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VÍRUS DO PAPILOMA HUMANO EM UNIVERSITÁRIAS EM MAPUTO, MOÇAMBIQUE: UTILIZAÇÃO DE UM MÉTODO DE AUTO-COLHEITA

Yara Priscilla Pedro Bule

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Dissertation application for the Master's degree in Molecular Medicine and Oncology, submitted to the Faculty of Medicine of the University of Oporto.

Supervisor: Rui Medeiros PhD.

Filiation: Portuguese Instituto of Oncology of Oporto.

Co-supervisor: Carla Carrilho MD. PhD.

Filiation: Department of pathology of the Central Hospital of Maputo, Mozambique

Co-supervisor: Jani Silva

Filiation: Biomedical Research centre (CEBIMED) of the Energy, Environment and Health Research Unit of the Fernando Pessoa University in Porto

DEDICATION

This thesis is dedicated to three women:

My mother, **Helena Francisco Xerinda**, who taught me how to be a warrior;
My aunt, **Sandra Margarida Xerinda**, who taught me how to grow; and
My grandmother, **Palmira de Jesus Cumbe**, who taught my mother and aunt all they know.

You are my light at the end of the tunnel.
Thank you.

In memory of my brother, Puchinho and my grandfather Francisco Uaca Xerinda.
May you keep watching over me.

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“It adds to the joy of discovery to know that your work may make a difference in people’s lives”

- Flossie Wong-Staal

NOMENCLATURE

bp – base pairs
DNA – deoxyribonucleic acid
dNTPs – deoxynucleotide triphosphate
CC – Cervical Cancer
HPV – Human Papillomavirus
LCR – Long Coding Region
STIs – Sexually Transmitted Infections
OR – Odds Ratio
ORF – Open Reading Frame
PBS – Phosphate Buffer Solution
PCR – Polymerase Chain Reaction
PVs – Papillomavirus

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RESUMO

Os países em desenvolvimento ainda carregam o fardo da infeção pelo Vírus do papiloma humano (HPV). Em Moçambique, as mulheres e raparigas sexualmente ativas são o grupo mais afetado por infeções sexualmente transmissíveis. Muitas destas mulheres e raparigas não são tratadas, principalmente, devido à falta de conhecimento da existência de programas de tratamento ou à deficiente triagem no sistema de saúde. Aplicar novos métodos de testagem e triagem de baixo custo pode ajudar a entender como ocorre a propagação, os tipos de HPV que as infetam, mas também compreender as práticas sociais e comportamentos nas comunidades, especialmente de mulheres e raparigas que são vulneráveis a fim de conter comportamentos de risco, essencial para o controle da infeção pelo HPV e consequentemente, reduzindo o risco de ter cancro cervical mais tarde nas suas vidas.

O presente estudo teve como objetivo caracterizar a infeção pelo HPV em estudantes universitárias, bem como identificar as práticas sociais e comportamentais que facilitam a propagação deste vírus.

Para avaliar a distribuição de HPV nesta população, um kit de auto-colheita (uma zaragatoa e um tubo de centrifuga 1.5mL) foi utilizado para colher as células cervicais de 504 mulheres e a genotipagem do HPV utilizando a técnica de PCR. A informação sociodemográfica, comportamentos sociais e dados clínicos foi obtida por questionário auto-administrado.

A prevalência do HPV no geral foi de 32.5% (164/504), e a do HPV 16 em particular foi de 6.3%. Após a análise estatística, os fatores de risco identificados, para a presença de HPV (qualquer genótipo), incluem o número de parceiros ao longo da vida (OR=1.65, *P*=0.010), número de parceiros no último ano (OR=2.44, *P*=0.001), prática de sexo oral (OR=1.79, *P*=0.006), uso de contracetivo oral (OR=1.64, *P*=0.011), não utilização de preservativo por motivos não reprodutivos (OR= 2.70, *P*=0.002), aborto (OR=1.66, *P*=0.010). Para o HPV 16, a prática de sexo anal (OR=3.55, *P*=0.011) foi identificada como um fator de risco.

Como conclusão, pode-se afirmar que há uma alta prevalência de HPV entre mulheres jovens universitárias na província de Maputo. O conhecimento das estudantes sobre HPV é insuficiente pelo que muitas delas têm comportamentos sociais que ajudam a propagar este vírus. A informação obtida através deste estudo é importante para elaboração de melhores métodos de rastreio e medidas preventivas nesta população.

ABSTRACT

Developing countries still carry the burden of human papillomavirus (HPV) infections. In Mozambique, sexually active women and young girls, are the group most affected by sexually transmitted infections. Many of these women are untreated mainly because of the lack of knowledge of treatment programs and deficient screening programs in the health system. Applying new treatment programs and low cost screening could help understand how the propagation of HPV occurs and which types of HPV are most prevalent in the population, along with understanding the social and behavioural practices in the communities, especially of vulnerable women and girls stifle risk behaviour, essential to control HPV infection and consequently, eliminate the possibility of succumbing to cervical cancer later in their lives.

The objective of this study was to characterize the infection of HPV in female university students, as well as identify the social and behavioural practices that facilitate the propagation of this virus.

To evaluate the distribution of HPV in this population, a self-sampling kit (a swab and a centrifuge tube 1.5mL) was used to collect cervical cells of 504 women and HPV genotyping was done using a PCR technique. The information on sociodemographic, social behaviours and clinical data was assessed by a self-administered questionnaire.

The prevalence of any HPV type was 32.5% (164/504) and HPV-16 was 6.3% (32/164). Following statistical analysis, the risk factors for the acquisition of any HPV type include, lifetime number of sexual partners (OR=1.65, *P*=0.010), number of sexual partners in the last year (OR=2.44, *P*=0.001), the practice of oral sex (OR=1.79, P=0.006), use of oral contraceptive (OR=1.64, *P*=0.011), non-condom use for reasons other than reproduction (OR=2.70, P=0.002), abortion (OR=1.66, *P*=0.010). An association was also found for HPV16 infection and participants who practiced anal sex (OR=3.55, *P*=0.011).

In conclusion, there is a high prevalence of HPV infection amongst university students in the province of Maputo. The students' knowledge on HPV is scarce and there are social and behavioural practices that aid in the dissemination of the virus. The information obtained through this study is important to help elaborate better testing and screening methods for this population.

I. INTRODUCTION

1. Human papillomavirus

Papillomaviruses (PVs) comprise a small, diverse group of double stranded deoxyribonucleic acid (DNA) viruses that infect various diverse hosts including birds, reptiles and mammals (Crosbie, Einstein et al. 2013). They are transmitted through skin-to-skin or mucosa-to-mucosa contact, causing them to be a common sexually transmitted infection (STI) (Crosbie, Einstein et al. 2013). The ubiquitous nature, host specificity and ability to infect but not cause disease of these viruses, suggest that the PV-host interactions are old. Indeed, their origin dates to around 350 million years ago.(Mendes de Oliveira and Levi 2016) To date more than 200 HPV types have been fully sequenced with more than 40 that infect the genital areas of men and women. (Doorbar, Egawa et al. 2015)

Human papillomaviruses are divided based on their sequence in the major capsid protein L1 and classified into five genera: alpha-, beta-, gamma-, mu- and nu- papillomavirus (Doorbar, Egawa et al. 2015).

The *Alpha-papillomavirus* genus is separated into two groups the mucosal type and the cutaneous type. The mucosal HPV types of the alpha-papilomavirus genus have been divided into four groups based on their oncogenic activity: high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), probable high-risk types (26, 53, 66, 68, 73, 82), low-risk types (6, 11, 13, 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89) and types of undetermined-risk (30, 32, 34, 62, 67, 69, 71, 74, 83, 84, 85, 86, 87, 90, 91) (Munoz, Castellsague et al. 2006, Nobre, de Almeida et al. 2008)

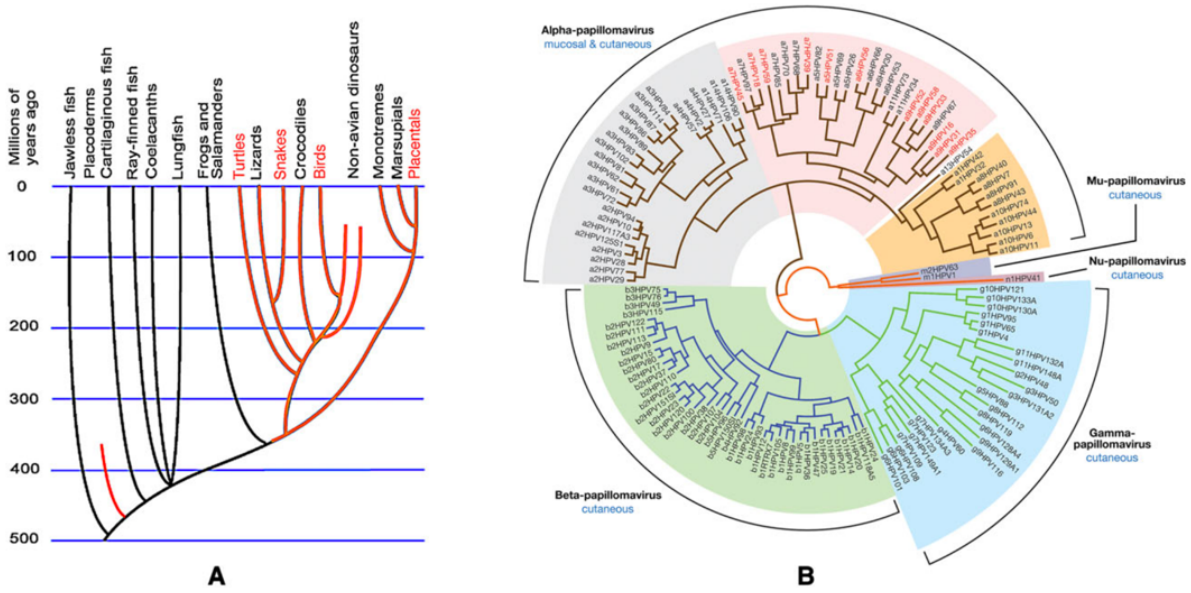

Figure 1: (A) Evolutionary tree showing the proposed appearance of an ancestral "papillomavirus" between the branch point leading to amphibians and reptiles (B) The human papillomaviruses types found in distributions of papillomaviruses in organisms. (Adapted from: Doorbar, 2015)

1.1. Structure and organization of the genome

In the mid-1950s to 1960s, observations of papillomaviruses by electron microscopy and nucleic acid analysis found that these viruses were small, non-encapsulated viruses (50-60nm diameter) with a double-stranded DNA circular genome of approximately 8000 bp. (Bravo, de Sanjose et al. 2010, Mendes de Oliveira and Levi 2016) This structure and organization is highly conserved through the entire HPV family.(Bruni, Serrano et al. 2015)

The HPV genome can be divided into three different regions: a region encoding the early genes, a region encoding the last genes and the non-coding region, termed long control region (LCR). Genes E1, E2, E4, E5, E6 and E7 are included in the early region and encode proteins intimately involved in the viral life cycle. L1 gene which encodes the major capsid proteins and L2 genes which encodes the minor capsid proteins are located in the late region. The LCR contains most of the regulatory elements involved in viral DNA transcription and replication and is located between open reading frames (ORFs) L1 and E6. (Tommasino 2014, Doorbar, Egawa et al. 2015)

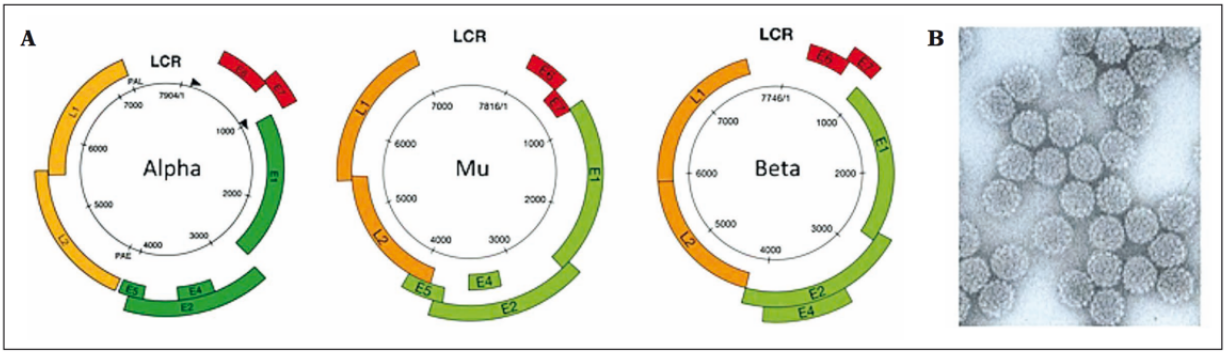

Figure 2: (A) Typical genome organization of the high-risk Alpha, Mu and Beta HPV genomes. (B) Electron micrograph of negatively stained papillomavirus particles. (Adapted from: Doorbar et al., 2015)

Papillomaviruses have well-conserved core genes, despite their variations in size and number of ORFs. E1 and E2 genes are involved in replication, L1 and L2 in packaging and E6, E7, E5 and E4 responsible for driving cell cycle entry, immune evasion, and virus release. The functions and features of the ORFs are decribed in Table 1. (Doorbar 2016).

Table 1: ORFs and their respective functions (Source: Doorbar, 2016)

Viral protein	Functions and features
E1	It forms a heterodimer complex with E2 and controls viral replication
E2	It regulates early gene promoter and together with E1 viral DNA replication
E4	It may mediate the viral particle release by destabilizing cytokeratin network
E5	It stimulates mitogenic signals of growth factors
E6	It inactivates many cellular proteins and is one of the major viral oncoproteins: p53
E7	It inactivates many cellular proteins and is one of the major viral oncoproteins: pRB
L1	It is the major capsid protein and is the component of the HPV prophylactic vaccine
L2	It is the minor capsid protein

1.2. Life cycle of HPV

The HPV life cycle can be separated into five phases: Infection and uncoating, genome maintenance, proliferative-phase, genome amplification, and virus synthesis. This organization is based on high-risk HPV (HR-HPV), particularly HPV 16 (Figure 3). (Doorbar 2005, Huh 2009)

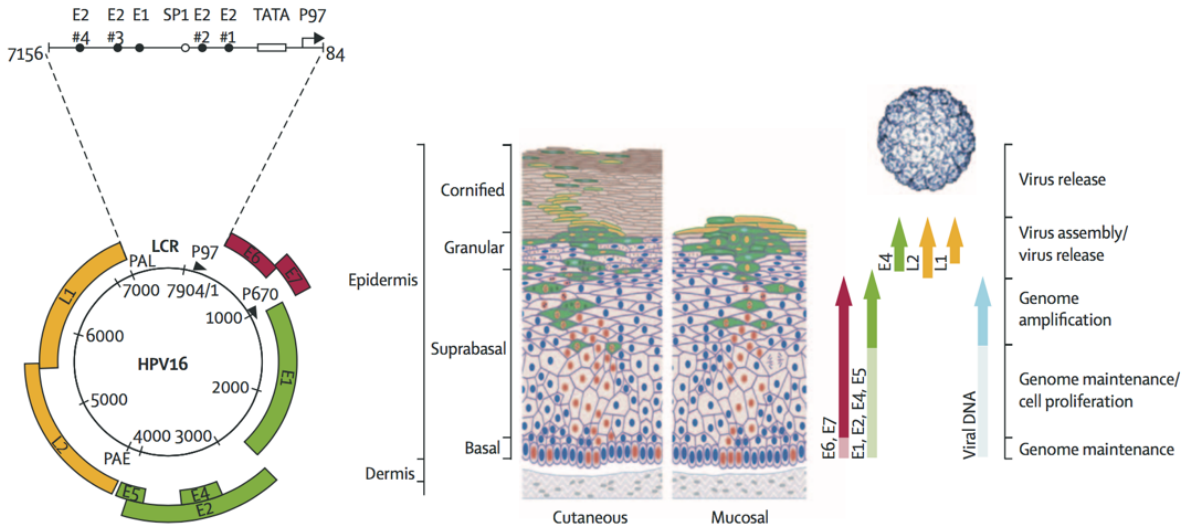

Figure 3: HPV genome and its expression withing the epithelium. (Adapted from: Schiffman, 2007)

The HR-HPV life cycle is closely linked to the differentiation programme of stratified epithelia and its ability to undergo a productive life cycle depends on the site of infection, the local microenvironment and the presence of hormones and cytokines (Tommasino 2014)

The first phase, infection and uncoating, is thought to begin with a wound or a form of epithelial trauma which might allow the access of virus particles to the basal lamina. Interaction with heparin sulphate proteoglygans might play a role in the attachment of the virus to the cell. Active cell division, prompted by active would healing, is necessary for viral genome entry into the nucleus. To assist the transfer to the secondary receptor on the basal keratinocyte, essential structural changes must occur in the virion capsid. These changes allow viral

internalization and integration of the viral genome in the nucleus. Once inside, virions undergo endosomal transportation, uncoating and cellular sorting. (Stubenrauch and Laimins 1999)

In the following phase, genome maintenance, the virus is thought to maintain it's a low copy number episome of its genome in the basal cells of the epithelium. It is believed that to maintain viral DNA as an episome and to facilitate genome segregation, the viral E1 and E2 proteins are expressed. In this phase viral early proteins are expressed at a low level, and viral genome is maintained in the basal cells at approximately 10-200 copies per cell.(Doorbar, Quint et al. 2012, Schiffman, Doorbar et al. 2016)

In the proliferative phase, E7 and E6 are expressed in basal migrating to the suprabasal cell layer, and normal terminal differentiation is hindered. E7 and E6 stimulate cell cycle progression and can associate with cell cycle regulators. E7 binds to pRB creating a cascade that leads to the expression of proteins necessary for DNA replication. Viral E6 binds to p53 complementing E7 by preventing apoptosis induction. Moreover, E6 allows the accumulation of chance errors to remain unchecked in the host's cell DNA.(Doorbar, Quint et al. 2012)

The amplification of the viral genome followed by infectious particle packaging is possible in the genome amplification phase. This process is described to occur in the upper epithelial layers following an increase in activity of the late (differentiation dependent) promoter, leading to increased expression of the E6 and E7 proteins, crucial for S-phase entry. In proliferative compartments in a subset of cells, amplification of viral genomes. Viral E4 and E5 gene expression is required, in order to occur the amplification. E2 binds to HPV upstream regulatory region and recruits E1 DNA helicase to form a complex E1/E2 that allows the viral genome replication to continue in the absence of cellular DNA synthesis.

Finally, in the virus synthesis phase, once viral genome amplification is complete, L1 (major capsid) and L2 (minor capsid) proteins are expressed in the upper layers of infected tissue forming a capsid. The virus is ready for re-infection and released from the cell.(Doorbar, Quint et al. 2012)

Given the diversity of HPV types, generalizations regarding infections routes must be avoided as multiple entry pathways have been cited depending on the virus types under study. Furthermore, the nature of the initially infected cell and how it relates to disease outcome is this still a matter of speculation (Doorbar, Quint et al. 2012))

1.3. HPV and cancer

HPV is now a well-established cause of cervical cancer (CC) and few other types of cancer: anogenital (anus, vulva, vagina and penis), head, neck, skin cancers and cancer of the conjunctiva.(Moscicki, Schiffman et al. 2006, Munoz, Castellsague et al. 2006, Carrilho, Gouveia et al. 2013)

Information on anogenital cancers is limited but there is a strong link with HR-HPV infection. Vulvar and vaginal cancers are rare and account for a small percentage of gynaecological cancers, yet vulvar cancers are more frequent in developed countries and vaginal cancers in developing countries. The incidence of penile cancers is low as well and usually affects men 50 years and older in less developed countries. Even though, majority of head and neck cancers are associated with tobacco and alcohol, current evidence suggest that infection with HPV 16 and other HR-HPV can lead to these types of cancer. (Blumberg, Monjane et al. 2015, Bruni, Serrano et al. 2015)

1.4. **HPV and cervical cancer**

Epidemiological studies in the 1990s, with the help of molecular technologies, provided evidence connecting HPV infections with the development of CC. (Moreno, Bosch et al. 2002)

Most HPV infections are harmless and can be cleared by the host's immune system in 1 – 2 years. A determining factor as to a whether an infection will evolve to cancer is persistent infections. Various studies have shown that persistent infections with oncogenic types of HPV are responsible for practically all cases of CC. (Schiffman, Castle et al. 2007, Crosbie, Einstein et al. 2013)

Four major steps are involved in the development of CC: HPV transmission or infection of the cervical transformation zone, viral persistence, progression of persistent infected epithelium to cervical precancerous lesions and invasion through the basement membrane of the epithelium (Figure 4). (Moscicki, Schiffman et al. 2006, Schiffman, Castle et al. 2007)

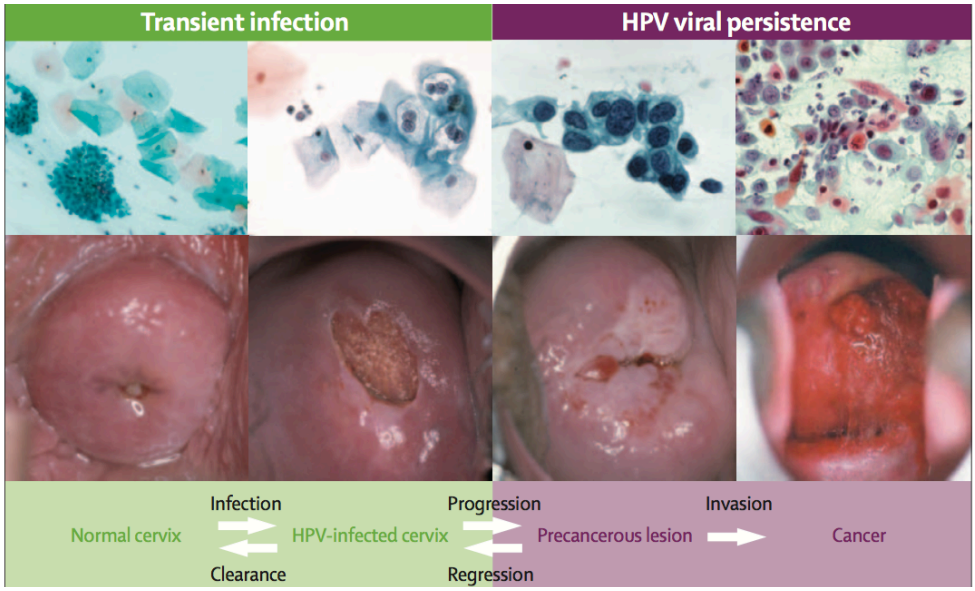

Figure 3 Major steps in the development of cervical cancer (Adapted from: Schiffman, 2007)

1.5. **Epidemiology of HPV and Cervical cancer**

Worldwide, there are 2,784 million women ages 15 years and older who are at risk of developing CC. (ICO HPV INFO CENTER). Women infected are between the ages of 15 to 44 years old, yet the majority of the infections will occur amongst sexually active young adults (Janet G baseman et al, 2004) (Emma Crosbie, et al. 2013). CC is classified as a type of cancer most frequently amongst women aged 15 to 44 years (Castellsagué, Menéndez et al. 2001). It is estimated that 8.5 million women aged 15 years and older are at risk of developing cervical cancer this year in Mozambique.(Bruni, Serrano et al. 2015)

CC is the fourth most common sort of cancer in women with an estimated number of 528,000 new cases and 266,000 deaths worldwide in 2012 and approximately nine out of ten CC deaths occur in developing countries (Watson-Jones, Baisley et al. 2013). Sub-Saharan Africa has one of the highest rates of CC in the world, with Mozambique having the second highest prevalence (Moon, Silva-Matos et al. 2012). In 2014, CC was responsible for 45,5% out of the 7,700 cancer related deaths in the Mozambican female population (Castellsague, Klaustermeier et al. 2008).

2. Detection and treatment

In Mozambique, the burden of STIs in supported, in majority, by women, nevertheless, they are the more reticent than men to seek treatment.(Cadman, Wilkes et al. 2015) Fear, shame, pain, and the doctor's sex are barriers women claim when looking for treatment. (Guan, Castle et al. 2012) Furthermore, poor health facilities and scarce heath personnel further hinders the country's capability to deal with cervical cancer. (Petignat, Faltin et al. 2007) Self-sampling circumvents majority of these difficulties, as well as eliminating the necessity of a speculum which adds costs to the exam. (Igidbashian, Boveri et al. 2011) Additionally, studies, (Shafer, Moncada et al. 2003) from developed and developing countries, have demonstrated that different self-sampling devices are just as sensitive as a physician collected sample to detect high risk HPV or HPV-DNA (Ortiz, Romaguera et al. 2013, Torre, Bray et al. 2015).

2.1 Self-sampling

Many aspects need to be taken into consideration when choosing a self-sampling device such as precision and cost (Torre, Bray et al. 2015). A meta-analysis by Petignant and co-workers (REF) demonstrated that the most common self-sampling tools used are the Dacron brush or they CytoBrush. Majority of the studies found good or strong concordance between the cervico-vaginal self-samples collected and the physician- collected samples using swabs/Dacron swabs or CytoBrush (Zhao, Lewkowitz et al. 2012).

In various developed countries, where many screening programs have been established, the CC rates decreased by 65% in the last 40 years (Davies, Kornegay et al. 2001). At the end of 2009, the ministry of health of Mozambique began a cervical cancer screening program using visual inspection with acetic acid (VIA) in four of the eleven provinces: Maputo, Sofala, Nampula, and Zambézia (Moon, Silva-Matos et al. 2012)

Self-sampling could be a low cost, low resource alternative to cytology based methods in developing countries (Silva, Cerqueira et al. 2014). However, unlike VIA, the HPV-DNA test provides high sensitivity, objective and reliable results based on the presence of high-risk HPV-DNA in samples. On the other hand, studies concluded that the HPV-DNA test could become a primary screening method for the prevention of CC in developing countries.

II. OBJECTIVES

General Objectives:

Determine the prevalence of Human papillomavirus (HPV) infection, in university students of Maputo, using a self-sampling method.

Specific Objectives:

- To characterize the population in terms of the sociodemographic data, risk assessment and clinical data in university women.
- Determine the presence of HPV using a Polymerase Chain Reaction (PCR) technique;
- Determine the presence and frequency of oncogenic HPV-16 using a PCR technique;
- Relate the HPV prevalence results with the information obtained through the questionnaires;
- Identify the social practices and behaviours that make the university students of Maputo vulnerable to HPV infection.

III. MATERIALS E METHODS

1. Ethics

The study was approved by the Ethics Committee of each university or institution selected for the study, as well as the National Committee of Bioethics of the Ministry of Health of Mozambique. The recommendations from the Declaration of Helsinki were followed in the elaboration of this project.

2. Study Population

Students from 2 public universities, 1 private university and 3 private academic institutions in the province of Maputo, were invited to participate in this study, between February and April of 2017. They were introduced to this study by means of a workshop, which divulged the objectives of the study and tried of sensitize the women about STI with focus on HPV and its consequences.

After the workshop, female students of any ethnicity, race or religion, aged between 18 to 30 were asked to partake in the study. The women who volunteered to participate were asked to sign a consent form so as to abide with ethical procedures. All the participants were given an explanation on how to collect cervicovaginal cells using a self-sampling tool. To safeguard the anonymity of the participants, a code, consisting of a letter and a number, associated to the sample and the questionnaire was randomly given to the participants.

Women who at the time of sampling were bleeding, had filled out the questionnaires incorrectly or incompletely and had poorly collected sample, were excluded from the study. A total of 526 samples were collected and after applying the exclusion criteria, 504 samples (95.8% of the samples collected) were included in this study.

3. Sample Collection (Self-Sampling Method)

The cervical cells were obtained using a sterile swab and a sterile centrifuge tube. In order to perform a self-collected vaginal swab the participant had to: inserted the swab into their vagina until they reached their cervix and rotated the swab for 30-40 seconds in the same direction. The swab was then removed from the vagina and the cotton tip placed in the centrifuge tube, the handle broken off and the centrifuge tube closed tight.

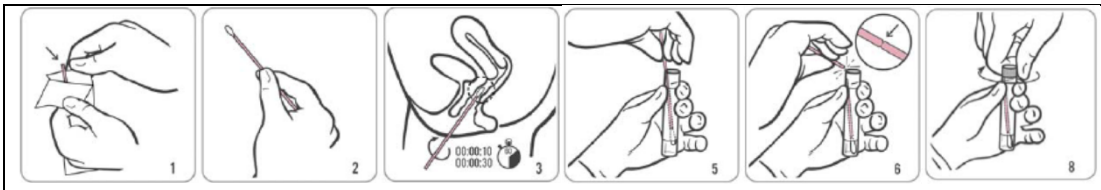

Figure 4. Instructions on how to perform a self-collected vaginal swab (Adapted from Gen-Probe Incorporated ®, San Diego CA.).

4. Laboratory Procedures

4.1. Sample Processing

The samples collected at the universities/institutions were transported, in a thermal bag with ice packs, to the Laboratory of Microbiology of the Faculty of Medicine of the Eduardo Mondlane university, in Maputo. Thereafter a 0,9% (w/v) saline solution was added to all samples and preserved at -80C.

To transport the samples from Maputo to Lisbon, the samples were placed in an arc with ice blocks. This arc was then transported under the same conditions to Biomedical Research centre (CEBIMED) of the Energy, Environment and Health Research Unit of the Fernando Pessoa University in Porto. There the samples were kept at -20C until they were processed.

For sample processing the swab was placed in a centrifuge tube (1.5 mL) and cells were concentrated by centrifugation (4500 rpm for 20 minutes). The supernatant was rejected and the precipitate suspended in 300µL of 1x phosphate-buffer saline (PBS). From the suspension, 100µL were added to the swab, which was used for DNA extraction, and the remaining sample (200 µL) was frozen at -80ºC.

DNA was manually extracted, from the samples, using the GRS Genomic DNA kit-BroadRange (Grisp®), according to the fabricant´s instructions.

4.2. **Polymerase Chain Reaction**

The PCR reactions were performed in a programmable *Bio-Rad MyCycler*® thermocycler with a final volume of 25µL for each reaction tube. To check the integrity of the DNA extracted from the specimens, a PCR protocol was used to amplify a region of 268 base pairs (bp) of the housekeeping gene, ß-globin, using the primers PCO_4 (5´- CAA CTT CAT CCA CGT TCA CC-3´) and GH_{20} (5´- GAA GAG CCA AGG ACA GGT AC-3´). (Resnick, Cornelissen et al. 1990) Each PCR amplification was carried out with; 1x Taq polymerase buffer (Grisp®), 2 mM of $MgCl_2$ (Grisp®), 0.2 mM of dNTPs (Grisp®), 0.2µM of each primer (©Metabion International AG), 1U Xpert Taq DNA polymerase (Grisp®)] and 10ng from the sample. The thermal cycling conditions were as follows: 4 minutes at 95˚C for pre-denaturation, followed by 35 cycles of 45 seconds at 95˚C, 30 seconds at 55˚C, and 45 seconds at 72˚C, with a final extension step at 72ºC for 5 minutes. All reaction sets had a negative control containing all PCR reagents, except DNA, to monitor for contamination.

4.2.1. HPV-DNA detection

HPV-DNA was detected using the degenerate primers, MY09 (5´-CGT CCM ARR GGA WAC TGA TC-3´) and MY11 (5´-GCM CAG GGW CAT AAY AAT GG-3´) (Irmiere, Manos et al. 1989), which amplify a region of HPV L1 gene of 450bp. Each PCR amplification included 1x Taq polymerase buffer (Grisp®), 3 mM of $MgCl_2$ (Grisp®), 0.2mM of dNTPs (Grisp®), 0.25 µM of each primer (©Metabion International AG), 1U Xpert Taq DNA polymerase (Grisp®) and 10ng of DNA. DNA of two HPV positive samples of Virology Service of Portuguese Institute of Oncology of Porto was used as positive control. A negative control containing all PCR reagents, except DNA, was added to monitor contamination. The thermal cycling was performed as follows: 4 minutes at 95˚C for pre-denaturation, followed by 35 cycles of 30 seconds at 94˚C, 30 seconds at 56˚C , 30 seconds at 72˚C, finalized by an extension step for 8 minutes at 72˚C.

4.2.2. HPV-16 DNA Detection

All HPV-positive samples were tested for the presence of HPV-16 DNA, using the primers HPV-16F (5'-GGA GGA GGA TGA AAT AGA TGG-3´) and HPV-16R (5'-GCC CAT TAA CAG GTC TTC CAA-3') (©Metabion International AG), which amplify a region of 157 bp (Della Torre, Donghi et al. 1992). The PCR reaction was performed with 1x Taq polymerase buffer (Grisp®), 2 mM of $MgCl_2$ (Grisp®), 0.2 mM of dNTPs (Grisp®), 0.3 µM of each primer (©Metabion International AG), 1U Xpert Taq DNA polymerase (Grisp®) and 10ng of DNA. DNA of two HPV-16 positive samples of Virology Service of Portuguese Institute of Oncology of Porto was used as positive control. The amplification conditions were as follows: 5 minutes at 94˚C for pre-denaturation, followed by 35 cycles of 45 seconds at 94˚C, 30 seconds at 55˚C for annealing and 45 seconds at 72˚C and a last step of extension at 72°C for 8 minutes.

4.2.3. Electrophoresis in agarose gel

To verify the amplification efficiency, 8µL of the amplified DNA, obtained from PCR, were analysed in a horizontal 1,5% (w/v) agarose gel stained with Midori Green (©NIPPON Genetics). Following electrophoresis, the gels were visualized using ultra violet (UV) light from the *Quantity one, Bio-Rad*® transilluminator and the *Quantity One*® analysis software version 4.6.1 for Microsoft Windows XP.

5. Statistical analysis

Data were analysed using IMB® SPSS® Statistics software version 23 for MacOS (Apple Inc.©). Age (mean age and mean age of first sexual intercourse), sociodemographic, social practices and clinical characteristics were categorised to explore the association of age and prevalence of HPV-DNA, HPV 16 DNA and non-HPV. To compare group frequencies, Chi-squared and Fisher's exact tests were used, using a 5% level of significance. The univariate analysis was used to estimate the odds ratio (OR) and 95% confidence interval as a measure of association between HPV-DNA presence and categorical variables.

IV. RESULTS

4. Characterization of the study population

4.1. Socio-demographic data

Five hundred and four women were included in this study, with ages between 18 and 30 years. The mean age was 22.2 years with a standard deviation of 3.27 years. The age distribution presented an asymmetrical distribution with a deviation to the left that indicates that majority of the participants were between the ages of 19 to 21 years (Figure 1).

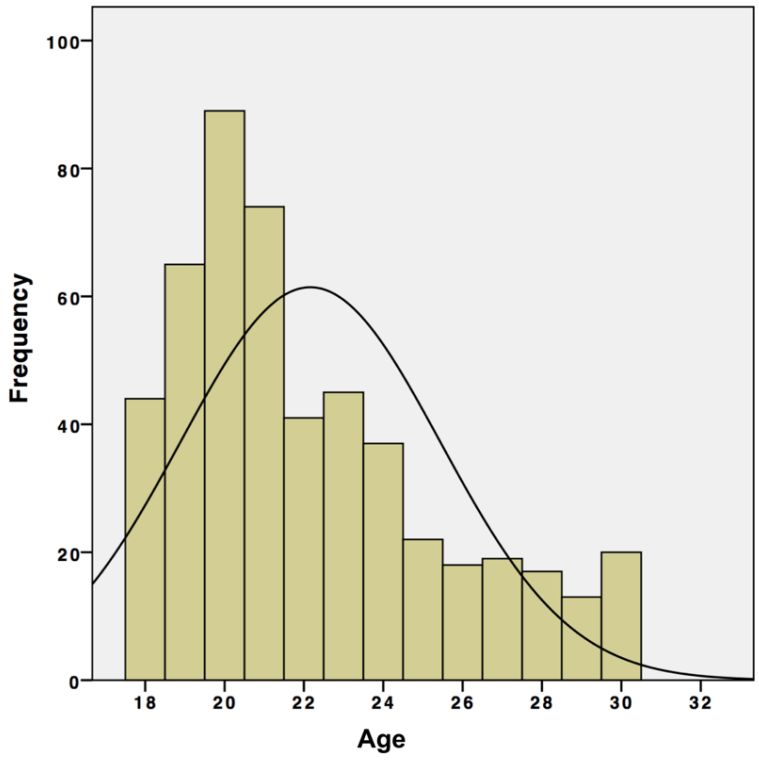

Figure 5 Age distribution frequency.

Further participant characteristics on sociodemographic data, such as nationality, marital status, professions, level of education and religion, are outlined in Table 2.

Table 2: Sociodemographic characteristics of the study population

CHARACTERISTIC	N (%)
Age (N=504)	
(mean ± SD)	22.16±3.273 (18-30)
Mean age, years (N=504)	
≤22	313 (62.1)
>22	191 (37.9)
Age, years (N=504)	
18-19	109 (21.6)
20-24	286 (56.7)
25-30	109 (21.6)
Nationality (N=504)	
Mozambican	499 (99.0)
Other	3 (0.6)
NR*	2 (0.4)
Marital status (N=504)	
Single	446 (88.4)
Cohabiting	30 (6.0)
Married	22 (4.4)
Divorced	1 (0.2)
NR*	5 (1.0)
Profession (N=504)	
Student	448 (88.9)
Worker	28 (5.6)
Unemployed	1 (0.2)
NR*	27 (5.3)
Level of education (N=504)	
12^{th} grade	485 (96.2)
Undergraduate	6 (1.2)
NR*	13 (2.6)
Religion (N=504)	
Catholic	224 (48.4)
Muslim	43 (8.5)
Other	198 (39.3)
NR*	19 (3.8)

*Not determined, unknown, or subject chose not to respond; SD, Standard Deviation.

4.2. Smoking, drinking, drug habits and risk assessment

The population was outlined in relation to behavioural characteristics. Out of the 504 participants, 94.9% referred to be non-smokers 53.0% non-drinkers and 89.7% never consumed drugs. Furthermore, the participants were asked where they had ever performed sexual favours 95.3% stated no, but 41.9% claimed to know that someone who has performed sexual favours., 8.9% claimed to be victims of sexual abuse and 38.3% claimed to know someone who has or had been a victim of sexual abuse.

Further population characterization, with variables detailing alcohol consumption and performance of sexual favours, is shown in Table 3.

Table 3: Smoking, drinking, drugs habits and risk assessment of the study population

CHARACTERISTIC	
Smoking status (N= 504)	**N (%)**
No	478 (94.9)
Yes	11 (2.2)
NR*	15 (3.0)
Alcohol consumption (N=504)	
No	267 (53.0)
Yes	231 (45.8)
NR*	6 (1.2)
Regularity (N= 229)	
Once a year	81 (35.5)
Once a month	94 (41.2)
More than once a month	35 (15.4)
Once a week	13 (5.7)
More than once a week	4 (1.8)
Once a day	1 (0.4)
Drinking circumstances (N=233)	
Socially	229 (98.7)
During meals	2 (0.9)
Both	1 (0.4)

(Continues)

Which drinks (N=224)	**N (%)**
Wine	44 (19.7)
Vodka	34 (15.4)
Whisky	7 (3.1)
Beer	73 (32.7)
Other	38 (17.0)
Two types	22 (9.9)
Three types	3 (1.3)
Four types	2 (0.9)
Time of day alcohol is consumed (N=225)	
Afternoon	51 (22.8)
Night	157 (70.1)
Both	16 (7.1)
Quantity of alcohol consumed (N= 232)	
1 cup or less	32 (13.9)
2 cups	87 (37.7)
3 to 6 cups	89 (38.4)
>=6 cups	23 (10.0)
Usually drinks with (N=228)	
Alone	2 (0.9)
Friends	95 (41.9)
Family members	74 (32.6)
Friends + Family	50 (22.0)
Alone + Friends	2 (0.9)
Alone + Friends + Family	4 (1.8)
Drug use (N=504)	
No	454 (89.7)
Yes	31(6.1) (0.2)
NR*	21 (4.2)
Performed sexual favours (N=504)	
No	480 (95.2)
Yes	9 (1.8)
NR*	15 (3.0)

(Continues)

Condom use (N=10)	
No	2 (20.0)
Yes	8 (80.0)
Age (N=9)	**N (%)**
15-18	7 (77.7)
20-24	2 (22.2)
Knows someone who performs sexual favours (N=504)	
No	269 (53.4)
Yes	212 (42.1)
NR*	23 (4.6)
Victim of sexual abuse (N=504)	
No	445 (88.3)
Yes	45 (8.9)
NR*	14 (2.8)
Knows someone who is/was a victim of sexual abuse (N=504)	
No	297 (58.9)
Yes	194 (38.3)
NR*	14 (2.8)

*Not determined, unknown, or subject chose not to respond; SD, Standard Deviation.

4.3. Clinical information

The mean age of menarche was 13.28 years with a standard deviation of 1.556 years. According to figure 2, the majority of participants reported the age of menarche between the ages 12 and 14 years, presenting a normal distribution (Figure 6).

Figure 6: Age distribution frequency for menarche.

The mean age of first sexual intercourse was 17.1 years with a standard deviation of 1.74 years. The age of first sexual intercourse varied from 11 to 25 years, with the highest frequency observed in participants aged 17 to 18 years. (Figure 7).

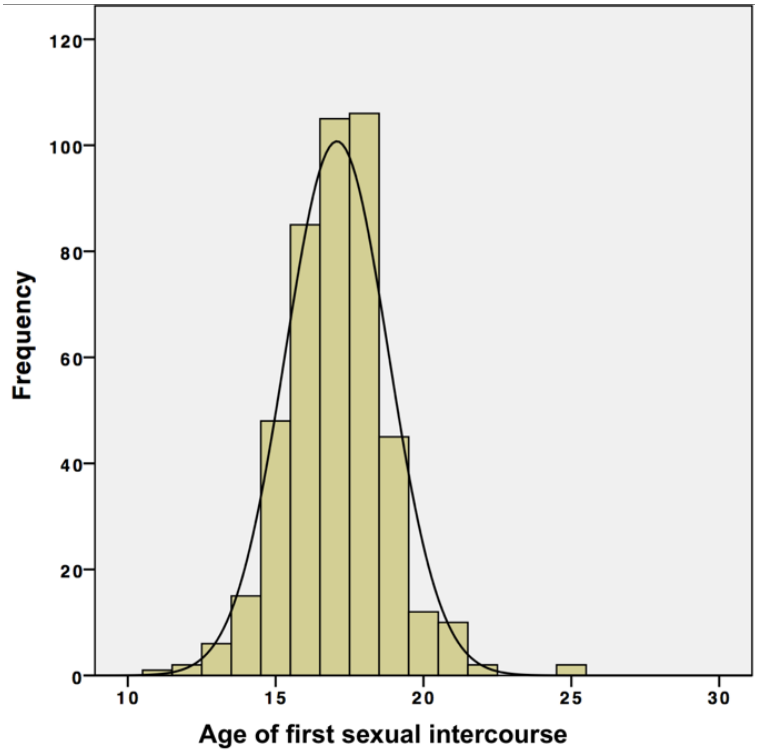

Figure 7 Age distribution frequency for first sexual intercourse.

The mean age of oral sex debut was 19.50 years with a standard deviation of 2.57 years. The age distribution frequency was normal with that majority of the participants having had their oral sex debut between the ages of 19 to 21 years. Out of the 504 women included in the study, 320 stated their oral sex debut, with the lowest age being 13 years and the highest being 29 years (Figure 8).

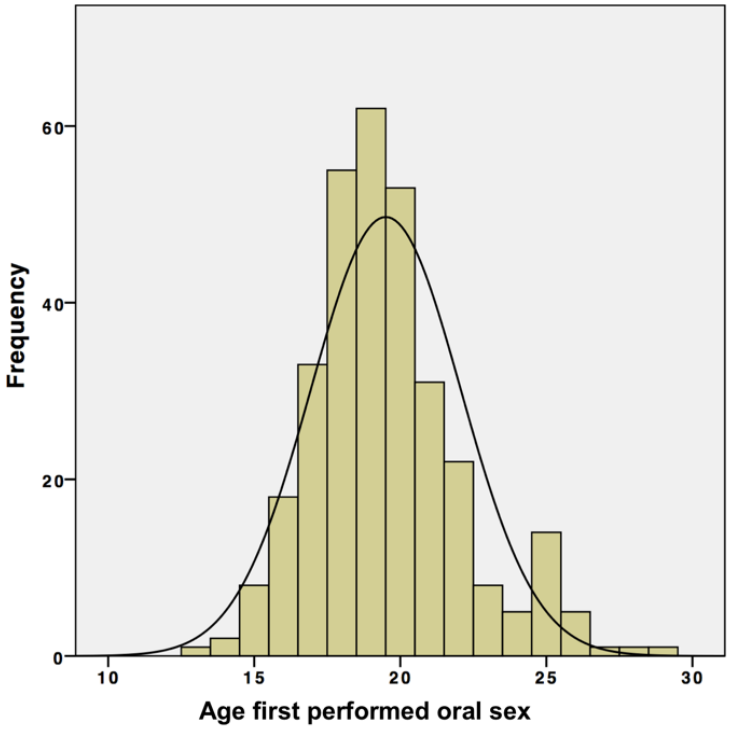

Figure 8 Age distribution frequency for oral sex.

The mean age of anal sex debut was 20.46 years with a standard deviation of 3.17 years. The age distributions was asymmetrical, with a deviation to the left, showing that majority of the participants has they anal sex debut between the ages of 19 to 20 years. Out of the 504 women included in the study, 57 stated their age of anal sex debut, with the lowest age being 13 years and the highest being 29 years (Figure 9).

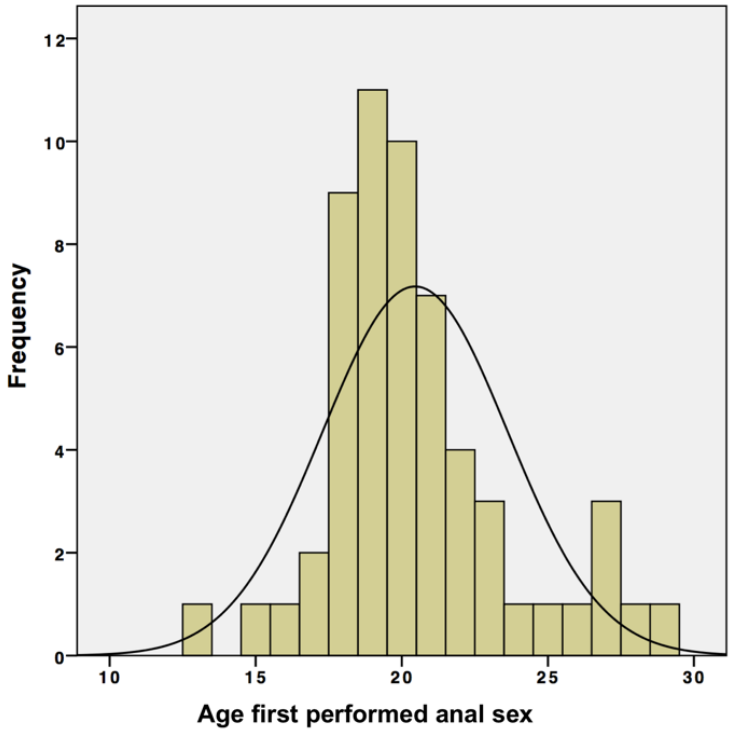

Figure 9: Age distribution frequency for anal sex.

Additional participant characteristics are shown in Table 4.

Table 4: Clinical characteristics of the study population

Characteristics	
Age of menarche (N=504)	
Mean ± SD	13.28 ± 1.556 (9-19)
NR*	5 (1.0)
Mean Age of menarche (N=499)	**N (%)**
≤13	278 (55.7)
>13	221 (44.3)
Age of menarche (N=499)	
9-11	52 (10.4)
12-15	408 (81.8)
16-19	39 (7.8)
Age of first sexual intercourse (N=504)	
Mean ± SD	17.07 ± 1.738 (11-25)
NR*	65 (12.9)

Mean age of first sexual intercourse (N=439)	N (%)
≤17	262 (59.7)
>17	177 (40.3)
Age of first sexual intercourse (N=439)	
11-13	9 (2.1)
14-16	148 (33.7)
17-19	256 (58.3)
20-25	26 (5.9)
Lifetime No. of sexual partners (N=504)	
Never had	53 (10.5)
1	150 (29.8)
2-5	251 (49.8)
≥6	40 (7.9)
NR*	10 (2.0)
No. of sexual partners in the last year (N=504)	
None	78 (15.5)
1	343 (68.1)
2-5	57 (11.3)
6-10	2 (0.4)
NR*	24 (4.7)
Frequency of coitus per month (N=504)	
None	90 (17.9)
1	53 (10.5)
2-5	219 (43.5)
6-10	72 (14.3)
>10	28 (5.6)
NR*	42 (8.3)
Oral sex (N=504)	
No	163 (32.3)
Yes	329 (65.3)
NR*	12 (2.4)
Anal sex (N=504)	
No	433 (85.9)
Yes	60 (11.9)
NR*	11 (2.2)

Oral contraceptive (N=504)	**N (%)**
No	295 (58.5)
Yes	197 (39.1)
NR*	12 (2.4)
Condom use (N=504)	
No	59 (11.7)
Yes	388 (77.0)
NR*	57 (11.3)
Reason for occasional or non-condom use (N=257)	
Lack of pleasure [A]	72 (28.0)
Pressured by partner [B]	36 (14.0)
Trying to get pregnant [C]	41 (16.0)
I trust my partner [D]	106 (41.2)
Two of the above option[(A+C/C+D)]	2 (0.8)
No. of pregnancies (N=504)	
None	223 (44.2)
1	98 (19.4)
2-5	98 (19.4)
>5	3 (0.6)
NR*	82 (16.3)
Parity (N=504)	
None	299 (59.3)
1	72 (14.3)
≥2	33 (6.5)
NR*	100 (19.8)
Abortion (N=504)	
No	283 (56.2)
Yes	193 (38.3)
NR*	28 (5.6)
Induced abortion (N=192)	
No	91 (47.6)
Yes	100 (52.4)
Ever been to a gynaecologist (N=504)	
No	264 (52.4)
Yes	220 (43.7)
NR*	20 (4.0)

Regularity (N=204)	**N (%)**
2 times per year	64 (31.4)
1 times per year	106 (52.0)
2 to 2 years	14 (6.9)
3 to 3 years	7 (3.4)
4 to 4 years	2 (1.0)
5 to 5 years	3 (1.5)
≥6 years	8 (3.9)
Ever performed a pap test (N=504)	
No	435 (86.3)
Yes	53 (10.5)
NR*	16 (3.2)
Pap test result (N=52)	
Normal	42 (80.8)
Altered	10 (19.2)
HPV test (N=504)	
No	445 (88.3)
Yes	23 (4.6)
NR*	36 (7.1)
HPV vaccine (N=504)	
No	411 (81.5)
Yes	8 (1.6)
Unknown	52 (10.3)
NR*	33 (6.5)
	(Continues)
STI history (N=504)	
No	386 (76.6)
Yes	111 (22.0)
NR*	7 (1.4)
Partner's STI history (N=504)	
No	146 (29.0)
Yes	41 (8.1)
Unknown	232 (45.8)
NR*	86 (17.1)

Mouth sores (N=504)	N (%)
No	365 (72.4)
Yes	128 (25.4)
NR*	11 (2.2)
Frequency (N=129)	
Frequently	7 (5.4)
Occasionally	27 (20.9)
Rarely	95 (73.6)
Skin warts (N=504)	
No	423 (83.9)
Yes	41 (8.1)
NR*	40 (7.9)
Areas (N=39)	
Hands	8 (20.5)
Feet	4 (10.3)
Other	27 (69.2)

*Not determined, unknown, or subject chose not to respond; STI, sexually transmitted infection; HPV, Human Papillomavirus; SD, Standard Deviation;

5. HPV distribution

5.1. Any HPV type

From the samples included in this study, 32.5% (n=164) were positive for HPV DNA. Within the women who stated to have never had sexual intercourse (n=53), 7.5% were positive for HPV infection.

HPV prevalence was similar in the 20-24 and 25-30 age groups (35.7% and 33.9%, respectively) and slightly lower in younger ages of 18-19 years (Table 5).

HPV was found with similar prevalences considering the smoking status, alcohol and drugs use, (Table 6).

Even though the majority of sexually active women stated to use a condom, The prevalence of HPV was similar considering users and non-users of condom (35.8% and 35.6%, respectively (Table 7)

The percentage of HPV infection in women who had had an abortion, 16.2% was comparable to the percentage of women had never had an abortion, 17.0%. From the women who had had a pap test (n=53), and reported a normal result, 38.1% were positive for HPV, and only 20.0% of women who reported an altered result were positive for HPV. Amongst women who reported having had an HPV vaccine, 37.5% were positive for HPV. (Table 7)

5.2. Oncogenic HPV 16

Out of the HPV-DNA positive samples, 6.3% (n=32) were positive for HPV16 DNA. Within the women who stated to have never had sexual intercourse (n=0) 0.0% were positive for HPV-16 infection.

Even though the majority of sexually active women stated to use a condom, the percentage of HPV infected women who use a condom was 20.1% (n=28). Women who identified as non-condom users and stated trust in their partner as a reason for not using a condom (n=12), continued to rank highest for HPV16 infection 12.2%. (Table 7)

The percentage of HPV infections among women who had had an abortion, 19.8% was comparable to the percentage of women had never had an abortion, 19.8%. From the women who had had a pap test (n=160), and reported a normal result, 25.0% were positive for HPV, and only 50.0% of women who reported an altered result were positive for HPV. Amongst women who reported having had an HPV vaccine, none were positive for HPV-16. (Table 7)

Table 5: Prevalence of HPV-DNA, HPV 16 DNA and non-HPV 16 DNA according to sociodemographic data

	No. of participants	Any HPV type N (%)	*P* value[(a,b)]	HPV 16 N (%)	Non-HPV 16 N (%)	*P* value[(a,b)]
SOCIODEMOGRAPHICS						
Age, years	504					
18-19	109	25 (22.9)	0.051	4 (16.0)	21 (84.0)	0.869[a]
20-24	286	102 (35.7)		21 (20.6)	81 (79.4)	
25-30	109	37 (33.9)		7 (18.9)	30 (81.1)	
Mean age, years	504					
≤22	313	96 (30.7)	0.252	20 (20.8)	76 (79.2)	0.612[a]
>22	191	68 (35.6)		12 (17.6)	56 (82.4)	
Marital status	499					
Single	446	146 (32.7)	0.802	28 (19.2)	118 (80.8)	0.986[a]
Cohabiting	30	11 (36.7)		2 (18.2)	9 (81.8)	
Married	22	6 (27.3)		1 (16.7)	5 (83.3)	
Religion	485					
Catholic	244	82 (33.6)	0.599	15 (18.3)	67 (81.7)	0.829[a]
Muslim	43	12 (27.9)		2 (16.7)	10 (83.3)	
Other	198	59 (29.8)		13 (22.0)	46 (78.0)	

Table 6: Prevalence of HPV-DNA, HPV 16 DNA and non-HPV 16 DNA according to risk assessment charateristics

	No. of participants	Any HPV type N (%)	*P* value[(a,b)]	HPV 16 N (%)	Non-HPV 16 N (%)	*P* value[(a,b)]
RISK ASSESSMENT						
Smoking status	489					
No	478	153 (32.0)	0.493	29 (19.0)	124 (81.0)	0.438[b]
Yes	11	4 (36.4)		0 (00.0)	4 (100.0)	
Alcohol consumption	498					
No	267	79 (29.6)	0.108	12 (15.2)	67 (84.8)	0.227[a]
Yes	231	84 (36.4)		19 (22.6)	65 (77.4)	
Drug use	483					
No	452	147 (32.5)	0.070	27 (18.4)	120 (81.6)	0.316[b]
Yes	31	15 (48.4)		4 (26.7)	11 (73.3)	
Performed sexual favours	489					
No	480	154 (32.1)	0.130	29 (18.8)	125 (81.2)	0.654[b]
Yes	9	5 (55.6)		1 (20.0)	4 (80.0)	
Victim of sexual abuse	490					
No	445	147 (33.0)	0.572	29 (19.7)	118 (89.3)	0.521[b]
Yes	45	13 (28.9)		2 (15.4)	11 (84.6)	

HPV, Human Papillomavirus; *P* value, level of significance;

[a]Pearson's Chi-Squared value; [b]Fisher's Exact Test;

Table 7: Prevalence of HPV-DNA, HPV 16 DNA and non-HPV 16 DNA according to sexual behaviour and clinical characteristics

	No. of participants	Any HPV type N (%)	*P* value[(a,b)]	HPV 16 N (%)	Non-HPV 16 N (%)	*P* value
SEXUAL BEHAVIOUR						
Age of first sexual intercourse	439					
11-13	9	5 (55.6)	0.223	0 (0.0)	5 (100.0)	0.051[a]
14-16	148	46 (31.1)		15 (32.6)	31 (67.4)	
17-19	256	100 (39.1)		15 (15.0)	85 (85.0)	
20-25	26	8 (30.8)		1 (12.5)	7 (87.5)	
Mean age of first sexual intercourse	439					
≤17	262	90 (43.4)	0.322	21 (23.3)	69 (76.7)	0.163[a]
>17	177	69 (39.0)		10 (14.5)	59 (85.5)	
Lifetime No. of sexual partners	494					
Never had	53	4 (7.5)	<0.001	0 (0.0)	4 (100.0)	0.802[a]
1	150	42 (28.0)		9 (21.4)	33 (78.6)	
2-5	251	100 (39.8)		20 (20.0)	80 (80.0)	
≥6	40	14 (35.0)		2 (14.3)	12 (85.7)	
No. of sexual partners in the last year	480					
None	78	15 (19.2)	<0.001	2 (13.3)	13 (86.7)	0.778[a]
1	343	112 (32.7)		24 (21.4)	88 (78.6)	
2-5	57	31 (54.4)		5 (16.1)	26 (83.9)	
6-10	2	1 (50.0)		0 (0.0)	1 (100.0)	

(Continues)

	No. of participants	Any HPV type	*P* value[a,b]	HPV 16	Non-HPV 16	*P* value
		N (%)		N (%)	N (%)	
Frequency of coitus per month	462					
None	90	20 (22.2)	0.057	0 (0.0)	20 (100.0)	0.094[a]
1	53	16 (30.2)		5 (31.3)	11 (68.8)	
2-5	219	85 (38.8)		15 (17.6)	70 (82.4)	
6-10	72	25 (34.7)		7 (28.0)	18 (72.0)	
>10	28	7 (25.0)		1 (14.3)	6 (85.7)	
Oral sex	492					
No	163	40 (24.5)	0.006	6 (15.0)	34 (85.0)	0.431[a]
Yes	329	121 (36.8)		25 (20.7)	96 (79.3)	
Anal sex	493					
No	433	141 (32.6)	0.527	23 (16.3)	118 (83.7)	0.011[b]
Yes	60	22 (36.2)		9 (40.9)	13 (59.1)	
Oral contraceptive	492					
No	212	83 (28.1)	0.011	17 (20.5)	66 (79.5)	0.422[a]
Yes	120	77 (39.1)		12 (15.6)	65 (84.4)	
Condom use	447					
No	59	21 (35.6)	0.972	4 (19.0)	17 (81.0)	0.586[b]
Yes	388	139 (35.8)		28 (20.1)	111 (79.9)	

(Continues)

	No. of participants	Any HPV type N (%)	*P* value[(a,b)]	HPV 16 N (%)	Non-HPV 16 N (%)	*P* value
Reason for occasional or non-condom use	257					
Lack of pleasure [A]	72	25 (34.7)	0.050	6 (24.0)	19 (76.0)	0.075[a]
Pressured by partner [B]	36	13 (36.1)		0 (0.0)	13 (100.0)	
Trying to get pregnant [C]	41	24 (58.5)		3 (12.5)	21 (87.5)	
I trust my partner [D]	106	35 (33.0)		12 (34.3)	23 (65.7)	
Two of the above option[(A+C/C+D)]	2	1 (50.0)		0 (0.0)	1 (100.0)	
CLINICAL CHARACTERISTICS						
No. of pregnancies	422					
None	223	62 (27.8)	0.104	10 (16.1)	52 (83.9)	0.538[a]
1	98	41 (41.8)		8 (19.5)	33 (80.5)	
2-5	98	32 (32.7)		9 (28.1)	23 (71.9)	
>5	3	1 (33.3)		0 (0.0)	1 (100.0)	
Parity	404					
None	299	92 (30.8)	0.817	15 (16.3)	77 (83.7)	0.814[a]
1	72	24 (33.3)		5 (20.8)	19 (79.2)	
≥2	33	9 (27.3)		2 (22.2)	7 (77.8)	
Abortion	476					
No	283	81 (28.6)	0.010	16 (19.8)	65 (80.2)	0.873[a]
Yes	193	77 (39.9)		16 (20.8)	61 (79.2)	
Induced abortion	191					
No	91	32 (35.2)	0.268	5 (15.6)	27 (84.4)	0.298[a]
Yes	100	43 (43.0)		11 (25.6)	32 (74.4)	

(Continues)

	No. of participants	Any HPV type N (%)	*P* value[a,b]	HPV 16 N (%)	Non-HPV 16 N (%)	*P* value
Ever been to a gynaecologist	484					
No	264	91 (34.5)	0.406	19 (20.9)	72 (79.1)	0.611[a]
Yes	220	68 (30.9)		12 (17.6)	56 (82.4)	
Ever performed a Pap test	488					
No	435	141 (32.4)	0.615	25 (17.7)	116 (82.3)	0.268[b]
Yes	53	19 (35.8)		5 (26.3)	14 (73.7)	
Pap test result	52					
Normal	42	16 (38.1)	0.244	4 (25.0)	12 (75.0)	0.490[b]
Altered	10	2 (20.0)		1 (50.0)	1 (50.0)	
HPV test	468					
No	445	140 (31.5)	0.918	28 (20.0)	112 (80.0)	0.582[b]
Yes	23	7 (30.4)		1 (14.3)	6 (85.7)	
HPV vaccine	471					
No	411	128 (31.1)	0.536	26 (20.3)	102 (79.7)	0.684[a]
Yes	8	3 (37.5)		0 (0.0)	3 (100.0)	
Unknown	52	20 (38.5)		4 (20.0)	16 (80.0)	
STI history	497					
No	386	119 (30.8)	0.117	21 (17.6)	98 (82.4)	0.263[a]
Yes	111	43 (38.7)		11 (25.6)	32 (74.4)	
Partner's STI history	418					
No	146	45 (30.8)	0.255	5 (11.1)	40 (88.9)	0.144[a]
Yes	41	16 (39.0)		3 (18.8)	13 (81.3)	
Unknown	231	90 (39.0)		23 (25.6)	67 (74.4)	

STI, sexually transmitted infection; HPV, Human Papillomavirus; *P* value, level of significance; [a]Pearson's Chi-Squared value; [b]Fisher's Exact Test

4.1. **Calculation of HPV infection risk**

Statistical significance was found between any HPV and number of sexual partners [OR 1.65, 95% Confidence Interval (95% CI) 1.08-2.55; *P* <0.010), number of sexual partners in the last year (OR 2.44, 95% CI 1.39-4.30; *P*=0.001), practices oral sex (OR 1.79, 95% CI 1.17-2.73; *P*=0.006), oral contraceptive use (OR 1.64, 95% CI 1.12-2.40; *P*=0.006), non-condom use for reasons other than reproduction (OR 1.64, 95% CI 1.12-2.40; *P*=0.002) and abortion (OR 1.66, 95% CI 1.13-2.44; *P* <0.010). (Table 8)

Statistical significance was found between HPV-DNA and practices anal sex (OR 3.55, 95% CI 1.36-9.28; *P*= 0.011). (Table 8)

Table 8: Risk factors for HPV-DNA and HPV-16 infection

CHARACTERISTICS	***P* Value** [(a,b)]	**OR**	**95% CI**
Any HPV type			
Lifetime number of sexual partners			
1 versus ≥2	0.010[a]	1.65	(1.08-2.55)
Number of sexual partners in the last year			
1 versus 2-10	0.001[a]	2.44	1.39-4.30
Oral sex			
No versus Yes	0.006[a]	1.79	1.17-2.73
Oral contraceptive			
No versus Yes	0.011[a]	1.64	1.12-2.40
Reasons for non-condom use			
To get pregnant versus other reasons*	0.002[a]	1.64	1.12-2.40
Abortion			
No versus Yes	0.010[a]	1.66	1.13-2.44
HPV-16			
Anal sex			
No versus Yes	0.011[b]	3.55	1.36-9.28

DNA, deoxyribonucleic acid; HPV, Human Papillomavirus; *p* = level of significance; OR, Odds ratio; CI, Confidence interval; [a]Pearson's Chi-Squared value; [b]Fisher's Exact Test**;** * lack of pleasure, pressured by partner, and trust in partner.

V. DISCUSSION

The objectives of this study were to determine the prevalence of infection by human papillomavirus, in university students of Maputo, using a self-sampling method, determine the presence of HPV and HPV-16 using a PCR technique, identify the social practices and behaviours that make the university students of Maputo vulnerable to infection and relate the results obtained from the study (HPV) with the information obtained through the questionnaires.

It is important to note that, there are studies detailing the prevalence and genotypes of HPV in Mozambique (Castellsagué, Menéndez et al. 2001, Naucler, Da Costa et al. 2004, Pizzol, Putoto et al. 2016, Edna Omar, Orvalho et al. 2017), but this is the first of its kind that focuses on female university students and uses a self-sampling method as a means to collect cervical cells.

HPV infection is the central cause of invasive CC and CC is the fourth most common cancer in the world. In Mozambique CC is the most common type of cancer amongst women 15 to 44 years old. The results from our study showed an overall prevalence of 32.5% (164/504) positive cases for any HPV type. The age of the participants in this study was suggestively significant for an infection with any HPV type with the ages 20 to 24 years old having the highest prevalence. Studies have shown that the highest rates of infection were found among sexually active young adults under 25 years of age (Burchell, Winer et al. 2006, Gerend and Magloire 2008). Similarly, a study by Menendez, et al., 2010, showed that in a rural area in Mozambique, the population most infected with HPV was between the ages of 21 to 30 years old. Even though a high prevalence was found in the population our study, this prevalence remains far less than the prevalence found in a recent study by Edna Omar and co-workers (Edna Omar, Orvalho et al. 2017). In their study, the prevalence amongst female young adults aged 18 to 24 years old was 6.8%. (Menendez, Castellsague et al. 2010)

According to various studies (Bosch and Munoz 2002, Burchell, Winer et al. 2006), the behavioural risk factors associated with HPV infection in adolescents and young adult women includes multiple sex partners and the number of lifetime sexual partners. The findings in our study show statistical significance between any HPV infection and the lifetime number sexual partners ≥2 (OR=1.65, *P*=0.010) which suggest that it is an important risk factor for HPV infection. Majority of women state that they have had 2-5 partners in their lifetimes with HPV infections rates in each group of 39.8%. In a study, women with more than one sexual partner

(2 to 5 partners) had a higher risk of HPV infection (Burchell, Winer et al. 2006). In sub-Saharan Africa, certain socio-cultural practices such as polygamy, increase the risk of HPV infection by 2-fold. (Anorlu 2008). An association was also found between number of sexual partners in the last year statistical significance was also found (OR=2.44, *P*=0.001).

In our study performance of oral sex was a risk factor for HPV infection (OR=1.66, *P*=0.010). yet evidence from other studies suggest that while infection with genital HPV types occurs oral sex or digital penetration, the risk of transmission is minimal. (Burchell, Winer et al. 2006).

It is thought that hormonal contraceptive act as a cofactor for cervical cancer, the similar connection was found in our study (OR=1.64, *P*=0.011). Ovaries, breasts, uterus and uterine cervix are hormone-responsive tissues that react to the presence of estrogens and progestagens. It is believed that these hormones enhance HPV gene-expression, however even though these associations are generally consistent across studies most are derived from retrospective case-control studies that did not always consider HPV (Moreno, Bosch et al. 2002, Munoz, Castellsague et al. 2006, Roura, Travier et al. 2016, Ramachandran 2017)

.

Our study presents data showing a statistical significance between abortions (OR=1.66, *P*=0.010) and HPV infection. Growing insights on human reproduction suggested a role for HPV infection and on infertility in humans (Jiao, Zhao et al. 2016, Pizzol, Putoto et al. 2016). In sub-Saharan African countries reproductive tract infections are endemic, and poor health care leads to chronic inflammation (Anorlu 2008). Inflammations and changes in vaginal bacterial microbiota are recognized as causes of major adverse pregnancy outcomes, for example, miscarriages (Niyibizi, Zanre et al. 2017). Studies connecting HPV infections and abortions, show contradictory results. Conversely, two studies have related male partner HPV infection with miscarriage rates (Huang, Zhong et al. 2014, Giakoumelou, Wheelhouse et al. 2016).

The results of this study age of first intercourse was suggestively significant when related to HPV16 infection (*P*=0.051). In contrast a study by R. Winer *et al.*, indicated that the percentage of HPV infection in university students, 18 to 24 years of age, from a first male sex partner was high, especially if said partner was sexually experienced (Burchell, Winer et al. 2006). This second study demonstrates that regardless of the age of first intercourse, the risk of HPV infection is elevated.

The prevalence of HPV 16 and or 18 in women in Mozambique is about 8.4% with women, aged 15 to 44 who present normal cytology and is similar to the percentage of HPV 16 (6.2%)

positive samples (HPV Info Centre). Our result is concordant with the result obtained from Edna Omar V. *et al.*, and Castellsagué *et al.*, et al. study which had a prevalence of 6.8% for HPV 16 in both studies (Castellsagué, Menéndez et al. 2001, Edna Omar, Orvalho et al. 2017).

In our study, anal sex was identified as a 3-fold risk factor for HPV16 infection with a frequency of 40.9% (9/22) within women who were positive for any HPV type. The incidence of anal cancer is on the rise in most of the developed countries. Certain sexual behaviours can influence the risk of HPV acquisition. A study found a connection between anal and cervical HPV infection in patients with severe cervical lesions, identifying sexual contact as the most important risk factor (Sehnal, Dusek et al. 2014). On the other hand, another study hypotheses that viral particles found in vaginal discharge of HPV infected women could infect the anus. Further studies must evaluate this link between HPV 16 and anal cancer (Burchell, Winer et al. 2006).

VI. CONCLUSION

In conclusion, this study shows that there is still a high prevalence of HPV amongst women in Maputo. A strong statistical significance was found with any HPV infection and lifetime number of sexual partners, number of sexual partners in the last year, oral sex, use of oral contraception and abortion.

The prevalence of HPV 16 is low but is in accordance to what is expected in the general population in Maputo. The HPV vaccine works as a protective factor against HPV16 in this population, but might be ineffective with other types of HPV.

The questionnaire was a useful tool to identify the social and behavioural practices that could be addressed in order to curb the dissemination of this virus. Having more workshops about sexually transmitted infections, giving young adults an open space free of judgement where they could speak openly and gain knowledge and information about intercourse and STI would be a crucial strategy to reduce further infection. Additionally, encouraging women to visit the gynaecologist and dismantling social and cultural taboos would contribute to hinder this infection.

We cannot rule out the influence that some limitations had on the study. Approximately, 4% of the samples collected were not included in the study due to difficulties from the participants to understand the questionnaire and to effectively communicate their thoughts. Moreover, most of the workshop participants were willing to answer the questionnaire but reluctant to collect a self-sample.

Nevertheless, this study allows a better comprehension of the epidemiology of HPV in young asymptomatic women in Maputo.

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VIII. TABLES

IX. FIGURES

X. APPENDIX

Appendix 1: Questionnaire used in this study.

Nº Amostra

Este é um questionário anónimo, pelo que agradeço a sua colaboração sincera para que a informação recolhida seja fidedigna.

A. DADOS SÓCIO-DEMOGRÁFICOS

1. Idade _____ anos

2. Nacionalidade _____

3. Estado civil ☐ Solteira ☐ União de facto ☐ Casada ☐ Divorciada ☐ Viúva

4. Profissão ☐ Estudante ☐ Trabalhador ☐ Desempregado

5. Nível de escolaridade ☐ Sem escolaridade obrigatória ☐ Obrigatória (9ª classe) ☐ Secundário (12ª classe)
☐ Licenciatura ☐ Pós-Graduação/Mestrado ☐ Doutoramento

6. Religião ☐ Católica ☐ Muçulmana ☐ Outra: _____

B. DADOS DE TABAGISMO, ÁLCOOL, DROGAS*

7. Fuma? ☐ Não ☐ Sim Idade início _____ anos Nº cigarros/dia _____
☐ Ocasionalmente Nº cigarros _____
☐ Ex-fumador(a) Nº cigarros/dia _____
Idade início _____ anos Idade fim _____ anos

8. Consome bebidas alcoólicas?

☐ Não ☐ Sim

8.1. Se sim, com que regularidade?

☐ Uma vez por ano ☐ Uma vez por mês ☐ Mais que uma vez por mês ☐ Uma vez por semana
☐ Mais que uma vez por semana ☐ Uma vez por dia ☐ Mais que uma vez por dia

8.2. Em que situações costuma consumir álcool?

☐ Socialmente (ocasiões festivas) ☐ Somente às refeições

8.3. Das seguintes bebidas alcoólicas qual bebe com maior frequência?

☐ Vinho ☐ Vodka ☐ Whisky ☐ Cerveja ☐ Outra. Qual?

8.4. Em que parte do dia consome álcool?

☐ Manhã ☐ Tarde ☐ Noite

8.5. Que quantidade costuma beber?

☐ 1 copo ou menos ☐ 2 copos ☐ 3 a 6 copos ☐ 6 ou mais copos

8.6. Costuma beber bebidas alcoólicas:

☐ Sozinho(a) ☐ Com amigos ☐ Com Familiares

8.7. Considera-se dependente do álcool? ☐ Não ☐ Sim

9. Consome drogas? ☐ Não ☐ Sim ☐ Já experimentei, mas não consumo

9.1. Se sim, que tipo de drogas costuma consumir?

☐ Haxixe ☐ Extasy ☐ Heroína ☐ Cocaína ☐ Anfetaminas ☐ Outra. Qual? _____

9.1.1. Qual a frequência de consumo?

☐ Socialmente (concertos, festivais, etc) ☐ Ao fim-de-semana ☐ 2-3 vezes por semana ☐ Diariamente

9.2. Considera-se dependente de drogas? ☐ Não ☐ Sim

10. Já aceitou dinheiro em troca de favores sexuais? ☐ Não ☐ Sim

10.1 Se sim, usou preservativo? ☐ Não ☐ Sim

10.2 Com que idade começou? _____ anos

11. Conhece alguém que aceita dinheiro em troca de favores sexuais?

☐ Não ☐ Sim

12. Já foi vítima de abuso sexual? *Por exemplo: ser tocada sem sua permissão, violada, estuprada, etc...*

☐ Não ☐ Sim

13. Conhece alguém que foi vítima de abuso sexual?

☐ Não ☐ Sim

C. DADOS CLÍNICOS E GINECOLÓGICOS

14. Idade da 1ªmenstruação _____ anos

15. Idade da primeira relação sexual _____ anos

16. Nº de parceiro (s) sexual (ais) ao longo da vida ☐ 0 ☐ 1 ☐ 2-5 ☐ 5-10 ☐ >10

17. Nº de parceiro (s) sexual (ais) no último ano ☐ 0 ☐ 1 ☐ 2-5 ☐ 5-10 ☐ >10

18. Frequência mensal de relações sexuais ☐ 0 ☐ 1 ☐ 2-5 ☐ 5-10 ☐ >10

19. Já praticou sexo oral? ☐ Nunca ☐ Ocasionalmente ☐ Regularmente

19.1. Com que idade praticou a primeira vez sexo oral? _____ anos

20. Já praticou sexo anal? ☐ Nunca ☐ Ocasionalmente ☐ Regularmente

20.1. Com que idade praticou a primeira vez sexo anal? _____ anos

21. Toma anticoncepcionais orais (pílula)? ☐ Não ☐ Sim Desde que idade? _____ anos

☐ Já tomei Quanto tempo? _____

22. Usa preservativo? ☐ Não ☐ Sempre ☐ Ocasionalmente

22.1 Se não ou ocasionalmente, porque?

☐ Não sinto prazer durante o acto com preservativo ☐ Fui pressionada pelo meu parceiro/marido

☐ Estava a tentar engravidar ☐ Confio no meu namorado/parceiro/marido por isso não preciso de preservativo

23. Quantas vezes esteve grávida? _____ Nº de filhos _____

24. Já fez/teve aborto? ☐ Não ☐ Sim

24.1 Se sim, foi induzido? ☐ Não ☐ Sim

25. Já foi ao ginecologista? ☐ Não ☐ Sim

26. Se sim, com que regularidade? ☐ 2 vezes por ano ☐ Uma vez por ano ☐ 2 em 2 anos

☐ 3 em 3 anos ☐ 4 em 4 anos ☐ 5 em 5 anos ☐ > 5 anos

27. Alguma vez fez papanicolau/citologia? ☐ Não ☐ Sim

27.1 Qual o último resultado? ☐ Normal ☐ Alterado Data _____

27.1.1. Se alterado, qual o grau?

☐ Atipia de células escamosas de significado indeterminado (ASC-US) ☐ Lesão escamosa de baixo grau (LSIL)

☐ Lesão escamosa de alto grau (HSIL) ☐ Atipia de células escamosas sem excluir HSIL (ASC-H)

28. Antes de hoje, sabia o que era HPV?

☐ Não ☐ Sim

28.1 Se sim, como soube?

29. Já fez o teste do HPV? ☐ Não ☐ Sim Resultado _____

30. Fez a vacina contra o HPV? ☐ Não ☐ Sim ☐ Desconheço

30.1. Qual? ☐ Gardasil® (HPV 6, 11, 16, 18) ☐ Cervarix® (HPV 16, 18) ☐ Desconheço Data _____

31. Sabe o que é uma infecção sexualmente transmissível (ITS)?

☐ Não ☐ Sim

32. Já teve alguma IST? *Por exemplo: Clamídia, HPV, verrugas genitais, gonorreia, tricomoníase, sífilis, Herpes genital, HIV/SIDA, etc…*

☐ Não ☐ Sim

32.1. Se sim, qual? _____ Data _____

32.1.1. Teve sintomas? ☐ Não ☐ Sim **Se sim, quais?** _____

31.1.2. Fez tratamento? ☐ Não ☐ Sim **Qual?** _____

33. O seu parceiro (a) já teve alguma IST? ☐ Não ☐ Sim ☐ Desconheço

33.1. Se sim, qual? _____

33.1.1. Fez tratamento? ☐ Não ☐ Sim ☐ Desconheço

Qual? _____

34. Já teve lesões na boca? ☐ Não ☐ Sim

34.1. Com que frequência aprecem? ☐ Frequentemente ☐ Ocasionalmente ☐ Raramente

35. História de verrugas na pele ☐ Não ☐ Sim

35.1. Se sim, em que zona? ☐ Mãos ☐ Pés ☐ Vários locais. Quais? _____

D. AUTO-COLHEITA CERVICO-VAGINAL

36. Sentiu dor ao realizar a auto-colheita? ☐ Não ☐ Alguma ☐ Muita

37. Como classifica a auto-colheita? ☐ Não-aceitável ☐ Neutro ☐ Aceitável

38. Voltaria a fazer o teste por auto-colheita? ☐ Não ☐ Sim ☐ Talvez

38.1. Porquê? _____

Se já realizou um exame ginecológico médico responda às seguintes questões:

39. Comparativamente ao exame ginecológico médico, como define a auto-colheita?

☐ Mais fácil ☐ Igual ☐ Mais difícil

40. Qual dos dois métodos de colheita acha mais doloroso? ☐ Colheita médica ☐ Auto-colheita

41. Considera a auto-colheita mais confortável que a colheita médica? ☐ Não ☐ Sim

42. De modo geral, prefere a auto-colheita ou a colheita médica? ☐ Colheita médica ☐ Auto-colheita

O questionário termina aqui. Agradeço a sua colaboração.