PREVALENCE OF HUMAN PAPILLOMA VIRUS AMONG ADOLESCENTS AT KENYATTA NATIONAL HOSPITAL

Dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine in Obstetrics and Gynecology at University of Nairobi

By

DR ERNEST MDACHI

DEDICATION

This book is dedicated to my children Esther, Ernest Jr and Erica and my wife Victoria for the time they had to do without me during the study period.

DECLARATION

This study was carried out as part fulfilment of the degree of Masters of Medicine in Obstetrics and Gynaecology at the university of Nairobi.

I certify that this dissertation is my own original work and has never been submitted for a degree in any other university.

Signed..... Date 5th JAN 2011.

DR. ERNEST MDACHI – PRINCIPAL INVESTIGATOR.

Registrar,

Department of Obstetrics and Gynaecology ,

University of Nairobi

CERTIFICATE OF AUTHENTICITY

This is to certify that this dissertation is the original work of Ernest Mdachi

Master of Medicine student in Department of obstetrics and Gynaecology, Registration number H58/70667/2007 University of Nairobi (2007-2011). The research was carried out in the department of obstetrics and Gynaecology, School of Medicine, College of Health Sciences. It has not been presented in any other university for award of a degree.

Signature.....

Date.....25/1/11.....

Prof. Koigi Kamau,

Associate Professor of Obstetrics and Gynaecology

Consultant Obstetrics and Gynaecology,

Chairman,

Department of Obstetrics and Gynaecology,

University of Nairobi

CERTIFICATE OF SUPERVSION

This is to certify that the thesis presented in this book was researched upon by Dr. Ernest Mdachi under my guidance and supervision, and that the thesis is submitted with my approval.

Signed..... Date.....6th. January 2011.

DR. OMONDI OGUTU

SENIOR LECTURER, DEPARTMENT OF OBSTETRICS/GYNAECOLOGY

SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF NAIROBI

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the following:

1. The Office of the President of the United Republic of Tanzania for sponsoring my postgraduate training at the University of Nairobi.
2. The chairman of the Department of Obstetrics and Gynaecology, University of Nairobi, Prof. Koigi Kamau for giving me an opportunity to do my masters degree in this University.
3. My Principal Supervisor, Dr. Omondi Ogutu,Consultant and Senior Lecturer at the Department of Obstetrics and Gynaecology, University of Nairobi for his tireless assistance, constant advice and direction , patience and moral support through all stages of this study.
4. My co-supervisor, the late Dr. Njoroge Waithaka, former Head of Department of Obstetrics and Gynaecology, Kenyatta National Hospital for his guidance in the proposal writing of this study, unfortunately he passed away while I was collecting data. May the almighty God rest his soul in eternal peace, Amen.
5. All the Consultants and Senior Registrars of the department of Obstetrics and Gynaecology for enabling me to acquire the necessary skills and knowledge during my training at Kenyatta National hospital.
6. Matron Nancy, Sr. Joyce and Sr.Rose for their assistance and support during data collection at the Youth Centre, Kenyatta National Hospital.
7. Mr. Allan King'oro, Incharge of HPV Laboratory at KEMRI , who did the HPV DNA testing and genotyping.
8. My fellow Registrars, Nursing staff and other medical cadres at Kenyatta National Hospital for their friendliness and cooperation during my stay at the hospital.
9. Mr. Robinson Njoroge for data entry and analysis.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ASCUS: Atypical Squamous Cells of Undetermined Significance. A category of abnormal cervical cells according to the Bethesda system of cytologic classification of cervical abnormalities.

CIN: Cervical Intra-epithelial Neoplasia. Also called Dysplasia-Is the disordered growth and development of the epithelial lining of the cervix. Divided into various degrees depending on severity such as CIN I, CIN II , CIN III and CIS.

CIN I: Mild dysplasia.Disordered growth involving the lower 1/3rd of the epithelial lining.

CIN II: Moderate dysplasia. Is disordered growth involving lower 2/3rd of the epithelial lining.

CIN III: Severe dysplasia.Is disordered growth involving more than 2/3rd of the epithelial lining.

CIS: Carcinoma in Situ; full thickness dysmaturity.

CD4-: A surface molecule expressed by cells of the immune system like T Lymbocytes, macrophages that binds to Class 11 Major Histocompatibility Antigen on Antigen presenting cells leading to secretion of cytokines. Is the major target by the Human Immunodeficiency Virus.

CERVICAL DYSPLASIA: Disordered growth involving the cervical epithelium.Characterised by loss of cell uniformity and architectural orientation, hyperchromatic nuclei and increased mitotic figures

CARCINOMA: Malignant tumor of epithelial origin derived from any of the 3 germ layers. Further classified as Adenocarcinoma (with glandular pattern) and Squamous cell carcinoma (with any recognizable squamous cell pattern).

DNA: Deoxyribonucleic Acid. A nucleotide chain containing bases that makes a double helical structure that carries the genetic code.

HIV: Human Immunodeficiency Virus; A retrovirus that is responsible for the Acquired Immunodeficiency Disease Syndrome (AIDS).

HLA: Human Leukocyte Antigen. Also called Major Histocompatibility Antigen (MHC). Consists of a group of genes located on chromosome 6 that binds antigens and presents to T Lymphocytes.

HPV: Human Papillomavirus. A DNA virus that is the causative agent for CIN and most invasive cervical cancer.

HYBRID II CAPTURE: A solution Hybridization method used to test for High risk Human Papillomavirus.

HSV: Herpes Simplex Virus; the etiological agent in most cases of genital herpes.

KNH: Kenyatta National Hospital.

PCR: Polymerase Chain Reaction.

SIL: Squamous Intra-epithelial Lesion; a cytological classification of cervical smears. Further sub classified into HSIL and LSIL.

HSIL/HGSIL: High Grade Squamous Intra-epithelial Lesion, cytologic changes consistent with CINII and CIN III;

SIL/LGSIL: Low Grade Squamous Intra-epithelial Lesion encompassing cytological changes consistent with koilocytic atypia or CIN I.

WHO: World Health Organization.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

FIGURES

TABLES

ABSTRACT

Background: Genital HPV infection is among the most common sexually transmitted disease worldwide. A clear causal relationship has been established between human papilloma virus (HPV) infection and the development of cervical cancer. HPV types 16 and 18 are implicated in over 70% of cancer of the cervix. Peer pressure among adolescents leads them to high risk sexual behaviors such as early sexual debut, unprotected sex, multiple sexual partners. These risky sexual behaviors among adolescents predispose them to HPV infection.

There is lack of concrete findings of prevalence and distribution of HPV types in Kenya. Some studies have demonstrated limited knowledge on the availability of HPV vaccine and negative attitude towards HPV vaccination by parents. This research will add to the existing knowledge by establishing HPV prevalence by types as well as knowledge gaps of HPV transmission and prevention among adolescents. Findings will inform decision makers about magnitude of HPV infection and effective strategies of preventing new HPV infections particularly scale up of HPV vaccination.

Main objective: To determine by DNA typing the prevalence of HPV infection among adolescent girls at Kenyatta National Hospital - Youth Clinic.

Methodology: This was a Cross Sectional Study carried out at Kenyatta National Hospital Youth clinic among a random sample of adolescent girls between 12-26 years between December 2009 and October 2010. Self-administered questionnaire was used. Cervical swab was taken from each respondent enrolled in the study. HPV-DNA was analyzed using PCR and type specific genotypes were isolated by Slit blot hybridization. The data obtained was analyzed using SPSS version 17 (SPSS, Chikago).

Results: A total of 264 female participants who met the eligibility criteria were enrolled into the study. The prevalence of cervical HPV infection was 9.8%. Of the study participants 72% (n=184) had knowledge of HPV and majority of respondents had knowledge that HPV is sexually transmitted. Majority (88%) of the study participants believed that HPV infection may lead to cancer of the cervix. Association between sexual behavior and HPV status was not statistically different between study participants who were HPV negative and HPV positive. There was no statistical significant association between HPV infection and coexistence of other sexually transmitted infections. Over 89% of participants who were HPV negative believed that condoms play a key role in reducing HPV transmission.

Conclusion: The prevalence of HPV among adolescent being cared for at Kenyatta National Hospital was (9.8%). Of the participants who were HPV DNA positive, 27% had co infection with type 18 and 66. This study has implication for HPV vaccination in Kenyan population in the prevention of cervical cancer.

CHAPTER 1

INTRODUCTION AND LITRATURE REVIEW

1.1 Introduction

Human papillomavirus is a double stranded DNA virus belonging to papovovirus family.

There are more than 100 HPV serotypes, about 40 of which infect the genital tract of both men and women. The virus infects both keratinized and non keratinized epithelia such as anogenital tract, oropharynx, lungs, prostate and skin. HPV can broadly be grouped into cutaneous and mucosal types according to their site of infection, and can be further subdivided into low-risk (LR) and high-risk (HR) types depending upon their association with malignancy. High risk HPV type 16 and 18 have been conclusively shown to be associated with invasive cancer of the cervix in 70% of cases. [71]

The main route of transmission of HR mucosal HPVs is through sexual contact, although the acquisition of the virus cannot be entirely explained by this mode alone. Evidence also exists for horizontal and vertical transmission by other routes . HR HPVs, particularly HPV-16, have been detected in oral swabs from new-borns, infants and children. [1, 2, 3]

HIV infection has been recognized and playing an important role in the progression of HPV infection to invasive cancer. [71] HPV is a known cause of benign and malignant disorders in both men and women. Premalignant and malignant diseases of the anogenital tract i.e. vulval, vaginal, anal, penile and cervical dysplasia and carcinoma, head and neck tumors, epidermodysplasia verruciformis, genital, palmer and planter warts, juvenile onset laryngeal papillomatosis. [4,5] HPV infection can now be prevented by the use of vaccine which is advised for pre pubertal girls and boys. The vaccine available are bivalent and quadrivalent types.

Classification

HPV is classified according to site of infection into:-

1. Cutaneous- those infecting skin.
2. Mucosal - those infecting mucus membranes

The mucosal type is further sub classified on the basis of pathogenicity into: [6]

1. High risk subtypes - include:

   16 ,18 ,31 ,33 ,35 ,39 ,45 ,51 ,52 ,56 ,58 ,59 ,68 ,73 ,82 .
2. Probably high risk:

   26, 53, 66.
3. Low risk subtypes:

   6 ,11 ,40 ,42 ,43 ,44 ,54 ,61 ,70 ,72 ,81 ,pap 155 ,pap 291

Most HPV infections are sub clinical and transient; about 90 % will resolve within nine months but may last up to two years [1, 7, 8, 9] due to development of cell mediated immunity. After infection HPV remains in a quiescent state that lasts two to twelve month then is either cleared or persists below sensitivity level (latent phase). About 10-20 % of infections may persist and develop into genital warts or CIN 1 within 3-6 months. Most of this regress but some may progress to pre-cancer lesions (CIN2, 3) in 4-5 years .[9, 10] Some high grade lesions may arise directly from initial HPV infection. [10]

Progression to invasive cancer takes 1- 15 years;the development of invasive cancer is age related with peaks at 50 years, CIN I at 28 years and CIN II, III at 42 years. [10, 11]

Risk factors that promote progression to dysplasia and invasion include:

a) Viral factors of HPV

Viral type, viral load ,co-infection with several viral serotypes. [10, 12, 13]

b) Environmental/external factors.

Oral contraceptive use, smoking, diet, trauma, other sexually transmitted infections

c) Host factors

Endogenous hormones, genetic factors, depressed cell mediated immunity like in HIV

disease.

1.2 Literature Review

Epidemiology

Human papilloma virus is the commonest viral sexually transmitted infection worldwide estimated to affect about 291 million women with an estimated 105 million being infected with high risk serotypes 16 and 18. [14] It is estimated that at least 50% of women will be infected with HPV in their lifetime. [15] The annual incidence is estimated at 371,000 worldwide. [7, 14]

The prevalence rates vary from region to region; with estimated rates of 1.4-25.6 % in asymptomatic women. [16] The World Health Organization estimates an overall prevalence of about 22 % for African countries. [17] Kenyan rates are unknown though studies in high risk men around Lake Victoria showed a rate of about 53 %. [18] The distribution of HPV types is also variable. The distribution in invasive lesions in order of reducing prevalence are: 16 ,18 ,33 ,45 ,31 ,58 , 52 , 35 , 59 , 56 , 51 , 39 , 6 , 68 , 73 , 66 , 70. [6,] HPV type 16 is consistently the most common serotype in high grade and invasive lesions. [19] The prevalence of high risk serotypes in most adolescent populations has been found to be low with rates of 3.4 % or less while low risk serotypes constitute the majority. [20, 21] Among the low risk serotypes, HPV 6 and 11 are the commonest estimated to cause more than 90 % of the genital warts [22, 7,16] and 25 % of CIN I, whereas high risk types account for the remaining 75 % of CIN I. Infection with multiple serotypes is common occurring in 20-30 % of women. [10, 23]

HPV Infection and Socio-demographic Characteristics

Studies have shown age, number of sexual partners and oral contraceptive use as risk factors associated with overall risk for HPV infection. In high risk HPV infections there is a trend of decreased risk among women with three or more parities and an increased risk among users of oral contraceptives, in women below age of 25 years, high educational level and multiple sexual partners. Young age and high level of education are predictors of multiple infections than single infection. For multiple infection, there is a trend for protective effect of parity (for three or more children compared to nulliparous).[24]

Age plays a role with the highest prevalence and incidence rates being among adolescents especially soon after initiation of sexual activity with rates exceeding 30% in most high risk adolescent populations.[1, 11, 23] There is a linear increase in prevalence by age from 14-25 years with the peak prevalence at 20- 24 years which then declines with advancing age though some societies demonstrate a second peak after the age of 60 years. [23, 25, 26] Most of these infections resolve with only about 5-10% persisting and progressing to premalignant and malignant lesions.

Relationship between HPV infection, sexual behavior and other STI

Studies have shown marked geographic differences in incidence of cervical cancers. These geographical differences in incidence are not only the result of differences in screening patterns but also differences in exposure to risk factors. Such as the very low incidence rates(2-4 per 100,000) reported from some areas in China and Spain where population based screening programs don't exist as a result of low exposure to HPV resulting from the conservative sexual behavior prevalent in the previous decades.[24]

In adolescent girls ,increased incidence is associated with early onset of sexual activity, multiple sexual partners, presence or a history of sexually transmitted infections, anal intercourse, smoking [1,23, 27] and less consistently : frequent sexual intercourse , use of combined oral contraceptives, pregnancy and abortions. [11,27,28,29,30] Having a sexual partner who has multiple partners, [31] homosexuals and non frequent condom users are also at increased risk. [32] Risk factors for infection in men are similar to those in girls. [33, 34] Lack of circumcision is an additional risk factor both for men and their sexual partners. [35, 36].

Co-existence of other sexually transmitted infections especially Chlamydia and herpes simplex type 2 are associated with persistence and progression of HPV disease. [37, 38] The association of HIV infection has been clearly documented, depressed immunity which occurs in HIV infection is associated with a high rate of persistence and invasion. [1, 12, 39, 40, 41] The role of other sexually transmitted infections e.g. gonococcus, trichomonas, bacterial vaginosis is not known.

Relationship between HPV & Cervical Cancer

A causal association between cervical cancer and HPV has been documented beyond reasonable doubt. [42] HPV has been demonstrated in over 99.7 % of all invasive lesions. [43, 44] The eight most common serotypes in invasive cancer in descending order of frequency include; HPV type 16 ,18 ,45 ,31 ,33 ,52 ,58 and 35 which are thought to account for more than 80 % of all invasive cancers worldwide with type 16 and 18 accounting for about 70 % of all invasive cancers. [22, 44] Type 16 is the predominant type in squamous cell tumors and adenocarcinomas while type 18 is more prevalent in adenocarcinomas. [45]

HPV alone does not cause invasion but it acts in concert with other co-factors such as Virulence, multiple HPV serotypes infection and presence of high genomic expression. [10, 23, 46, 47, 48] HPV persistence and invasion is more common in cigarette smokers as compared to the general population [39, 48] probably due to a possible

association with sexual behavior. [39] However, nicotine has been found in cervical mucus of cigarette smokers and these could have a direct carcinogenic effect. [49] Smokers are also more likely to have high grade lesions as opposed to low grade lesions. [39, 50]

The role of combined oral contraceptives is controversial .Several studies have shown a high risk of high grade and invasive lesions up to 4- fold in prolonged current users for more than 5 years but not in past users. [1, 51, 52] They are mainly associated with adenocarcinoma as opposed to squamous cell carcinomas. The estrogen and progesterone in oral contraceptives has been thought to promote HPV integration into the host genome. [53] The high prevalence of high grade and invasive lesions could be due to detection bias arising from more frequent examinations and pap smears in clients using oral contraceptives. [54]

Adolescents' knowledge on HPV, its transmission and prevention

Studies have shown that knowledge about HPV in the general public appears to be low. Using the open question 'what causes cervical cancer', the percentage mentioning HPV was less than 2 % in surveys in the United Kingdom and Mexico.The potential for a prophylactic vaccine has attracted a great deal of press coverage and this may have increased knowledge of HPV. However, internet and newspaper articles are most likely to be assessed by higher SES groups. So educational impact could be stronger in more educated groups. [55]

Acceptability and effective immunization against Human papillomavirus depends on adolescents and their parents' knowledge on HPV, its transmission, prevention and sequelae. Studies assessing these aspects among adolescents and their parents have been disappointing with most displaying limited knowledge on the subject. [57, 58, 59] The acceptance of vaccination by parents have shown varying results. Omondi-Ogutu found an acceptance rate of only 58% by parents. [72]

The relationship between HPV infection and cervical cancer is unknown by most women with the knowledge being more scanty in high risk groups. [57] However, adolescents with prior treatment for sexually transmitted infections, genital warts and previous treatment for dysplasia have been found to be more knowledgeable. [57] Almost all infections are acquired through vaginal or anal intercourse, but rarely HPV may be transmitted by oral-genital sex. The male condom is not as effective at preventing HPV transmission as it is for the prevention of other STDs; the male condom does not prevent all skin-to-skin contact during sex. [56] The role of condoms in HPV prevention is exaggerated among adolescents most of whom believe it is fully protective while some believe oral contraceptives are protective. [60] Some studies have also demonstrated limited knowledge on the availability of HPV vaccine and negative attitude towards HPV vaccination by parents. [61]

Human papilloma virus screening/ diagnosis

Various methods have been used to screen for HPV infection. This include: clinical examination, cytology, colposcopy, histology, Electron microscopy, Immunoperoxidase, DNA Hybridization and antibody detection.

Cytology has been used as the standard for secondary prevention as it is cost effective resulting in a marked reduction in cancer deaths. [62] The sensitivity of conventional pap smears is low approaching 57.7 % hence the need for recurrent screening. [62, 63] Use of liquid based cytology increases the sensitivity to 84.4 % while colposcopy has a 70 % sensitivity. [63, 64]

DNA based methods have a high sensitivity with a low specificity and a high negative predictive value. [63, 65] It is recommended by the American Society of Colposcopy and Cervical Pathology (ASCCP) for triaging patients with Atypical Squamous Cells of Unknown Significance (ASCUS) so as to reduce the number of unnecessary colposcopies. [66] It is also recommended for primary screening in

conjunction with cytology in women more than 30 years. [65, 67, 68] Use of HPV DNA analysis to routinely screen adolescents is not recommended since most of the infections are transient. [8, 9, 11, 23]

The sensitivity and specificity for recurrence in patients with previous ablative or surgical treatment is better with HPV DNA as opposed to Pap smear hence is a better diagnostic method for recurrences. [68, 69]

The common HPV DNA detection methods PCR and Hybrid II Capture methods have been shown to have similar sensitivity and specificity [70] though Hybrid II Capture can detect individual genotypes. [69]

1.3 Justification

There is a worldwide variation in HPV prevalence across age groups which may reflect differences in sexual behavior between different regions thus studies of HPV prevalence in adolescents are needed for all geographic regions.

The prevalence and distribution of HPV types in Kenya adolescent population is unknown. The attendants of youth clinic represent the high risk group. HPV screening is expensive thus not routinely recommended before vaccination. Results from this study will be useful in setting up criteria that will be used to triage adolescents who will benefit from the HPV vaccine.

This study will help to determine the knowledge on HPV transmission and prevention among adolescents in the KNH clinic. The findings will be used to formulate and implement measures for advocacy in prevention of HPV infection.

1.4 Research Questions

1. What is the Prevalence of HPV among adolescents attending Youth clinic at KNH?
2. What is the level of Knowledge on HPV transmission and prevention among high risk adolescents?

1.5 Objectives

Broad objective

To determine the prevalence of HPV infection by DNA typing and knowledge on HPV transmission and prevention among adolescents attending Youth clinic at Kenyatta National Hospital.

Specific objectives

1. To determine by DNA typing the prevalence of HPV infection among adolescent girls at Kenyatta National Hospital Youth Clinic
2. To determine the socio-demographic characteristics of HPV positive adolescents at Kenyatta National Youth Clinic
3. To determine the relation between HPV infection and coexistence of other sexually transmitted infections
4. To determine the relationship between HPV infection, number of sexual partners and sexual debut
5. To determine the adolescents' knowledge on HPV, its transmission and prevention

CHAPTER 2

METHODOLOGY

2.1 Study design

This was a hospital based cross sectional study .The study set out to determine the point prevalence of HPV among adolescents hence a cross-sectional study was appropriate . The study population was girls who attended the Youth clinic.

2.2 Study site

The study was undertaken at Kenyatta National Hospital Youth clinic. KNH is a national teaching and referral hospital in Nairobi, Kenya, situated about three kilometers from the city centre.

2.3 Study population

All adolescent girls aged 12-26 years who attended the Youth clinic within the study period. Youth clinic is a youth friendly clinic that was established in 1989 to cater for special needs of adolescents. It attends to an average of 25 youths per day who present for various reasons including treatment for sexually transmitted infections, drug abuse, teenage pregnancies, and consultation for menstrual abnormalities among others. All adolescents girls who were eligible and gave an informed consent or those whose parents or guardians were available to give the consent were recruited in the study.

2.4 Respondent Selection

2.4.1 Inclusion criteria

1. All adolescents aged 18 years and above who consented to the study and those under 18 years parental consent was requested.

2.4.2 *Exclusion criteria*

1. Clients who had active vaginal bleeding
2. Clients who were not psychologically or physically sound to make an informed consent
3. Clients who were expectant at the time of the study
4. Clients who were virgins

2.5 Sample Size Calculation and Sampling Procedure

Sample size calculation

This was done using the formula for prevalence rates:

$N= (Z^2pq)/d^2$

Where N=sample size

P=prevalence rates, taken to be 22 %; the estimated rate for African countries

q = 1-p

d = level of significance at 95 % confidence interval, 0.05

Hence $N = [(1.96)^2*0.22*0.78]/0.0025 =264$

Sampling Procedure

Simple random sampling was used. All adolescent girls who met the study inclusion criteria and consented to the procedure were selected. This represented a random sample of the adolescent girls that attended the Youth clinic between December 2009 and October 2010.

2.6 Data collection methods & Procedure

Materials/Tools

Questionnaires,pens, private room, sterile normal saline, cervical brushes, speculums, specimen transport media and cold box.

Recruitment and consenting of study patients

264 girls were recruited in the study from the adolescent clinic. Recruitment was done from Monday to Friday. Screening of clients for fulfillment of inclusion/exclusion criteria was done through direct interviewing of respondents. This was done by the Principal investigator.

Adolescent girls who attended the clinic within the study period and who met the study criteria were informed about the study. A written informed consent was sought from those aged 18 years and above. For those aged less than 18 years, the parents or guardians were approached for the consent.

Type of Data collected

Respondent's characteristics- age, marital status, level of education, gynaecological history, sexual history and social history. Knowledge on HPV transmission, consequences of HPV infection and Prevention. Number of patients with HPV infection- Low risk and high risk genotypes.

Methods of Data Collection

Administration of a questionnaire- all information collected from each respondent was filled in a questionnaire; this included respondent's characteristics, knowledge on HPV transmission and prevention and number of respondents with HPV infection.

The specimen collection procedure was explained and the respondents were directed to a private room for specimen collection.

Respondents were put in lithotomy position, sterile procedure were observed, speculum was introduced into the vagina and the cervical swab was taken by using a cervical brush. The swabs were placed in a labeled glass tube containing a preservative and stored in a cold box.

The cold box containing specimens was transported to KEMRI HPV Laboratory for DNA analysis on the same day of collection before 15 HRS. Laboratory forms accompanying participant's specimen were given unique number for each of the respondents. This unique number was also labeled clearly in the respondent questionnaire.

All specimens collection were done by the Principal investigator.

DNA analysis was done by using PCR and Positive samples were genotyped by Slit blot Hybridization.

Details of DNA analysis for HPV is as follows:-

- **Processing of the cervical swab for HPV**
  - **DNA Extraction**

The extraction of the DNA was carried out using the AL Buffer as a lytic buffer to the cervical cell previously obtained from the respondent. The proteinase K was introduced into the sample for the purpose of destruction of the RNA found in the cell contents to it's respective bases.

Introduction of absolute ethanol brought about precipitation of all the proteins in the mixture that was then followed by centrifugation of the same so as to sediment the proteins. The supernatant which contains the DNA templates and the suspended bases of the destroyed RNA were then be subjected to the spin column onto which the DNA adsorbed as the rest of the stuff is washed down the column into the collecting tube. After washing the adsorbed DNA twice with AW1 and AW2 respectively, The DNA was then eluded into a 1.5ml eppendoff tube using the AE buffer or distilled water.

The sample so collected was introduced into the next step of amplification or stored under -30^{0}C for later use.

- **HPV amplification**

  The samples having been removed from the -30^{0}C fridge, where they had been stored immediately after DNA extraction, a mixture of the sample with a volume of the Master mix which comprises of; PCR water, buffer gold, dNTP mix, GP_5, GP_6. Taq polymerase, was made and dispensed into 0.2ul PCR tubes. These were then loaded into the thermocycler that uses the pre-programmed thermocyclic cycles ranging in temperatures from 95^{0}C, 50^{0}C, 72^{0}C, and 4^{0}C respectively, thus amplifying the DNA templates of interest till the desired detectable range was attained by the end of the 35 thermocyclic cycles.

  - **Electrophoresis**

    2% (0.8g)or 3% (1.2g) agarose gel was dissolved in to 40ml of 1% TAE buffer and warmed or micro waved to dissolve and was allowed to stand till it attained the temperature of approximately 56^{0}C.Ethidium bromide added and then it was dispensed onto the setting tray and left to stand un-interrupted till it solidified.

    The comb for setting the wells was removed, leaving back the impressions of the wells on the gel and the gel tray was transferred to the electrophoresis

tank. The samples DNA mixed with the loading buffer was then loaded on to the gel in readiness for electrophoresis ran.

- **Detection**

At the end of the electrophoresis run the gel was transferred into the UV hood that was interlinked with the monitor and a printer that then recorded the image detected and subsequently printed it.

❖ **Typing of HPV**

- **Hybridization:**

The HPV typing by slit blot method involves the following standardized procedure;

The DNA so amplified, was then mixed with denatured 0.4M NaOH, incubated at 37^{0}C for a period of 5min, then embedded onto the hybond N+ nylon membrane in readiness for hybridization.

The pre-hybridization was first undertaken without type specific probes at 37^{0}C for only 5min then the pre-hybridization solution was replaced with the hybridization solution mixed with type-specific hybridization probes. The temperature of hybridization under this condition was 50^{0}C for over 30min or overnight.

Rinsing was done thereafter using buffer A and later followed with buffer A mixed with Tween 20 twice at the interval of 10min each.

- **Blocking**

The membrane was rinsed with buffer A and incubated in a tray containing 20-50ml of blocking solution at room temperature for 30min.

- **Incubation**

The membranes were then incubated in a tray containing 20ml of reaction solution with 8ul of anti-alkaline phosphate antibody for one hour at room temperature.

- **Detection**

1. After removing all traces of buffer using a paper towel, the strips were arranged on the polythene paper and 1-2ml of CDP- Star detection reagent added on the membrane and left for 2min.
2. All the membranes were set on polythene bag in the Lumishot Camera and an acquiring period to 10-30 sec was set, then the film was pulled out and let to develop for 2sec then ripped open to observe the results

Quality Assurance

Pre analytical

Cervical specimens were collected by the principal investigator himself who ensured that the standard procedure of collection was observed.

The samples were put into labeled glass tubes containing preservative and were stored in a cold box.

All specimens were transported to the HPV lab for analysis on the same day.

Laboratory forms accompanying the participant's specimen were given unique numbers for each of the respondents. This unique number was also labeled in the respondent's questionnaire.

Analytical

The laboratory number of the specimen was counterchecked to ensure correspondence. The standardized protocol from QIAGEN that is internationally recognized was used for processing the samples for DNA extraction, amplification,

ophoresis and detection of HPV. Genotyping of various types of HPV was done ing standardized protocol for hybridization.

analytical

rding of the result to the questionnaire was carefully done, ensuring spondence between assigned laboratory number and client's questionnaire.

Data Management

generated from the study was stored in a computer under password protection backed up on an external hard drive and CD. The hard drive and CD was under safe custody of the principal investigator. Each entry had the unique study ber so as to protect the privacy of the study participants. The data collection tionnaires were filed and stored in a safe cabinet where verification of results can done whenever necessary. The primary data was captured as MS Excel adsheets. The data was then exported into a Statistical Package for Social tists version 17 (SPSS, Chicago) software, which was used for data analysis. A p- e of <0.05 was considered statistically significant.

Data analysis

ographic data on study participants was presented in tables. This includes such as age, occupation, educational level and marital status. Sexual history he study participants was presented in numbers and percentages. The ciation between the outcome variable (HPV status) and categorical pendent variables such as concurrence, circumcision etc. was determined g either the Pearson Chi Square test or the Fishers Exact test. The strength of tatistically significant associations ($p<0.05$) will be measured using logistic ession. Logistic regression modelling was used to measure the strength of ciation between the HPV status (outcome variable) and the following

continuous independent variables; age of first intercourse, number of lifetime sexual partners. HPV infection and coexistence of other sexually transmitted infections has been displayed in table form as absolute numbers and percentages.

2.9 Ethical considerations

Authority was sought from Kenyatta National Hospital ethical and research committee. The study was undertaken after a formal approval by the committee. A written and informed consent was obtained before clients participated in the study. No client was forced, coerced or financial inducements used to influence her choice to participate in the study. Clients who were not willing to participate in the study were assured of good services as any other clients. The process of specimen collection was not harmful to the clients. All information obtained from clients has been handled with confidentiality.

The clinic number and contacts appears on the questionnaire for the purpose of follow up. Result of the test was communicated to the patient for further appropriate interventions and follow up.

2.10 Study Limitations

The main study limitation in this study was recall and information bias with regard to sexual history. In addition 7 patients did not have HPV results.

The study being a cross-sectional, it lacks power to determine associations between independent and dependent variables.

CHAPTER 3

RESULTS

3.1 Demographic Characteristics

A total of 264 female participants were enrolled into the study. Out of all the enrolled participants, 7 did not have HPV results. The mean age was 22.6 years (SD=23 years)(as shown in Table 1 below).

Table 1: Demographic characteristics of the adolescents that participated in the study

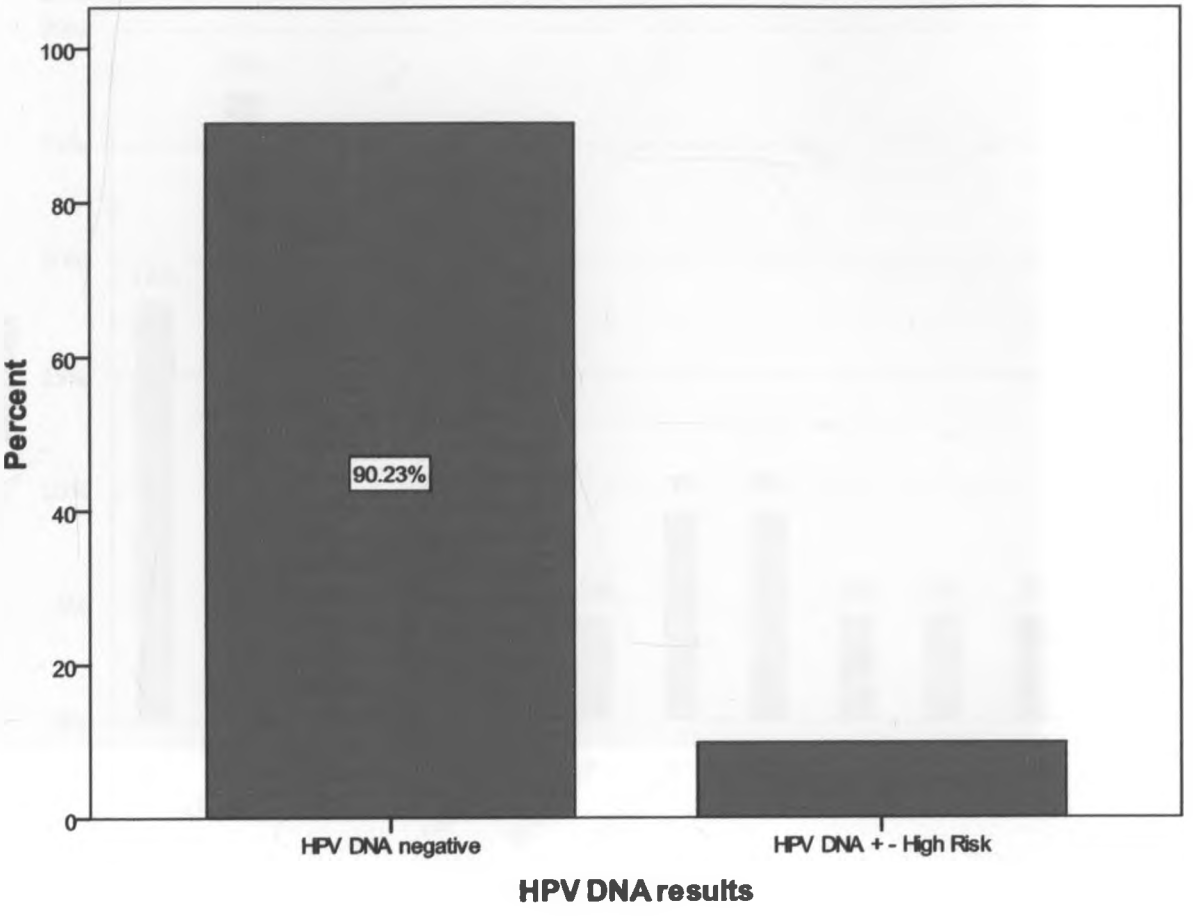
Patient characteristics	HPV negative	HPV positive
Median age (Min, Max) n=256	22.7 (16,25)	22.0 (17,25)
Marital status	n,(%)	
Single cohabiting	79(34)	10(40)
Singe not cohabiting	119(52)	13(52)
Married	28 (12)	0
Divorced	3(1)	1(4)
Widowed	2(1)	1(4)
Education level		
Primary	19 (8.3)	1(4)
Secondary	19(8.3)	2(8)
College	185(81.1)	21(84)
University	5(2.2)	1(4)
Spouse's Education level		
None	2	0
Primary	6	0

Patient characteristics	HPV negative	HPV positive
Secondary	12	0
Tertiary	48	8
University	3	0

Most of the study participants had college education or more. Those who are single and yet cohabiting were 34.8%. The majority of the respondents (86.3%) were single.

Prevalence of HPV infection and distribution of HPV strains among study rticipants

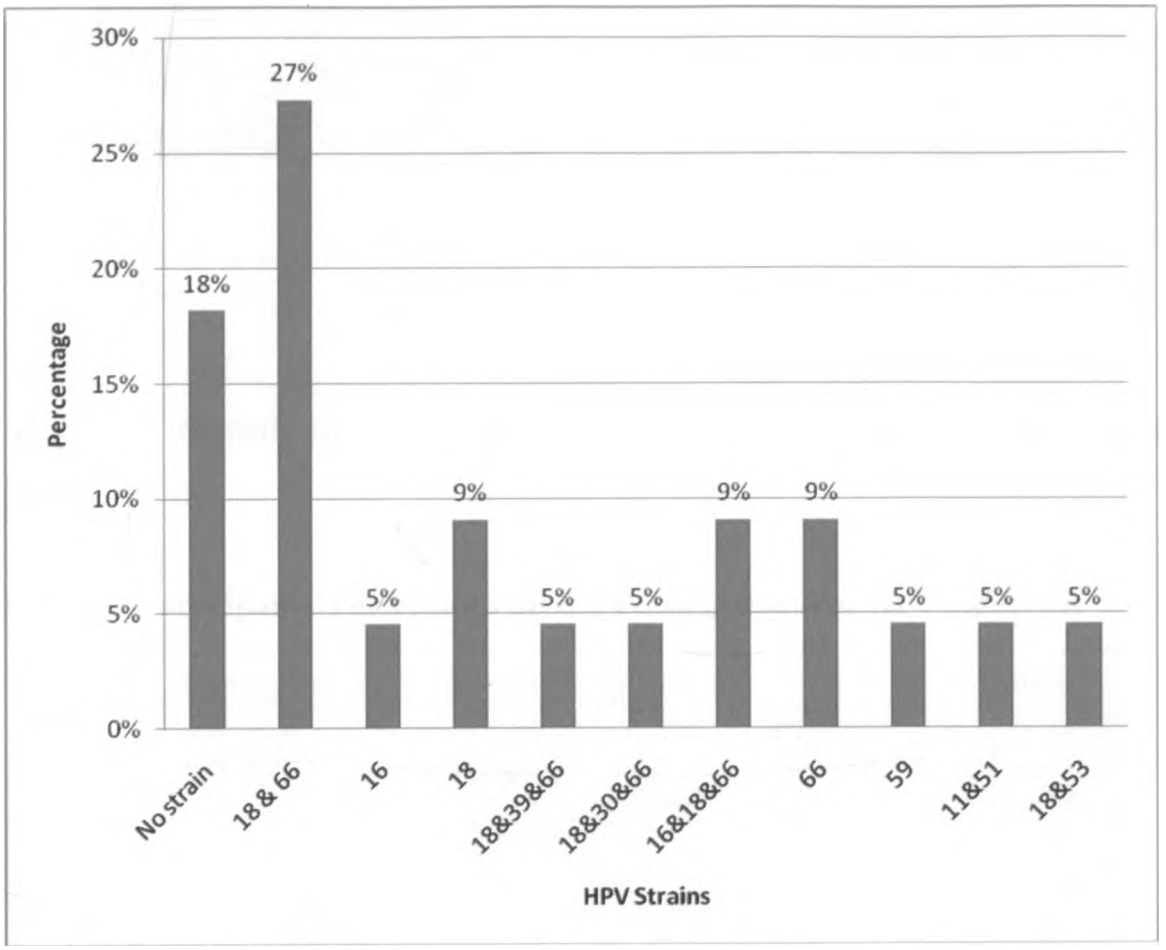
gure 1: Prevalence of HPV infection among adolescents at KNH (n=256)



ut of the 256 participants who were tested, 25 (9.77 %) had positive HPV DNA sults. The prevalence of HPV infection was found to be 9.77 % (95% CI: 6.13 to 41). This has been illustrated in figure 1.

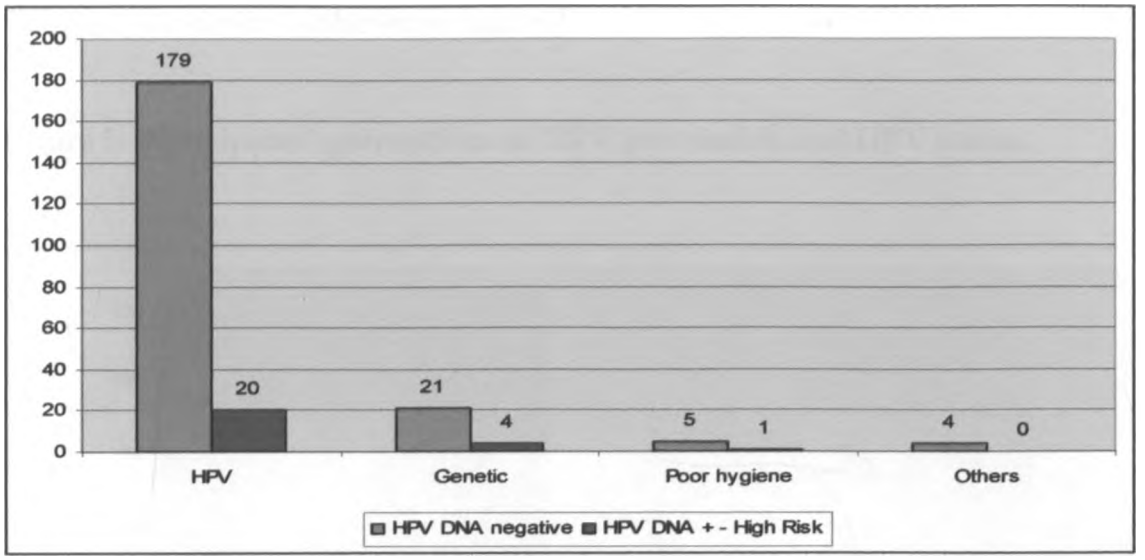
Out of the 256 participants who were tested, 25 (9.77%) had positive HPV DNA results. The prevalence of HPV infection was found to be 9.77% (95% CI: 6.13 to 13.41).this has been illustrated in the figure 1 above.

Figure 2: Distribution of the HPV strains



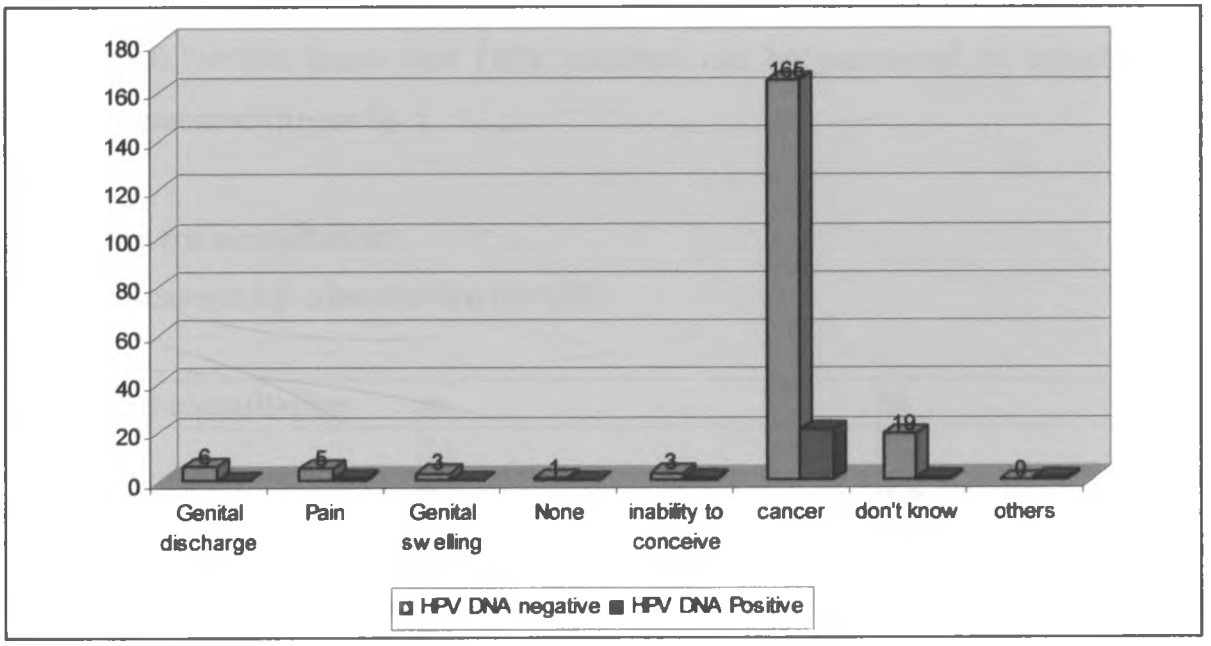
Most of the participants, who were positive, had multiple serotypes with majority 27% infected with HPV strain type 18 and 66. There was no Low risk strains isolated and 18% of the respondents who tested positive for HPV DNA had uncharacterised strains.

Figure 3: Participants' Perception of What Causes Cervical Cancer



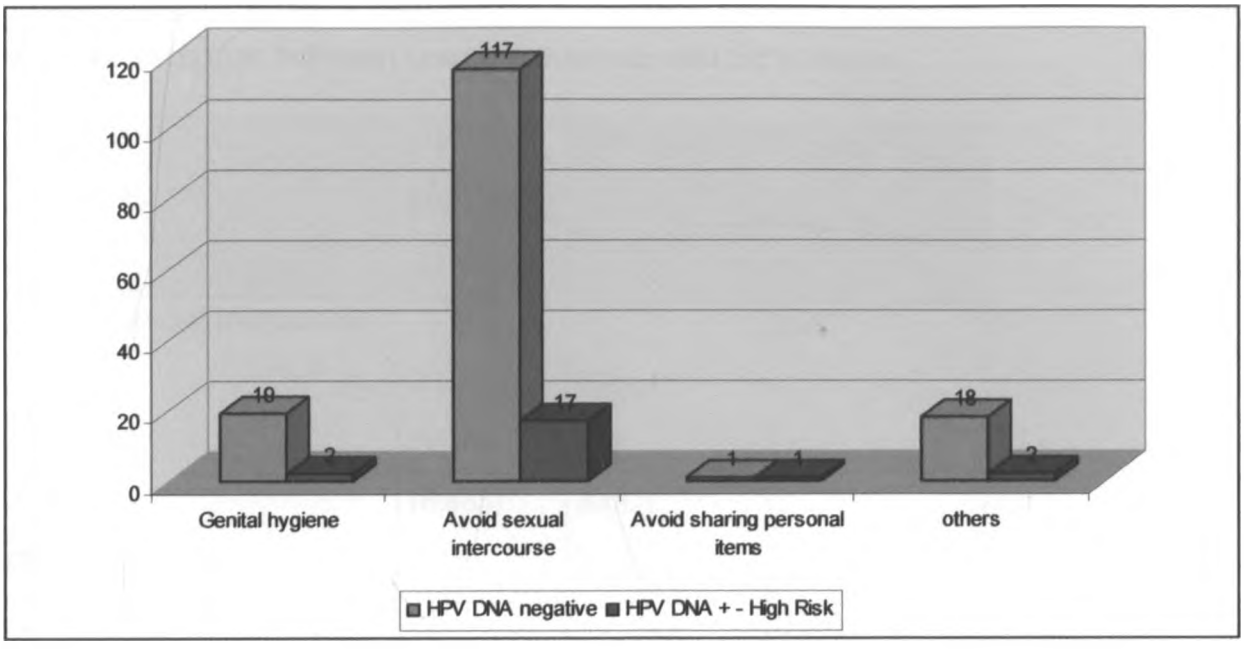
Majority of the study participants recognize that HPV is the cause of cervical cancer (figure 3).

Figure 4: Participants' Perceptions on the Consequences of HPV Infection



More than 88% (n=165) of the participants knew that HPV infection may lead to cancer of the cervix as shown in figure 4.

Figure 5: Participants' perceptions of HPV prevention and HPV status.



Of study participants who gave their perceptions of HPV prevention, majority of them 79.8% (n=134) knew that HPV infection can be prevented by avoiding sexual intercourse (figure 5).

3.3 Reason for consultation

Table 2: Reasons for consultation (n=165)

Reason for consultation	n	%
STI	13	7.9
Drug abuse	2	1.2
Teenage pregnancy/puerperium	4	2.4
Counseling	94	57.0
Others	52	31.5

Total	165	100.0
Missing	98	

Majority of the consultations were for counseling 57% (n=94) as shown in table 2.

3.4 Association between sexual behavior and HPV status

Table 3: Association between sexual behaviour and HPV status

	HPV negative n,(%)	HPV positive n,(%)	P value
Age at first sexual intercourse			0.273
<10 years	2(1)	1(4)	
10-15 years	31(14)	2(9)	
>15 years	189(85)	20(87)	
Missing/Unknown	9	2	
Number of lifetime partners			0.882
<2	138(63)	14(67)	
3-5	60(27)	6(28)	
>5	22(10)	1(5)	
Missing/Unknown	11	4	
Sexual partners in the past 6 months 2	189(88)	20(91)	>0.999
3-5	21(10)	2(9)	
>5	4(2)	0	
Missing/Unknown	17	3	

	HPV negative n,(%)	HPV positive n,(%)	P value
Frequency of intercourse per week			
<=1	147(69)	17(89)	0.164
2-3	42(19)	1(5.5)	
>3	25(12)	1(5.5)	
Missing/Unknown	17	6	
Practice douching after sex			0.446
Yes	46(22)	6(32)	
No	161(78)	13(68)	
Missing/Unknown	24	6	
Ever treated for an STI			0.951
Yes	31(14)	3(12.5)	
No	139(62)	16(67)	
Not sure (Discharge)	55(24)	5(20.5)	
Missing/Unknown	6	1	
Does current partner have another partner			0.574
Yes	14(7)	3(13)	
No	100(47)	10(43.5)	
Do not know	99(46)	10(43.5)	
Missing/Unknown	18	2	

	HPV negative n,(%)	HPV positive n,(%)	P value
Is your partner circumcised			0.810
Yes	178(84.4)	20(87)	
No	26(12.3)	3(13)	
Did not know	7(3.3)	0	
Missing/Unknown	20	2	

Association between sexual behavior and HPV status was not statistically different between study participants who were HPV negative and HPV positive. The factors were: age at first sexual intercourse, number of lifetime partners, sexual partners last 6 months, frequency of intercourse per week, practice douching after sex, ever treated for an STI, if current partner has another partner, if partner was circumcised (table 3).

3.5 Association between HPV infection and STIs

Table 4: HPV Infection and Coexistence of other Sexually Transmitted Infections

	HPV negative n,(%)	HPV positive n,(%)	P- value
Past or current history of STIs			
Yes	31(18)	3(16)	0.951
No	139(82)	16(84)	
Unknown	61	6	

There was no statistically significant association between HPV infection and coexistence of other sexually transmitted infections as shown in table 4.

Table 5: Association Between HPV status and Knowledge About HPV and Its acquisition.

Patient characteristics N=255	HPV negative n,(%)	HPV positive n,(%)	P value
Know about HPV			0.018
Yes	161(70)	23(92)	
No	70(30)	2(8)	
Know how HPV is acquired			0.068
Poor hygiene	5 (3)	0	
Sexual intercourse	159 (96)	22(92)	
Sharing instruments	0	1(4)	
Other	1(1)	1(4)	
Unknown	66	1	

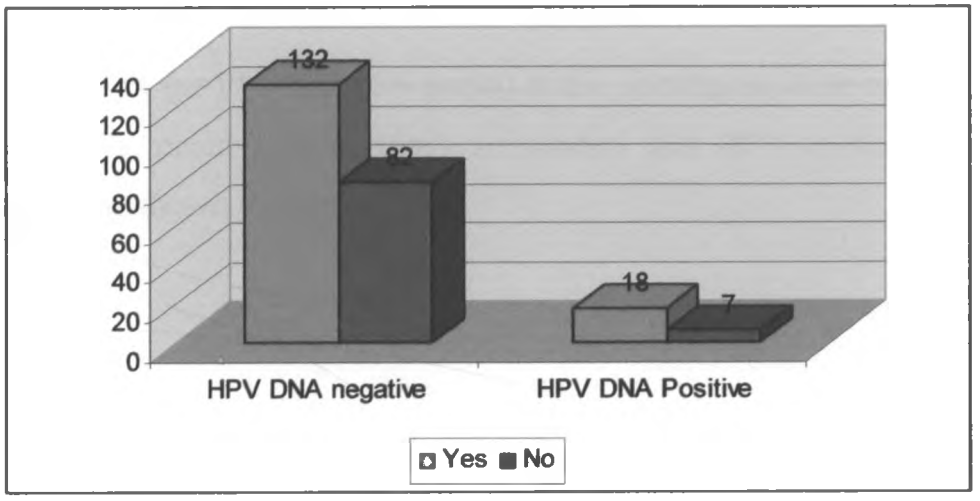
Of the study participants 72% (n=184) had knowledge of HPV and majority of participants had knowledge that HPV is sexually transmitted. The knowledge of HPV by the adolescents and infection status was statistically significant (p=0.018). None of the other knowledge factors were significantly associated with the infection status. Majority of the participants responded that HPV infection is acquired through sexual intercourse, this was however not significantly associated with HPV infection (p=0.068).

Table 6: Association between Perceptions of the Role of Condoms and HPV transmission

Role of condoms in HPV transmission	HPV DNA results		P value
	HPV DNA negative n%	HPV DNA positive n%	
Eliminates/Reduces HPV transmission	177(88)	19(79)	0.423
Has no effect	24(12)	5(21)	
Unknown	30	1	

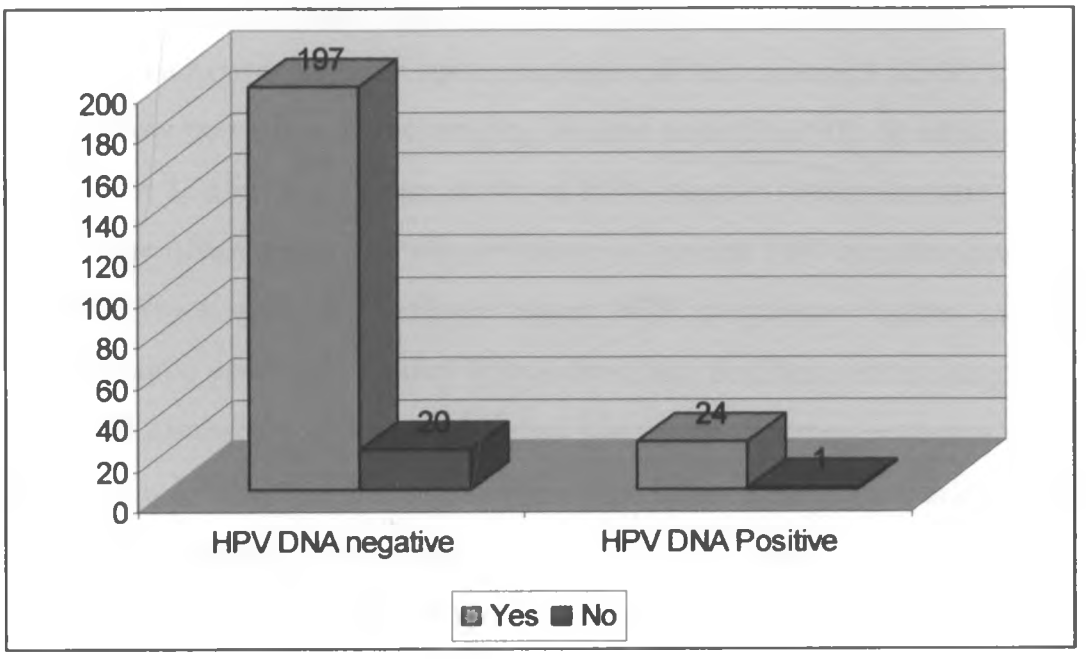
88% of participants who were HPV negative believed that condoms play a key role in reducing HPV transmission. This association with HPV infection status was however not statistically significant (p=0.423) as shown in table 6.

Figure 6: Association between knowledge of HPV vaccine and HPV status



Majority of the respondents (n=150) had knowledge on HPV vaccine .The association between this knowledge and HPV status was not statistically significant (p=0.385) as illustrated in figure 6.

Figure 7: Association between knowledge of HPV as a Cause of Cancer of the Cervix and HPV status



About 93% (n=197) and 96% (n=24) of the participants who were HPV negative and HPV positive respectively responded that HPV is the cause of cervical cancer as shown in figure 7.

CHAPTER 4

DISCUSSION

The prevalence of cervical HPV infection as defined by a positive HPV DNA test was 9.8% among adolescent girls at Kenyatta National hospital. This prevalence is lower than World Health Organization estimates for African countries of 22 % [17] and that from a Kenyan study done among high risk men around Lake Victoria of 53 %. [18] This low prevalence is encouraging because more than 90% of adolescent girls aged between 12 and 26 years are at risk of HPV infection. A similar study done in Gambia, West Africa shown that the prevalence of cervical HPV infection among girls aged 15-24 years was 15%[78]. Much higher HPV prevalence figures have been reported in some unselected studies from Eastern and Southern Africa, ranging from 34% in rural Zimbabwe [74] to 44% in urban Kenya. [73] Human immunodeficiency virus (HIV) infection and concomitant immune suppression is an acknowledged cofactor in the progression of cervical cancer [75,76] and such high HPV prevalence may be due to the high rates of HIV infection in these regions. [77]

Twenty five samples (9.8%) were HPV-DNA-positive by PCR. The prevalence of HPV types is described in Figure 1. A total of 8 different HPV types were detected. Of the positive samples, 4 (16%) were positive for a single HPV type, 17 (68%) were samples containing more than one HPV type (mixed: 32%, two types and 16%, three types). The most prevalent HPV types were HR types HPV 18 and 66. An uncharacterized type was found in 16% of the HPV-positive samples. In a study to assess distribution of HPV in family planning population in Nairobi, Kenya shown that the most prevalent HPV types were HR HPV 16, 18, 31, 35, 52, 53, 58, and 66. [73] This study has shown that HPV 16 was not among of the most frequently HPV sub type.

Sexual behavior is the key to HPV infection. In this study there was no statistical difference between those with a positive HPV test and negative HPV test with regards to sexual behavior, however this study being a cross-sectional has inadequate power to determine and detect associations between different characteristics and HPV status. Other studies have demonstrated that, in adolescent girls ,increased incidence of HPV infection is associated with early onset of sexual activity, multiple sexual partners, presence or a history of sexually transmitted infections, anal intercourse, smoking [1,23,27] and less consistently : frequent sexual intercourse , use of combined oral contraceptives, pregnancy and abortions. [11,27,28,29,30] Lack of circumcision is an additional risk factor both for men and their sexual partners. [35, 36]

The knowledge of HPV was 71.8% (Table 4), this was high unlike earlier studies that showed low knowledge in the general public. [55, 57, 58, 59] This is attributed to the fact that most study participants have a college education and more. These findings are encouraging because acceptability and effective immunization against HPV depends on adolescents and their parents' knowledge on HPV, its transmission, prevention and sequelae.

Most of the HPV negative participants who have heard about the HPV vaccine were HPV negative. The association between this knowledge and HPV status was not statistically significant (p=0.385) as illustrated in figure 5. A causal association between cervical cancer and HPV has been documented beyond reasonable doubt [42] and for this reason HPV vaccination is one of the most feasible ways of primary prevention of cervical cancer. The biggest challenge impeding most health care systems in scaling up HPV vaccination as a way of primary prevention of cancer of the cervix is the cost of the currently available vaccines.

Majority (70 %) of the study participants had knowledge that condoms reduce transmission of HPV. More education is needed regarding condoms to avoid

misconception that it prevents transmission. Studies have shown that the role of condoms in HPV prevention is exaggerated among adolescents most of whom believe it is fully protective. [60] The male condom is not as effective at preventing HPV transmission as it is for the prevention of other STDs; the male condom does not prevent all skin-to-skin contact during sex. [56]

CONCLUSION

The prevalence of HPV among adolescent being cared for at Kenyatta National Hospital was (9.8%) and most prevalent subtypes were HR HPV types 18 and 66 . The knowledge of HPV transmission and prevention was high.

RECOMMENDATIONS

- National study should be conducted to determine the national prevalence of HPV infection.
- Advocacy on HPV and its etiological association to cancer of the cervix
- HPV vaccine as a primary prevention method should be introduced in the National vaccination programme.

REFERENCES

1. Winner RL, Lee SK, Hughes JP et al. Genital HPV infection: Incidence and risk factors in a cohort of female University students. Am J E pidemiol 2003; 157; 218-228.
2. Giles. Transmission of HPV. Can Med Assoc 2003; 168(11):1391-139
3. Watts DH, Koutsky LA, Holmes KK. Low risks of perinatal transmission of Human Papillomavirus: results from prospective cohort .Am J Obstet Gynecol 1998; 178(2):365-373
4. Parkins DM.The global health burden of infections associated cancers in the year 2000. Int J Cancer 2006; 118 (12):3030-44
5. Zur H H. Papillomavirus infection-a major cause of human cancers.Biochim Biophys Acta 1996; 1288(2):F55-78
6. Nubia M, Xavier FB. Epidemiologic classification of HPV type associated with cervical cancer E J M 2003; 348:518-527
7. Stanley M. Prophylactic HPV vaccines. Journal of Clinical Pathology 2007; 60:961-965
8. Weaver BA. Epidemiology and Natural history of Genital HPV infection. J Am Osteopath Assoc 2006; 106(suppl 1):S2-S8
9. Merck medicus Modules. HPV disease: Natural History of HPV infection; 2006
10. Sciffman M, Herrero R, Desalle M at el. The carcinogenicity of HPV types reflects viral evolution. Virology 2005; 337 (1):76-84
11. Scheurer ME, Tortolero LG, Adler SK. HPV infection: Biology, epidemiology and prevention. International Journal of Gynecological Cancer 2005; 15(5):727-746
12. Moscicki AB, Ellenberg JH, Farhat S. Persistence of HPV infection in HIV infected and non-infected adolescent girls: risk factors and differences, by phylogenetic type .J Infec Dis 2004; 190 (1): 37-45
13. Lorincz AT, Castle PE, Sherman ME et al. Viral load of HPV and the risk of CIN3 or Cervical Cancer. Lancet 2002; 360(9328):228-29

14. Burchel AN, Winner RL. Epidemiology and transmission dynamics of genital HPV infection. Vaccine 2006(suppl 3):52-61
15. Villa LL. Prophylactic HPV vaccine: reducing the burden of HPV related diseases. Vaccine 2006; 245:23-28
16. Cliff GM, Gallus S, Herrero R. World wide distribution of HPV types in cytologically normal women. Lancet 2005; 366:991-8
17. Aslori G,Castellsague X,Chaouki N et al.WHO/ICO information Centre for HPV and Cervical Cancer; WHO 2007
18. Ngayo MO, Bukusi E, Rowhani AR at al. Epidemiology of HPV infection among fishermen along Lake Victoria Shore in Kisumu District ,Kenya. Sex. Transm. Inf; 2008; 84(1):62-66
19. Smith JS, Lindsay L, Keys J et al. HPV type distribution in invasive cervical cancers and high grade cervical neoplasia: an update of meta-analysis and identification of global data gaps. Br J Cancer 2007
20. Dunne EF, Unger ER, Sternberg M et al. Prevalence of HPV infection among females in the U.S. JAMA 2007;297: 813-19
21. Gostin LO, Deangellis CD .Mandatory HPV vaccine. JAMA 2007; 297:1921-23
22. Clifford G, Franceschi S, Diaz. HPV type distribution in women with and without neoplastic disease. Vaccine 2006; 24 (suppl 3): 26-34
23. Moscicki AB. Natural history of HPV in adolescents and relationship to cervical cancer. Clinical Oncology 2007; 103-111
24. M. Molano, H. Posso, E. Weiderpass et al. Prevalence and determinants of HPV infection among Colombian women with normal cytology. British Journal of cancer (2002)87,324 -333
25. Gloria YF, Bierman R, Beardsley L. Natural history of cervicovaginal Papillomavirus infection in young women. NEJM 1998; 338:423-428
26. Herrero R, Hildesheim A, Bratti C at al. Population based study of HPV infection and cervical neoplasia in rural Costarica .J Natl Cancer Inst 2000; 92:464-74

27. Pham TH, Nguyen TH, Herrero R et al. HPV infection among women in south and North Vietnam .Int J Cancer 2003; 104:213-20
28. Tarkowski TA, Koumans EH, Sawyer Met al. Epidemiology of HPV infection and abnormal cytologic test results in an urban adolescent population. J Infect Dis 2004; 189:46-50
29. Karlsson R, Johnson M, Edlund K et al. Lifetime number of sexual partners as the only independent risk factor for HPV infection: a population based study. Sex Trans Dis 1995; 22:119-27
30. Bauer HM, Hildesheim A, Shiffman MH et al. Determinants of genital HPV infection in low risk women in Portland; Oregon. Sex Transm Dis 1993; 20:274-78
31. Burk RD,Ho GY,Beardsley L.Sexual behavior and partner characteristics are the predominant risk factors for genital HPV infection in young women Infect Dis 1996;174(4):679-89
32. Winer RL, Hughes JP,Feng Q at al. Condom use and risk of genital HPV infection in young women. NEJM 2006; 354(25):2645-2654
33. Svare EI, Kjaer SK, Worm AM et al. Risk factors for genital HPV DNA in men resemble those found in women; a study of male attendees at a Danish STD clinic :Sex Trans Dis 2002; 78 :215-18
34. Lajous M, Mueller N, Aurelio CV et al. Determinants of prevalence, acquisition and persistence of HPV in healthy Mexican Military men. Cancer Epidem Biomarkers and Prevention 2005; 14: 1710-16
35. Xavier C, Bosch FX, Nubia M et al. Male circumcision; penile HPV and cervical cancer in females.NEJM 2002; 346:1105-12
36. Casellsaque X, Ghaffari A, Daniel RW et al. Prevalence of penile HPV DNA in husbands of women with and without cervical neoplasia:a study in Spain and Columbia. J Infect Dis 1997; 176(2):353-61

37. Anttila T, Saikku P, Koskela P et al. Serotype of Chlamydia trachomatis and risk of development of cervical squamous cell carcinoma.JAMA 2001; 185(1):47-51

38. Smith JS, Herrero R, Bossett C et al. HSV-2 as a cofactor in the etiology of invasive cervical carcinoma. Journal of National Cancer Inst 2002; 94(21):1604-1613

39. Minkoff H, Fieldman JG, Strickler HD. Relationship between smoking and HPV infection in HIV infected and non-infected women. J Infect Dis 2004; 189(10):1821-8

40. Heard I, Tassie JM, Schmitz V et al. Increased risk of cervical disease among HIV infected women with severe immunosuppression and high viral load. Obstet Gynecol 2000; 96:403-9

41. Garcia-Pineres AJ, Hildesheim A, Herrero R et al. Persistence of Human papillomavirus is associated with generalized decrease in immune responsiveness in older women. Cancer Res 2006; 66: 11070-11076

42. Bosch FX, Lorincz A, Munoz N.The causal relation between HPV and cervical cancer: Journal Clinical Pathol 2002; 55: 244-65

43. Munoz N, Castellsague X, Berrington A et al. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006; 24(suppl 3):1-10

44. Schiffman M, Castle PE, Jevonimo J et al. HPV and cervical cancer. The Lancet 2007; 370:890-907

45. Castellsague X, Diaz M, Munoz N et al. World wide Human Papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. J Natl Cancer Inst 2006; 98: 303-15

46. Hubert NK, Sedman KM, Shiller JT. HPV type 16 E6 increases degradation rate of P53 keratinocytes.Journal of Virology 1992; 66: 6237-6241.

47. Moscicki AB, Hills N, Shiboski S et al. Risk of incidence HPV infection and Low grade squamous intra-epithelial development in young females. JAMA 2001; 285(23):2995-3002

48. Brisson J, Bairati I, Morin C. Determinants of persistent detection of HPV in cervix. J Infect Dis 1996; 173(4):794-9

49. Prokopczyk B,Cox JE,Hoffman D et al. Identification of tobacco specific carcinogen in cervical mucus of smokers and non smokers Natl Cancer Inst 1997;89:868-73

50. Ho GY, Kadish AS, Burk RD. HPV 16 and cigarette smoking as risk factors for high grade intra-epithelial noaplasia.Int J Cancer 1998;78:281-5

51. Lacey JV, Brinton LA, Abbas FM et al. Oral contraceptives as risk factors for cervical adenocarcinomas and squamous cell carcinoma. Cancer Epidemiol Markers Prev 1999; 8:1079-85

52. Xavier C, Nubia M. Cofactors in HPV carcinogenesis –Role of parity: Oral contraceptives and Tobacco smoking.JNCI 2003; 31:20-28

53. International Agency for Research on Cancer .IARC monograph on the evaluation of carcinogenic risk to humans. Hormones may promote integration of HPV into host genome; resulting in dysregulation of E6 and E7. HPV 1995, vol 64.

54. Vaccarella S,Herrero R,Dai M et el. Reproductive factors; Oral contraceptive use;HPV infection; pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol. Biomarkers Prev; 2006; 15(11):2148-2153.

55. L.A.V Marlow, J Waller and J Wardle. Public Awareness that HPV is a risk factor for cervical cancer. British Journal of Cancer(2007)97,691-694

56. Burk RD, Ho GY, Beardsley L, Lempa M, Peters M, Bierman R. Sexual behavior and partner characteristics are the predominant risk factors for genital HPV infection in young women. J Infect Dis 1996; 174(4): 679-689

57. Waller J, Caffery KM,Forrest S et al. Awareness of HPV among women attending a well women clinic : Sex Transm Infect 2003; 79: 320-322

58. Dell DL, Chen H; Ahmad F et al. Knowledge about HPV among adolescents; Obstet Gynecol 2000; 96:653-56

59. Zoë P; Stacy J, Avis M et al. HPV and the value of screening; young women's knowledge of cervical cancer. Health Education Research 2003; 96: 653-56
60. Adams PJ; Kahn JA. Understanding and preventing HPV infection during adolescence and young adulthood; Journal of Adolescent Health 2005; 37: S1-2
61. Olshen E, Woods ER, Austin SB et al. Parental acceptance of HPV vaccine. J Adolescent Health 2005; 37: 248-51
62. Peto J, Gilhan C, Fletcher O et al. The cervical cancer epidemic that screening has prevented in the UK. Lancet 2004; 364 (9430):240-56
63. Solomon D. Comparison of three management strategies for patients with ASCUS. Baseline results from a randomized trial. JNCI 2001; 93:293-299
64. Guido R, Schiffman M, Solomon D et al. Post colposcopy management strategies for women referred with low grade SIL or HPV DNA positive ASCUS: a 2 year prospective study .AMJ Obstet Gynecol 2003; 188:1401-5
65. Sherman ME, Lorincz AT, Scott DR et al. Baseline cytology; HPV testing and risk of cervical carcinoma; a 10 year cohort study. J Natl Cancer Inst 2003; 95(1):46-52.
66. ASCCP: American Society for Colposcopy and Cervical Pathology Practice Recommendations: HPV testing; clinical uses of HPV DNA testing; 2006
67. Cox JT, Lorincz AT, Reid R. Clinical role of HPV testing .HPV 2nd Edition 1996.Obstet Gynecol 23(3):811-851
68. Arbyn M,Paraskevaidis E, Hirsh M et al. Clinical utility of HPV detection ; triage of minor cervical lesions; follow up of women treated for high grade CIN;an update of pooled evidence.Gynecol Oncol 2005; 99:S7-11
69. Qureshi MN, Rudelli RD, Biscotti CV et al. Role of HPV testing in predicting cervical intra-epithelial lesions .Diagnostic Cytopathology 2003; vol 29, No 3
70. Ann SS, Per R, Pia A et al. Comparison between Hybrid Capture 11 test and PCR-based HPV detection methods for diagnosis and post treatment follow up of CIN. J Clin Microb 2005; 43(70:3260-66)

71. Xavier Bosch, Jorma. Paavonen, David. Jenkins, Paulo. Naud J, et al (2007).Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomized controlled trial *The Lancet*, Volume 369, Issue 9580, (2161-2170).
72. Omondi Ogutu (2010): Parental acceptance of HPV vaccination to their pubertal daughter in Nairobi Kenya. PGDRM UNITID, Nov. 2010.
73. De Vuyst H, Steyaert S, Muchiri L, Sitati S , et al (2003): Distribution of HPV in family planning population in Nairobi, Kenya. Journal of the America Sexually Transmitted Diseases Association : Vol 30. 2 (137-142)
74. Baay M, Eyrun K, Patricia N, et al (2004): Human papillomavirus in rural community in Zimbabwe: the impact of HIV co-infection genotype distribution . Journal of Medical Virology, Volume 73, issue 3, 481-485
75. Feingold AR, Schreiber K, Munk G, Schrager LK, Klein RS (1990): Risk of human papillomavirus (HPV) and cervical squamous intraepithelial lesions (SIL) highest among women with advanced HIV disease .International Conference on AIDS. 1990 Jun 20-23; 6: 215 (abstract no. S.B.517).
76. Moscicki A, Ellenberg J, Farhat S and Jiahong Xu (2004): Persistence of HPV infected and unaffected adolescent girls, risk factors and differences by Phylogenetic type. Journal of infectious diseases Vol: 190, no 1 (37-45).
77. UNAIDS, 2004: Report on global AIDS epidemic, Geneva.
78. Wall S.R, Scherf C.F, Morison L, et al (2005):Cervical human papillomavirus infection and SIL in rural Gambia, West Africa. Viral sequence analysis and epidermiology. British Journal of Cancer (2005) 93,1068-1076.

APPENDICES

APPENDIX I: PATIENT INFORMATION AND CONSENT FORM.

nformation Sheet:

Human Papilloma Virus (HPV) is the virus known to be the causative agent for ervical cancer. The main route of transmission of HPV infection is through sexual ontact. Preventive measures include abstinence from sexual contact and Vaccination. This study aimed at determining the magnitude of HPV infection and level of nowledge on HPV transmission and prevention among adolescents attending dolescent clinic at Kenyatta National Hospital.

Participation in this study is voluntary, selected candidates who met study's inclusion riteria will be required to sign the consent form and thereafter filling of a uestionnaire. Then speculum will be introduced into your vagina, cervical swab will e taken by using a cervical brush and sent to Laboratory for analysis.

Risks and benefits of this study- risks anticipated are slight discomfort during ntroduction of the speculum and slight pain during cervical brushing . The benefits nclude detection of HPV infection status and any cervical lesion. The results obtained n this study will be communicated to the clinic for further appropriate actions.

Ethical issues- This study has been approved by the ethical and research committee of his hospital. The results of this test will be sent back to you through the clinic.

You will not be charged for the Laboratory test involved in this study. The cervical ample as well as results from this study will only be used for the above purposes and o other.

Confidentiality- your identity will be kept strictly confidential throughout the study s well as during the publication of the study findings. Your decision to participate or

not to participate in this study will not affect the quality of your required medical service at this clinic or KNH at large. No financial or any other kind of inducement will be given to anyone who chooses to take part in this study.

Kindly fill the consent form below:

Consent Form

(a). English Version:

I, _____ of out-patient clinic number_____agree to participate/ to allow my daughter to participate in the study of HPV testing, the nature of which has been explained to me by DR/MR/MRS _____. I understand that a cervical specimen will be taken and transported for analysis. I understand that the procedure will not be harmful to me/my daughter and that I can withdraw from the study if I wish. I know that this is a study and will not alter the quality of services I receive from this clinic.

Signature: Health care Provide_____Date_____

Client: _____Date_____

Parent/Guardian _____Date_____

During this study, if you have any concerns contact me Dr. MDACHI, E. (Principal investigator) on telephone number 0724 076013 or my supervisors Dr OMONDI OGUTU (telephone number 0722 510215) and Dr. NJOROGE WAITHAKA (telephone number 0736 818078) .

If you have further questions about the study you can contact Prof .BHATT, K. The chairperson of Ethical and Research Committee KNH on telephone number 02726300 Ext 44102

(b). Swahili version:

Mimi, _____wa Nambari ya Kliniki_____.Ninakubali kuhusika/binti wangu kuhusika kwenye utafiti wa kupimwa viini vya HPV, ambayo namna yake imeelezwa na Daktari/Bwana/Bi _____. Nimeelezwa kuwa kipimo kitachukuliwa kutoka sehemu yangu ya uzazi na itapelekwa kupimwa. Pia ninaelewa ya kwamba huu uchunguzi hautakuwa na madhara yeyote kwangu/kwa binti na kuwa niko huru kujiondoa kwa utafiti huu kama nikitaka. Ninaelewa ya kwamba huu ni utafiti na hautabadilisha kiwango cha huduma ninayostahili kupata katika Kliniki hii.

Sahihi: Mhudumu wa Afya: _____Date_____

Mteja: _____ Date_____

Mzazi/Mlezi: _____Date_____

Wakati wa utafiti huu, iwapo una maswali/ maoni wasiliana nami Dk. MDACHI, E. (Mtafiti Mkuu) kwa nambari ya simu 0724076013 au Wasimamizi wangu Dk. OMONDI OGUTU (0722 510215) na Dk. NJOROGE WAITHAKA (0736818078).

Iwapo una maswali zaidi kuhusu utafiti huu unaweza kuwasiliana na Prof. BHATT, K. Mwenyekiti wa Kamati ya Utafiti na Maadili KNH kwa nambari ya simu 02726300 Ext 44102.

Assent Form

(a). English Version:

I, _____ of out-patient clinic number_____agree to participate in the study of HPV testing, the nature of which has been explained to me by DR/MR/MRS _____. I understand that a cervical specimen will be taken and transported for analysis. I

understand that the procedure will not be harmful to me and that I can withdraw from the study if I wish. I know that this is a study and will not alter the quality of services I receive from this clinic.

Signature: Health care Provide_____Date_____

Client: _____Date_____

Parent/Guardian _____Date_____

During this study, if you have any concerns contact me Dr. MDACHI, E. (Principal investigator) on telephone number 0724 076013 or my supervisors Dr OMONDI OGUTU (telephone number 0722 510215) and Dr. NJOROGE WAITHAKA (telephone number 0736 818078) .

If you have further questions about the study you can contact Prof .BHATT, K. The chairperson of Ethical and Research Committee KNH on telephone number 02726300 Ext 44102

(b). Swahili Version:

Mimi, _____wa Nambari ya Kliniki_____.Ninakubali kuhusika kwenye utafiti wa kupimwa viini vya HPV, ambayo namna yake imeelezwa na Daktari/Bwana/Bi _____. Nimeelezwa kuwa kipimo kitachukuliwa kutoka sehemu yangu ya uzazi na itapelekwa kupimwa. Pia ninaelewa ya kwamba huu uchunguzi hautakuwa na madhara yeyote kwangu na kuwa niko huru kujiondoa kwa utafiti huu kama nikitaka. Ninaelewa ya kwamba huu ni utafiti na hautabadilisha kiwango cha huduma ninayostahili kupata katika Kliniki hii.

Sahihi: Mhudumu wa Afya: _____Date_____

Mteja: _____ Date_____

Mzazi/Mlezi: _____Date_____

Wakati wa utafiti huu, iwapo una maswali/ maoni wasiliana nami Dk. MDACHI, E. (Mtafiti Mkuu) kwa nambari ya simu 0724076013 au Wasimamizi wangu Dk. OMONDI OGUTU (0722 510215) na Dk. NJOROGE WAITHAKA (0736818078).

Iwapo una maswali zaidi kuhusu utafiti huu unaweza kuwasiliana na Prof. BHATT, K. Mwenyekiti wa Kamati ya Utafiti na Maadili KNH kwa nambari ya simu 02726300 Ext 44102.

APPENDIX II: DATA COLLECTION INSTRUMENT (QUESTIONNAIRE)

Study Title: Prevalence of Human Papiloma Virus among adolescents at KNH.

1. Study Number

2. Clinic Number

3. Physical Address/Tel No.

1. Age .....Yrs

Tick one appropriate option:

5. Marital Status .

(a) Single- i. cohabiting

-ii. Not cohabiting

(b) Married

(c) Divorced/Separated

(d) Widowed

(e) Others (Specify) /

6. If (a.i) or (b) above, for how long?

(a) Less than 1 year (b) 1-3 years (c) More than 3 years

7. Level of Education:

(a) Primary (b) Secondary (c) Tertiary/College (d) Others (Specify)

8. Education status of spouse if married:

(a) Primary (b) Secondary (c) Tertiary/College (d) Others (Specify)

C.OBSTETRIC& GYNAECOLOGIC HISTORY

1. Parity.....

2. Age at 1st menstrual period

(a) Less than 10 years (b) 10-15 years (c) 15-17 years (d) More than 17 years

3. Date of last menstrual period .../ ...../ 2010.

4. Have you used any Family planning method?

(a) Yes (b) No

5. If yes to 4 above, which method (s) and for how long? When did you stop?

Method	(tick)	Duration of use	Year stopped
Pills	( )	_____	_____
Implant	( )	_____	_____
3 monthly injection	( )	_____	_____
Condoms	( )	_____	_____
Coil	( )	_____	_____
Natural	( )	_____	_____

Others (Specify) _____

6. Have you ever done a pap smear ?

(a) Yes (b) No

7. If yes to 6 above, what were the results?

(a) Normal (b) Abnormal

8. If (b) in number 7 above, were you treated?

(a) Yes (b) No

9. Have you had or been treated for genital warts before(describe)?

(a) Yes (b) No

D.SEXUAL HISTORY

1. Age at 1st sexual intercourse

(a) Less than 10 years (b) 10-15 years (c) More than 15 years

2. Number of lifetime partners

(a) Two or less (b) 3-5 (c) More than 5

3. Number of sexual partners in the last 6 months

(a) Less than 2 (b) 2-3 (c) More than3

4. How often do you practice sexual intercourse per week?

(a) Once or less (b) 2-3 times (c) More than3 times

5. Do you douche after sexual intercourse (describe to the client what is douching)?

(a) Yes (b) No

6 If yes in 5 above, what do you use? _____

7. Have you been treated for a sexually transmitted infection before?

(a) Yes (b) No (d) Not sure (treated for abnormal discharge/pain but not sure if it was sexually transmitted).

8. Does your current partner have another sexual partner?

(a) Yes (b) No (c) Don't know.

9. Is/Are your current sexual partner(s) circumcised ?

(a) Yes (b) No (c) Don't know

E. SOCIAL HISTORY:

1.Have you ever smoked cigarettes ?

(a) Yes (b) No

2. If yes to 1 above, for how long?

(a) Less than 1 year (b) 1-5 years 9c) More than 5 years

3. If yes to 1 above, how many sticks in a day?

(a) Less than 3 (b) 3-5 (c) 5-10 (d) More than 10

3. Do you take alcohol?

(a) Yes (b) No

4. If yes in 3 above, for how long have you taken?

(a) Less than 1 year (b) 1-5 years (c) More than 5 years

5. How often do you take alcohol?

(a) 1-2 times per month (b) Every weekend (c) More than 3 times per week (d) Everyday

F. KNOWLEDGE ON HPV

1. Have you heard of HPV before?

(a) Yes (b) No

2. If yes to 1 above, how is it acquired?

(a) Poor hygiene (b) sexual intercourse (c) sharing instruments (d) others (specify)

3. If yes to 1 above, how do you reduce or prevent HPV infection?

(a) Through genital hygiene (b) Avoiding sexual intercourse (c) avoiding sharing of personal items (d) Others (specify)

4. In your view, what are the consequences of HPV infection?

(a) Genital discharge (b) pain (c) Genital swelling (d) None (e) inability to conceive (d) cancer (d) Don't know (e) Others (specify).

5. In your view, what is the role of condoms in HPV transmission?

(a) Eliminates transmission (b) Has no effect (c) Reduces transmission

6. Have you heard of HPV vaccine?

(a) Yes (b) No

7. Have you heard of cervical cancer before?

(a) Yes (b) No

8. In your views what is the cause of cervical cancer ?

(a) HPV (b) genetic (c) Poor hygiene (d) Poverty (e) others(specify)

G. REASON FOR CONSULTATION

Confirm from clinical records:

1. Sexually transmitted infection
2. Drug abuse
3. Teenage pregnancy/pueperium
4. Counseling
5. Others (specify).

H. HPV DNA RESULTS

1. HPV DNA NEGATIVE: ( )
2. HPV DNA POSITIVE: ( )

(a) HIGH RISK HPV PRESENT: ( )

GENOTYPES:

(b) LOW RISK HPV PRESENT: ( )

GENOTYPES:

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/ A/506

15th June 2010

Dr. Ernest Mdachi
Dept. of Obs/Gynae
School of Medicine
University of Nairobi

Dear Dr.Mdachi

RESEARCH PROPOSAL: "PREVALENCE OF HUMAN PAPILLOMAVIRUS AMONG ADOLESCENTS AT KENYATTA NATIONAL HOSPITAL" (P90/3/2010)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 15th June 2010 to 14th June 2011

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

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

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

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. Prof. K. M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept. of Obs/Gynae, UON
The HOD, Records, KNH
Supervisors: Dr. Omondi Ogutu, Dept. of Obs/Gynae, UON
Dr. Njoroge Waithaka, Dept. of Obs/Gynae, KNH