Human papilloma virus (HPV). Kuna haina nyingi za hivi virusi vya HPV lakini aina inayosababisha huu ugonja ni HPV 6(sita) na HPV 11(kumi na moja). Aina ya virusi ambavyo mgonjwa ameambukizwa huwa inaelekeza namna ugonjwa utakavyo endelea na makali yake. Watoto huabbukizwa hizi virusi wanapozaliwa na mama ambaye pia ana hivi virusi kwa nzia yake ya uzazi. Kwenye sehemu ya uzazi,hivi virusi husambabisha ugonjwa wa genital warts. Watu wazima huambukizwa virusi kutokana na kushiriki gono ya kutumia mdomo.

Mtafiti anachiguza aina ya hivi virusi ambavyo viko kwa ugonjwa wa RRP na vile zinaelekeza makali ya ugonjwa.Katika fomu hii, habari amabayo inahitajika kwa utafiti wa huu ugonjwa, imeelezwa. Tunakuomba upitie fomu hii, na uulize maswali yoyote unayoweza kuwa nayo kabla ya kukubali kushiriki katika utafiti huu.Kwa kuwa habari fulani inaweza kuwa nyeti, unaweza kuuliza faragha zaidi na kupunguza mwingiliano kwa watu wawili tu - wewe na msimamizi mkuu.

Sababu za utafiti

Matokeo ya utafiti yatatoa taarifa kuhusu aina ya virisi vya HPV katika ugonjwa wa recurrent respiratory papillomatosis na vile vinavyo elekeza makali ya ugonjwa. Matokeoa pia yatakua muhimu kwa utumizi wa matibabu zaidi na badala katika huu ugonjwa**.**

Matokeo ya utafiti yanaweza kutumika kama jukwaa la kuingizwa kwa mipango ya chanjo dhidi ya HPV, kwa kusudi la kupunguza idadi ya matukio ya papillomatosis ya kupumua kwa muda mrefu.

Maelezo ya Utafiti

Kabla ya kukubali kushiriki katika utafiti huu, utapewa nafasi ya kuuliza maswali juu ya huu utafiti. Maswali yako yote yatajibiwa na kama utaridhika, basi utapewa fomu ya idhini ambayo utatia sahihi ya kukubali. Mtafiti mkuu au mtafiti msaidizi atachukua taarifa binafsi inayohusu historia ya matukio ya ugonjwa,umri,umri wakati ugonjwa ulipotabulika, upasuaji uliofanywa na vile ugonjwa umeendelea. Wakati wa upasuaji ili kutoa papillomas, nyama ndogo(biopsy) itatolewa na kupelekwa kwa mahabara hii kupima aina ya virusi vya HPV.

Hatari zinazohusika

Utafiti huu utafanywa kwa njia ya kimatabu inayokubalika Hutatumia pesa zozote zaidi katika utafiti huu, ila tu ile ya matibabu yako ya kawaida. Kama utakataa kushiriki, matibabu hayataondolewa.

Faida

Matokeo ya utafiti yatatoa taarifa kuhusu aina ya virisi vya HPV katika ugonjwa wa recurrent respiratory papillomatosis na vile vinavyo elekeza makali ya ugonjwa. Matokeoa pia yatakua muhimu kwa utumizi wa matibabu zaidi na badala katika huu ugonjwa**.**

Matokeo ya utafiti yanaweza kutumika kama jukwaa la kuingizwa kwa mipango ya chanjo dhidi ya HPV, kwa kusudi la kupunguza idadi ya matukio ya papillomatosis ya kupumua kwa muda mrefu.

Siri

Utapewa nambari ambayo itatumika kukutambulisha kama mshiriki wa huu utafiti. Jina lako halitatumika, ili kuhakikisha usiri.

Pesa

Hutapata gharama yoyote zaidi ya ile ya matibabu yako, wala kupokea faida yoyote ya kifedha. Pesa ya kugaramia utafiti wa virusi vya HPV sitagaramiwa na mutafiti mkuu.

Matumizi ya Data

Habari itkayopatikana kutoka kwa utafiti huu itashirikiwa tu, baada ya kuidhinishwa na kamati ya Maadili ya KNH-UON. Habari hiyo itashirikiwa katika vikao vya kisayansi kama majarida, mikutano na mikutano ya utaalamu.

Uhuru wa mshikiri.

Uta shiriki kwa huu utafiti kwa hiari yakao na Unauhuru wa kujiondoa kutoka kwa utafiti kwa hiari yako wakati wowote bila adhabu yoyote.

Tamko la Mtaalamu

Mimi kama mchunguzi mkuu natangaza kuwa hakuna malipo ya kifedha niliopokea, wala wasimamizi au hospitali ya Taifa ya Kenyatta, kutoka kwa kampuni yoyote ya dawa, au kampuni nyingine yoyote, ili kufanya utafiti huu.

Tafadhali jisikie huru kutafuta maelezo ya ziada kupitia anwani zilizopewa hapa chini;

Mtafiti Mkuu:

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Tofuti :www.erc.uonbi.ac.ke

Hospitali Kuu ya Kenyatta

Saduku la posta: 20723 code 00202

Simu: 726300-9 Ext 44355, 44102.

Appendix II (b): Fomu ya Idhini (swahili)

Fomu ya Makubaliano

Tamko la mshiriki

Mimi nimesoma au nimesomewa yaliyochapishwa katika fomu hii. Nimeweza kupata maelezo kutoka kwa mtaalamu wa utafiti. Maswali yangu yamejibiwa kwa lugha ninayoelewa. Ninaelewa ya kwamba, kushiriki kwa utafiti huu ni kwa hiari yangu na kwamba ninaweza kujiondoa kwa utafiti huu kwa wakati wowote. Nimekubali kwa hiari yangu kuhusishwa katika utafiti huu Kwa kutia sahihi, sitakuwa nimekata tamaa ya haki zangu kama mhusishwa katika utafiti huu.

Sahihi ya Mshiriki/ Kidole gumba ……

Tarehe ……

Jina la mshuhudia …….

Sahihi / kidole cha gumba …….

Appendix III: Data Collection Sheet

Study no ……... Age……. Sex: Male ☐ Female ☐

PART A: HISTORY

1. Age at diagnosis/ first excision……...

2. Number of surgical excision procedures in last 1 year……

3. Total number of surgical excision procedures since diagnosis……

4. Have you had tracheostomy done Yes ☐ No ☐

5. Age of mother at time of delivery…… Unknown ☐

6. History of maternal genital warts Yes ☐ No ☐

7. Mode of delivery Vaginal ☐ Caesarian section ☐ Unknown ☐

8 Order of birth ……

9. Other affected siblings Yes ☐ No ☐

10. History of oral sex Yes ☐ No ☐

PART B: CLINICO-HISTOLOGIC DATA

Extra laryngeal spread

1. Oral cavity Yes ☐ No ☐
2. Oropharynx Yes ☐ No ☐
3. Hypopharynx Yes ☐ No ☐
4. Trachea Yes ☐ No ☐
5. Bronchi Yes ☐ No ☐

Pathological Data

6. Histological diagnosis: Previous…… Current……

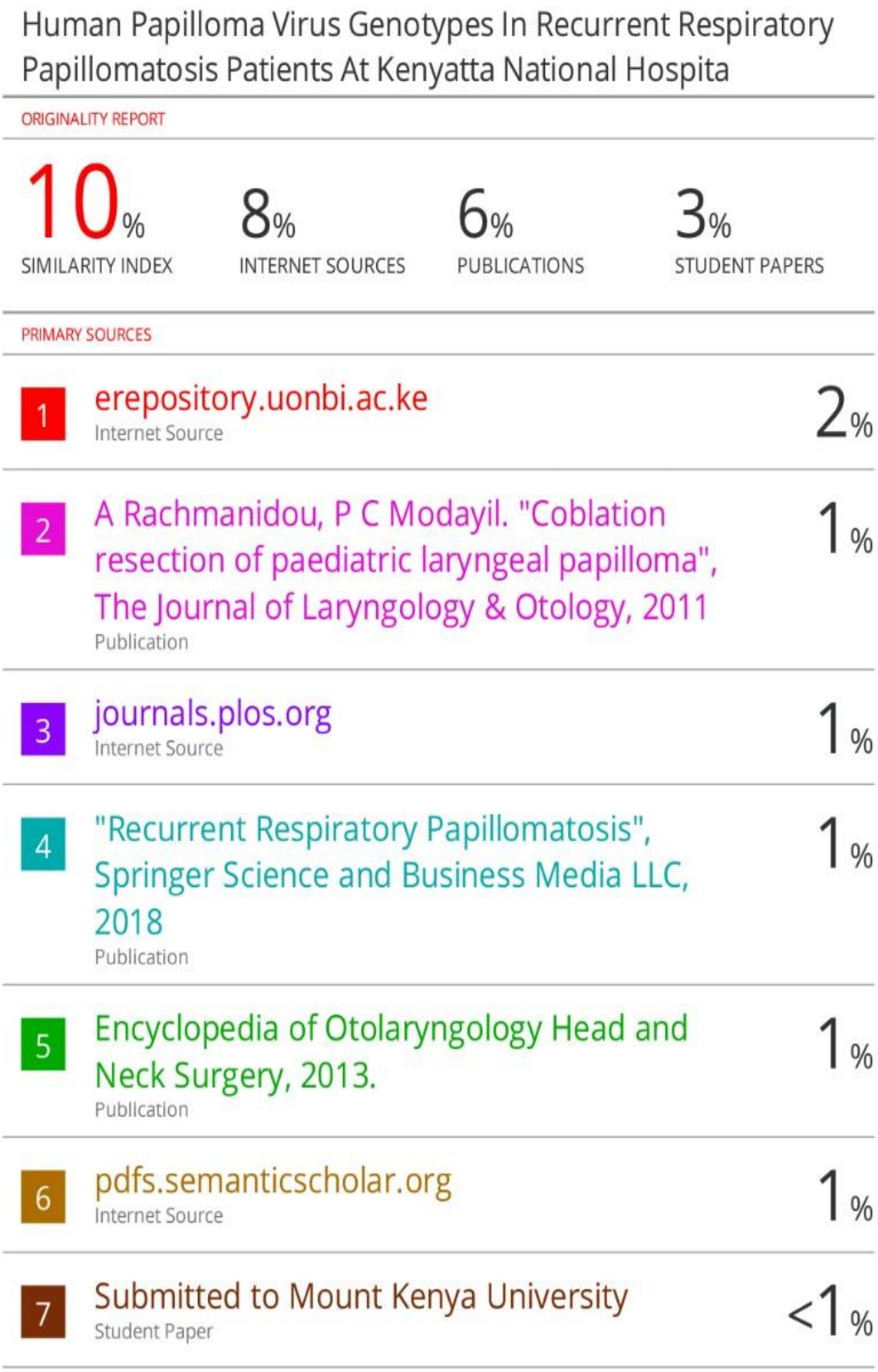
7. HPV genotype 6 ☐ 11 ☐ No HPV 6/11 ☐

Appendix IV: Doyle Scoring Criteria

a) Ten or more total surgical papilloma excision procedures
b) Three or more surgical papilloma excision procedures in one year
c) Distal extension of papilloma into subglottic area and trachea
d) Need for tracheostomy

Aggressive disease is described as presence of one or more of the above criteria.

Appendix V: Antiplagiarism Report



Human Papilloma Virus Genotypes In Recurrent Respiratory Papillomatosis Patients At Kenyatta National Hospita

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Appendix VI: KNH/UON ERC Letter of Approval

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Ref: KNH-ERC/A/391

25th October, 2021

Dr. Charles Matheka David
Reg.No.H58/87416/2016
Dept. of Surgery
Faculty of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 25 OCT 2021 P.O. Box 20723 - 00202, NAIROBI KNH/UoN-ERC

Dear Dr. Matheka

RESEARCH PROPOSAL: HUMAN PAPILLOMA VIRUS GENOTYPES IN RECURRENT RESPIRATORY PAPILLOMATOSIS PATIENTS AT KENYATTA NATIONAL HOSPITAL (P642/07/2021)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and approved your above research proposal. The approval period is 25th October 2021 – 24th October 2022.

This approval is subject to compliance with the following requirements:

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

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

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

Yours sincerely,

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

c.c. The Dean-Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Chair, Dept. of Surgery, UoN
Supervisors: Dr. Joyce Awani, Dept. of Surgery, UoN
Dr. John Ayugi, Dept.of Surgery, UoN
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