HUMAN PAPILLOMAVIRUS DETECTION AND TYPING IN PATIENTS WITH ABNORMAL PAP SMEARS

by

DR. ELIZEBETH FREISLICH

RESEARCH DISSERTATION

Submitted in fulfillment of the requirements for the degree of

MASTER OF MEDICINE

in

OBSTETRICS AND GYNAECOLOGY

in the

FACULTY OF MEDICINE

at the

MEDUNSA CAMPUS

UNIVERSITY OF LIMPOPO

SUPERVISOR: DR. T.L. MSIBI

CO–SUPERVISOR: PROF. T.S. MONOKOANE

2010
DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of M Med (Obstetrics and Gynecology) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Dr. E. Freislich Date: November 2009
Student Number: 210437402
**Index:**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>5</td>
</tr>
<tr>
<td>Study Rationale</td>
<td>6 - 9</td>
</tr>
<tr>
<td>Literature Review</td>
<td>9 - 33</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>34</td>
</tr>
<tr>
<td>Objectives of the study</td>
<td>34</td>
</tr>
<tr>
<td>Methods</td>
<td>34 - 38</td>
</tr>
<tr>
<td>Data analysis</td>
<td>39</td>
</tr>
<tr>
<td>Ethical considerations</td>
<td>39</td>
</tr>
<tr>
<td>Results:</td>
<td></td>
</tr>
<tr>
<td>(a) Demographics</td>
<td>40</td>
</tr>
<tr>
<td>(b) Characteristics</td>
<td>41</td>
</tr>
<tr>
<td>(c) Examination</td>
<td>42</td>
</tr>
<tr>
<td>(d) Co-morbidities</td>
<td>42</td>
</tr>
<tr>
<td>(e) Menarche, coitarche and interval</td>
<td>43</td>
</tr>
<tr>
<td>between menarche and coitarche</td>
<td></td>
</tr>
<tr>
<td>(f) Use of contraceptives</td>
<td>43 - 44</td>
</tr>
<tr>
<td>(g) HIV Status</td>
<td>44 - 45</td>
</tr>
<tr>
<td>(h) Co-infection with HIV and HPV Genotypes</td>
<td>46</td>
</tr>
<tr>
<td>(i) Pattern of HIV and HPV Infections in relation to patients’ ages</td>
<td>47</td>
</tr>
</tbody>
</table>
(j) Results of Colposcopy and Punch biopsies

(k) The relationship between Histology and Punch biopsy, HIV and HPV genotypes.

Discussion

References

Data Sheet

Consent Form
ACKNOWLEDGEMENTS:

I want to thank Prof. T.S. Monokoane for all his advice, Dr T.L. Msibi for all her advice and hard work, Dr D.S. Beltchev for his advice and hard work and my fellow registrars for their hard work. I want to thank Prof. F. Guidozzi and dr. W.W. Edridge for their advice.

I want to acknowledge Dr. Gerhard Weldhagen from AMPATH, who enabled me to do this study, by organizing the donation of a Linear Array HPV Genotyping Test kit from Roche. I want to thank Roche for their generosity in donating the test kit. I want to thank Dr. Cornelius Clay and the staff of the Special Biochemistry Laboratory at AMPATH for their ever helpful, cheerful work in doing the actual HPV Genotyping and helping me keep track of the results.

At Medunsa, I want to thank Mr Isaac Mandiwana of Cytology and the other staff of the Cytology Laboratory for interpreting the Pap smears fast and helping me get the results. I also want to thank the Pathologists and Registrars of Anatomical Pathology who did all the histology and enabled me to get the results.

I want to thank Prof O.A Towobola and Mrs. M.A. Potgieter for the statistical calculations.
Without them all, I would never have been able to do this research.

**STUDY RATIONALE:**

Cervical cancer is the most common cancer of women on the African continent and the second most common cancer of women worldwide and in South Africa\(^1\)\(^2\). It has been estimated in 1997 that, among women who received no cervical screening in South Africa, 1 in 26 women were likely to develop cervical cancer\(^2\).

Screening will probably decrease the incidence of cervical cancer by 60% or more\(^2\). There is a direct relationship between the number of women screened by Pap smears and the decreased incidence of cervical cancer. In Iceland, where more than 90% of women were screened in that time, the incidence decreased by 80%. In Norway, where only 5% of the women were screened, the incidence only decreased by 10%\(^2\). In South Africa, it is estimated that Pap smears were taken in 18.8% of white women and only 2.6% of black women in 2002\(^2\).

Real-world obstacles to successful cervical cancer prevention in developing countries involve people more than technologies\(^3\). This can be managed by focusing on system quality management\(^3\). The root causes of poor quality must be examined. Suba et al\(^3\) found causes such as obso-
lete supplies, poorly maintained microscopes, insufficient training and suboptimal working conditions. Successful follow-up for screen-positive women has been achieved through the allocation of budgets for dedicated personnel to recontact women with positive test results.

Human Papillomavirus (HPV) infection is known to cause cervical cancer. Human Papillomavirus (HPV) infection is also regarded as the most common sexually transmitted infection worldwide, with an estimated lifetime risk of 79% for women to contract at least one infection between the ages of 20 and 79 years. Although some men have anal or genital lesions associated with HPV 16 and 18, most men serve as vectors of oncogenic HPV. Male partners may be important contributors to their female partners’ risk of cervical cancer.

The 15 HPV types, which are classified as high risk virus types, cause 95% of all cervical cancer. The High Risk HPV Genotypes are: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. HPV 16 and 18 together cause around 70% of all cervical cancer.

Squamous cell cervical cancer constitutes approximately 80% of cervical cancers. Adenocarcinoma is the second most common histological type and shows a rising incidence, even in developed countries.
There is geographical variation in type-specific HPV prevalence\(^9\). HPV16 is the most common type associated with adenocarcinomas, except in Southeast-Asia, where the prevalence of HPV 18 exceeds that of HPV 16. HPV 16, 18, 35, 45 and 59 are present in 96% of adenocarcinomas of the cervix\(^{10}\).

A pooled analysis by Clifford et al\(^9\) showed that the prevalence of high risk HPV types is around 18% in sub-Saharan Africa, with HPV 16 and HPV 35 present in 8% of women. HPV 31 and HPV 33 were present in 7% of women and HPV 18 was present in 4% of women. Sub-Saharan Africa had the highest prevalence of all HPV types and Europe the lowest. The variation in prevalence of HPV 16 across regions was smaller for HPV 16 than for the other high-risk types. The next common high-risk types were HPV 33 and HPV 56 in Asia, HPV 58 in South America and HPV 31 in Europe\(^9\).

This study’s rationale was to ascertain the HPV types prevalent in patients with abnormal Pap smears seen at the Gynaecological Outpatients Clinic at Dr. George Mukhari Hospital, the Gynaecological Oncology Clinic at Dr. George Mukhari Hospital, the Tshepang Clinic at Dr. George Mukhari Hospital and the Setshaba Research Centre of the University of Limpopo – Medunsa Campus in Soshanguve.
This study can also act as a pilot study for future studies to test the effectiveness of using high risk HPV types screening as a primary screening method, instead of Pap smears, to identify patients who are at a higher risk to develop cervical cancer and who need further investigations such as Colposcopically directed biopsies.

**LITERATURE REVIEW**

**Incidence:**

The incidence of HPV virus infections vary according to age, sexual activity, the number of times tested and the laboratory technique used \(^{11}\).

Acquisition of high risk HPV genotypes (HR HPV) is age dependant, with the highest frequency being amongst the youngest women \(^{12}\).

**Incident v Persistent HPV Infections:**

An incident HPV infection may regress spontaneously. A persistent HR HPV infection is one of the causative factors of cervical intraepithelial neoplasia \(^ {12}\).

Franco et al calculated a monthly incidence rate of 1.3% for new infections resulting in 38 % cumulative HPV positivity after 18 months \(^{12}\).
Syrjänen et al found a monthly rate of acquisition of incident HR HPV infections of 1.0% in women who were HR HPV DNA negative and Pap smear negative at baseline. In these women, time of acquisition of a HR HPV infection preceded an abnormal Pap smear by approximately 3 months (16.6 and 19.4 months, respectively)\textsuperscript{13}.

The time to acquisition of an incident abnormal Pap smear was significantly longer in women who were HR HPV DNA negative at baseline (19.4 months v 9.2 months in women who were HR HPV DNA positive at baseline). The rate of acquisition of an abnormal Pap smear was significantly higher in the women who were HR HPV DNA positive at baseline (3.1% v 1.5% in women who were HR HPV DNA negative at baseline)\textsuperscript{13}.

Schlecht et al found an incidence rate of SIL by Pap smear of 8.68 per 1000 women-months among women with HPV type 16 or 18 infections that persisted over 2 visits\textsuperscript{14}.

Sherman et al reported that the prevalence of HR HPV infections declines with age: only 31.2% among women with ASCUS who were 29 years or older, compared with 65% in those aged 28 and younger\textsuperscript{15}.
The majority of HPV infections are transient and are not clinically evident with 70-90% of infected women spontaneously clearing their infections within 12-30 months \(^{16}\).

Women with persistent HR HPV infection have the greatest risk of developing cervical precancer and cancer \(^{17}\). The longer an HPV infection persists, the less likely a patient is to clear her infection \(^{18}\). In a population-based study, women with type-specific persistence for more than 2 years were 800 times more likely to develop a high-grade cervical lesion \(^{19}\). The progression from HPV infection to HPV persistence to the development of high-grade CIN and ultimately invasive cervical cancer appears to take, on average up to 15 years, although cases of rapid-onset cancers do occur \(^{20}\).

In light of the high prevalence of HPV in young women, screening strategies have focused on women 30 years of age or older in an attempt to minimize the identification of transient HPV infections \(^{21}\).

**Infections with Multiple HPV Genotypes:**

Levi \(^{22}\) found that of 208 HIV positive women, 79% had multiple HPV genotypes. Trottier found that at individual visits, 1.9 - 3.2% of women
had multiple HPV infections \(^{23}\). Cumulatively during the first year and the first 4 years of follow-up, 12.3% and 22.3% were infected by multiple types, respectively \(^{23}\). HSIL risk markedly increased with the number of types. [OR 41.5 for single-type infection, OR 91.7 for two to three types, OR 424.0 for four to six types, relative to women consistently HPV negative during first year of follow-up] \(^{23}\). Co-infections with HPV 16 and 58 seemed especially prone to increase risk \(^{23}\).

Wheeler et al \(^{24}\) found a non-significant greater risk for \(\geq\) CIN III in women with multiple HR HPV types without HPV 16 than women with single HR HPV types without HPV 16 (10.9\% v 7.9\%). They found that the HR HPV types other than HPV 16, had a collective risk of \(\geq\) CIN III of 7.9\%. Multiple infections with HPV types of different risk classes resulted in a risk similar to, and not significantly different from, the risk observed for the highest class \(^{24}\).

**Pathophysiology:**

The HPV gets access through scratches, scars or at the transformation zone of the cervix, infecting the basal and parabasal cellular layers, where latent infection ensues \(^{5}\). Integration of highly oncogenic HPV DNA into host-cell chromosomes of the basal cells of cervical squamous epithelium is followed by the binding of HPV E6 and E7 oncoproteins to tumour-
suppressor genes p53 and RB, respectively \(^\text{25}\). This HPV DNA integration precedes the transformation from low grade to high grade cervical lesions \(^\text{26}\).

In non-infected cells: the p53 tumour suppressor gene levels increase in response to cellular or DNA damage or aberrant cell proliferation signals. High levels of p53 cause the cell to stop growing in the G1 phase of the cell cycle and allow it to either repair damaged DNA before the next round of DNA synthesis or be eliminated through apoptosis \(^\text{22,}\ 27\).

The E6 and E7 gene of the high risk HPV genotypes encode main transforming proteins. The E6 gene protein binds to the p53 tumour suppressor protein and promotes its rapid proteolytic degradation. The decreased p53 levels diminishes the cell’s ability to control the cell cycle and repair DNA damage and ultimately leads to uncontrolled cell growth \(^\text{12,}\ 26,\ 27\).

The E7 gene protein forms a complex with the retinoblastoma protein (pRB) and disrupts the complex between the cellular transcription factor E2F-1 and pRB. This results in the release of E2F-1, stimulating cellular DNA synthesis and uncontrolled cellular growth \(^\text{12,}\ 26,\ 27\).

In summary: the above processes result in impaired tumour-suppressor-gene function, involving DNA repair, decreased apoptosis and eventual
cell immortalisation 25.

HPV 16 E7 protein also induces centrosome-related mitotic disturbances that are potentiated by HPV 16 E6 protein 26, 27. The above results in the desegregation of the chromosome during mitosis leading to numerical and structural chromosomal aberrations 5.

Mutations causing chromosomal alterations, loss of heterozygosity, genetic instability and proto-oncogene and telomerase activation in immunopermisive individuals have important roles in virus-induced carcinogenesis 25.

Co-factors such as genetic or environment factors, such as smoking, may also be necessary for progression to the invasive stage 26. The so-called non-European variants of HPV 16 and 18 may increase the degradation potential of p53. HPV 16 is polymorphic and the Arg / Arg genotype of p53 could have greater susceptibility to HPV – E6 degradation than the other genotypes. The coincident interplay between the non-European genomic variants of HPV 16 / 18 and p53 Arg / Arg may explain, at least in part, the persistence of HPV infection and tumour progression in women with cervical neoplasia 25.
HPV persistence in HIV positive patients has been linked to a reduction in HLA class II molecules and a greater number of immature Langerhans cells within the cervix \(^{26}\).

Evidence-based epidemiological and molecular data suggest that persistent infections with HR HPV types are the intermediate endpoints, leading to both intraepithelial and invasive cervical neoplasia \(^{25}\).

The multihit, multistage model of carcinogenesis is a physiologically based quantitative model uniting the processes of mutation, cell growth and turnover. It also accounts for human heterogeneity for inherited traits and environmental experiences. It is an attempt to explain the relationship between the molecular mechanisms of mutagenesis and the actual processes by which most people get cancer \(^{28}\).

Age-incidence relationships and experimental evidence suggest that cancer is a multi-stage disease \(^{29}\). Tumours are monoclonal implying that multiple hits need to affect a single clone of cells \(^{30}\). Genes may interact in an unordered or ordered fashion along a polygenic pathway. Cancers almost always are heterogenous \(^{31}\).

Hanahan and Weinberg argued that most cancers have to achieve six essential alterations on the way to malignancy: self-sufficiency in growth
signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis. However, the number of stages cannot be deduced this way, because some of the acquired capabilities probably interact.

Herrero-Jimenez et al developed a model to compute the essential parameters of the two-stage initiation promotion model, using colon cancer as an example. Their work was based on the work of Nordling, Armitage, Doll, Moolgavkar and Knudson. When Hemminki et al tested the model on cervical cancer, they found that the number of initiation mutations required for cervical cancer are 5 stages.

In cervical cancer, immune surveillance plays an important role. Immunosuppressed patients are at a marked risk for many types of squamous cell carcinomas. Suppressed immune function is also likely to modulate host response to virus, such as HPV.

Hemminki et al found the effect of nonshared environmental factors (sporadic causes of cancer) to be 80% for cervical cancer. Shared environmental effects between twins were shown to be 20%. This suggests that the genetic effects are masked by strong environmental influences, such as HPV.
The Pap smear as primary screening for Cervical cancer and its precursors:

The goal of cervical screening is the detection of cervical cancer and precursor lesions\(^{35}\).

Papanicolaou showed that exfoliated cervical cells could be reliable harvested and spread, screened and stained on a glass plate. With the Pap smear, he laid the foundations of cervical screening\(^ {36}\).

Organized Screening versus Opportunistic screening Programmes:

During the 1960s, it became apparent that a population screening programme could reduce both the incidence and death rate from cervical cancer, as first demonstrated in British Columbia\(^ {37}\). Until the 1980s, cervical screening was not applied in a systematic fashion in the UK, with the result that many women at greatest risk were not screened\(^ {38}\). The death rate from cervical cancer was essentially unchanged until the national call and recall program was instituted in 1988 in the UK\(^ {39}\).

The program originally involved every woman between the ages of 20-64 years (20-60 years in Scotland) being called and recalled for a Pap smear every 3-5 years. The death rate from cervical cancer is now 50% of what
it was in 1988 with 2 700 cases of invasive cancer, 19 000 cases of carcinoma in situ and approximately 1 200 deaths each year \(^{39}\). Similar falls in death rates have been seen in Finland, Iceland and the USA \(^{38}\).

In 1990, target payments were introduced for GPs in the UK to do Pap smears of 80% or more of their female patients. The national coverage has risen to 85.3% in the UK, because of the call and recall system and the target payment to GPs \(^{39}\). This must be compared to the estimated coverage in South Africa, where Pap smears were done on an estimated 18.8% of white women and 2.6% of black women in 2002 \(^2\).

The National Cervical Screening Policy (SA Department of Health, 2000) in South Africa allows for female public health care patients to have 3 Pap smears at ten year intervals from age 30 years.\(^1\) The aim is to reduce cervical cancer incidence rates by 60% (Department of Health, 2000). It is an opportunistic screening program.

Miles et al compared organized screening programs with opportunistic screening programs and identified seven lessons learnt:

1) Organized screening has greater potential ability to reduce cancer incidence and mortality due to higher achievable levels of population coverage, follow-up and quality compared with opportunistic
screening\textsuperscript{40}.

2) Organized screening programs aim to achieve a population-level benefit and a balance of benefits and harms; as a result, organized programs may not provide screening that offers maximum protection to each individual but offer them greater protection from harms\textsuperscript{40}.

3) Equality of access is often a key principle of health care provision in countries with organized screening\textsuperscript{40}.

4) In organized programs, the opportunity to be screened is determined by health policy and by the adequacy of the call-recall system; in opportunistic screening, the opportunity is determined to a greater extent by individual factors, such as the knowledge and behaviour of patient and provider, insurance coverage, and the patient’s pattern of encounters with health services\textsuperscript{40}.

5) Cost of screening as a barrier is largely remedied by organized programs, but limitations in terms of access remain\textsuperscript{40}.

6) Organized programs do not eliminate socioeconomic and ethnic disparities in the uptake of cancer screening, and each model faces challenges related to informed consent\textsuperscript{40}.
7) Introducing an organized system of screening presents many challenges related to existing and required infrastructure, resources, vested interest, public and provider acceptance of centralized health care.

To achieve the goal of reducing South African cervical cancer incidence by 60%, our national screening policy will have to be changed to an organized screening policy. To introduce a call and recall system, a reliable centralised data base must be used. The National Electoral Rolls are the biggest South African centralised population data base, but presently are not up to date. In 2008, however, it will be up to date, because, a national election is due to be held.

Pilot programs can be initiated in the Primary Health Clinics of the larger metropolitan areas, using the local electoral rolls for a call and recall program. This can be done by the Municipal Health Departments. Primary Health Care Physicians and sisters can be paid a target payment to motivate the taking of Pap smears.

If the pilot programs are proven to be cost-effective, the program can be extended to smaller towns and ultimately to rural areas. In the rural areas, the traditional leaders can be asked to facilitate the call and recall
program amongst their people. Mobile clinics can be used to reach areas where there are no permanent Primary Health Care clinics.

The cost of the target payment, diverse costs of the program and the cost of the cytology screening can be offset against the cost of treating patients with cervical cancer and its precursor lesions.

In South Africa, 5203 cervical cancer cases were reported in 1999. This amounts to an estimated average of 26.1 per 100 000 women (National Cancer Registry)\(^41\). If a Call and recall program is started in South Africa, the cervical cancer incidence can be reduced by 50% as per the UK example.

A South African example of a successful Public Health National Programme is the National Immunisation Programme where 84% of all infants were fully immunised during 2006 (Every Death Counts Report)\(^42\). The Immunisation Programme is a hybrid call and recall programme, where the infants are immunised at birth and the mothers are then given a return date for the next appointment. At each immunisation, a return date for the next appointment is given.

A possible Call and recall program for cervical screening in South Africa can be started at the 6 weeks post partum appointment at the Post natal
clinics, where a Pap smear or a liquid-based cytological screening could be done on every woman of 30 years and above, who haven’t had a Pap smear in the past. Their results could be given to the patients on the date of the next appointment for immunisation for their infant. A card could be given to the patients, similar to the immunisation cards, with a perforated section for notification of change of address. They could be informed that, should they move in the next ten years, they should send the perforated section with the correct contact details to the Health Department of the municipality where they move to. In this way, a national data base could be started, supplementing the Electoral voters rolls.

**Current challenges in cervical screening:**

**Sensitivity:**

The Pap smear has a low sensitivity of 58% to detect CIN 3 lesions. The Pap smear has a high false-negative rate. The specificity of the Pap smear is 94.2%. The majority of missed lesions are due to failure to sample the lesion. In order to achieve maximum sensitivity, it is necessary to act on the most minor abnormalities.

This creates one of the major difficulties in cervical screening – the management of low-grade abnormalities, which carry a very low positive predictive value for the presence of CIN, yet are associated with a
significant number of underlying high-grade CIN lesions\textsuperscript{38}.

Where the cytology is reported as unsatisfactory, the Pap smear needs to be repeated. Liquid-based cytology involves a fluid suspension of exfoliated cells being placed in a liquid medium. The cell suspension is aspirated through a filter and the resulting thin layer of cells is deposited on a glass slide. This provides cleaner preparations, which are easier to read.

Large pilot studies in the UK found that inadequate cytology would be cut by 80%, laboratories could process the slides more quickly, and that, despite increased costs per slide, overall liquid-based cytology would be cost-effective. NICE agreed and liquid-based cytology is being implemented across the UK\textsuperscript{38}. Similar studies need to be done in South Africa to establish the most cost-effective technology to be used as part of our National screening program.

\textbf{Shortages of cytoscreeners and consultant cytopathologists.}

By using liquid-based cytology techniques, the need for repeat Pap smears will reduce\textsuperscript{38}. Automated reading of cytology slides has been approved by the FDA in the USA. The most abnormal appearing cells are then presented to the cytoscreeners using a computer-guided microscope platform. Using computerised algorithms, that the least abnormal 25% of
slides can be passed negative without being seen by a cytoscreener. Such technology has the potential to make screening more efficient, reducing adequate staffing pressures.

**HPV testing:**

Quantitative real-time PCR assays for diagnosis of high risk HPV types are available in South Africa. A sample from a cervical brush or spatula can be tested for the presence or absence of specific high risk HPV types. The sensitivity and negative predictive values for the test are 94%.

The specificity for the test is a concern and false-positive rates of 5-20% have been reported. Schiffman found a specificity for HSIL or cancer of 89%, which was lower than the specificity for cytology (94.2%). This would result in excessive patients who would need to be referred for colposcopy, many of which who could be false-positive results.

The combination of the Pap smear and HPV testing attain very high sensitivity and negative predictive values (approaching 100%). Restriction to older women seem to improve the specificity of the HPV test, but this also improves the specificity of cytology.

Wright et al found that HPV testing of self-collected vaginal swabs is less specific than Pap smears (false-positive rates of 17.1% v 12.3%), but as
sensitive as Pap smears to detect HSIL in women aged 35 years and older. (66.1% v 67.9%)\(^49\). The self-collected samples were performed under optimal conditions. (in the examination room after specific instruction for its use. Performance of a self-collected sample under more realistic conditions (e.g., community distribution) needs to be evaluated\(^49\).

An accurate self-sampled HPV test creates the possibility to evaluate women who are unwilling or unable to submit to pelvic examination\(^47\).

**HPV testing: adjuvant or primary screening?**

HPV testing has been used in the study of the etiology of cervical cancer. It has also been used for three main screening or management-related purposes:

1) Primary screening: for the detection of cervical cancer or its precursor lesions among asymptomatic women without a referral diagnosis, i.e., as true population screening, either opportunistic or systematic. HPV testing is usually used to complement a screening Pap smear or as a screening tool in isolation\(^44\). A single HPV test cannot distinguish between prevalent or incidental infections, limiting its use as a meaningful screening tool\(^44\).

2) Secondary triage: for the detection of cervical cancer or its pre-
cursor lesions among women who have an abnormal Pap smear requiring further evaluation. Here HPV testing is used as a substitute for a repeat Pap smear as part of a management algorithm to triage women who should undergo immediate colposcopy and biopsy. It can also be used to complement the result of a repeat Pap smear in a more controlled environment 44.

3) Follow-up of treated cases – for improved surveillance of recurrent cervical lesions after treatment to permit more aggressive management of cases that are likely to recur, because of persistent HPV infection 44.

The South African Women’s Health Advisory Board have suggested that women < 35 years of age with ambiguous Pap smears such as Atypical Glandular Cells of Undetermined Significance (AGUS), Atypical Cells of Undetermined Significance (ASCUS) and Low Grade Squamous Intra-epithelial lesions (LGSIL) should receive a HPV test. In the presence of HR-HPV DNA, these patients are then referred for colposcopy and appropriate treatment 1,50. If the HPV test is negative, the Pap smear should be repeated after one year 50.

The South African Women’s Health Advisory Board have recommended that women from age 35 to 65 years have a HPV test with a Pap smear as primary screening for cervical cancer or its precursors 50.
If both are negative, the screening interval should be increased to 10 years. If the HPV test is positive, but the cytology is negative, the HPV test should be repeated after one year. If both are positive, the patient should be referred for colposcopy and appropriate treatment \(^{50}\).

The patients with ambiguous Pap smears such as Atypical Glandular Cells of Undetermined Significance (AGUS), Atypical Cells of Undetermined Significance (ASCUS) and Low Grade Squamous Intraepithelial lesions (LGSIL) with a positive HPV test, whose initial colposcopy do not reveal CIN II or CIN III, pose a difficult clinical problem.

In the ALTS trail, only 10% of these women were found to have CIN II or CIN III after 2 years follow-up period \(^{24}\). The ASCCP consensus guidelines recommended either HPV testing at 12 months or cytology at 6 and 12 months in these cases \(^{51,52}\).

The American Cancer Society and the American College of Obstetricians and Gynecologists now recommend combined HPV and Pap smear testing for women age 30 and older as primary screening for cervical cancer. For women younger than 30 years, screening is still every year with conventional Pap smears and every two years with liquid-based (Thin-prep) cytology \(^{26,52}\).
If both HPV and Pap smears are negative, the screening interval can be extended to every 3 years. If both are negative, the negative predictive value that CIN III or cancer is absent, is almost 100%. If a woman has a positive HPV test, but a negative Pap smear, she should repeat both tests \(^{26,52}\).

A few large randomly controlled trials of HPV testing are presently ongoing. The HART (HPV in Addition to Routine Testing) trial in the UK, the ARTISTIC (A randomized Trail in Screening To Improve Cytology) also in the UK and the CCast (Canadian Cervical Cancer Screening Study) in Canada \(^{44}\).

**HPV testing as a cure test.**

The ASCCP consensus conference recommended that HPV testing could be used as a test for cure for women with CIN II or CIN III at least 6 months following excision or ablation of the transformation zone. The women with HR HPV would then be referred for colposcopy \(^{51,52}\).

Coupe et al found that HPV testing at 6 months and both HPV and cytological testing at 24 months after treatment did not lead to an increase in colposcopy rate and was cheaper than and just as effective as the current European protocol \(^{53}\).
HIV and HPV co-infection.

South Africa is experiencing a very serious HIV pandemic, with an estimated 6 million people living with HIV/ AIDS. Around 87% are in the age group 15 – 45 years, of which around 50 % are women. HIV positive women are more likely to have HPV infections of any type than the HIV negative women \(^{24,54}\). In a study done in Zimbabwe, it was found that HPV types 11, 39, 43, 51 and 59 occurred more frequently in HIV positive women \(^{55}\).

HIV positive women with HPV are also more likely to have Cervical Intraepithelial lesions (CIN) lesions on Pap smear \(^{54}\). CIN lesions are independently associated with HPV infections (OR 9.8), HIV infection (OR 3.5) and CD4 count < 200 (OR 2.7) \(^{56}\).

With the reality of a large percentage of patients getting infected with HIV as teenagers, the onset of HPV Screening at age 30 in South Africa, may be too late \(^1\). Lomalisa et al found that HIV positive patients presented with invasive cervical carcinoma almost 10 years earlier than HIV negative patients \(^{57}\).
Treatment of HR HPV infection:

There is international consensus that, where there is a positive HR HPV test with positive cytology, patients should be referred for colposcopy and appropriate treatment should be given, usually by Large Loop Excision of the Transformation Zone (LLETZ) or cone biopsy.\(^1,2,6\)

The screen-and-treat approach has the maximum benefit in settings where compliance is poor and no facilities or expertise exists for performing colposcopies and histology.\(^44\).

Denny et al found that the prevalence of high-grade cervical intraepithelial neoplasia and cancer was significantly lower in 2 groups of patients who were screened by using HPV DNA testing and visual inspection of the cervix with acetic acid and then treated with cryotherapy than in the delayed evaluation group. At 6 months, CIN II or a higher grade of intraepithelial neoplasia or cancer was diagnosed in 0.8% of the women in the HPV group compared to 3.55% in the delayed evaluation group.\(^58\).

This approach was criticised by Suba\(^3\) who emphasized that the root causes of poor quality must be examined and corrected and not compensated for by screen-and-treat approaches.
HPV Vaccine.

In the FUTURE II trial, a quadrivalent recombinant vaccine (Gardasil) was tested that is effective against HPV types 6, 11, 16 and 18$^{35,59}$. With immunisation against HPV 16, there is also some cross–effective-ness against HPV 52.

Biopsy–proven disease, including CIN, vulvar intraepithelial neoplasm (VIN), vaginal intraepithelial neoplasm (VAIN), genital warts and invasive cancer was reduced by 100% for type-specific HPV’s. With 30 months of follow-up, the incidence of persisting HPV 6, 11, 16, 18 infections, was decreased by 89% in women who received at least 1 dose, compared to those who received placebo$^{35}$. Vaccination is preventative and not therapeutic against existing HPV infections of HPV 6, 11, 16 and 18$^{35}$.

The FDA has approved it for the prevention of HPV 16 and 18 related cervical cancer, CIN II/III, AIS, VAIN, VIN and genital warts and CIN I caused by HPV 6, 11, 16 and 18$^{5,35}$.

The vaccine has also been approved by the FDA for use in adolescent girls 9 – 15 years of age.
The CDC’s Advisory Committee on Immunization Practices has recommended that 9 and 10 year old girls be vaccinated at the discretion of their physician. The American Advisory Committee on Immunization practices (ACIP) endorses immunization before the onset of sexual activity and recommends routine vaccination from 11 to 12 years in females. It can be commenced as young as 9 years. Females 13 to 26 years, not previously vaccinated, can also be vaccinated. Three doses are given. In the United Kingdom, teenage girls of 12 – 13 years will be offered HPV vaccine from September 2008.

The bivalent vaccine (Cervarix) was shown to be effective (over 88%) against incident and persistent HPV 16 and HPV 18 infections up to 4 years following vaccination. It demonstrated significant protection against cytological abnormalities and 100% efficacy against CIN associated with HPV 16 and / or 18. There was also some evidence for vaccine-related crossprotection against incident HPV 45 and 31 infections. Cervarix has been approved in Europe and the UK and is awaiting FDA approval.

The quadrivalent vaccine is available in South Africa. A South African Vaccination program could reduce the incidence of and mortality of cervical cancer and may reduce the costs of maintaining screening pro-
grammes.

The vaccine is well tolerated with the most common adverse effect being a headache $^{35}$.

Vaccination may be offered to immunosuppressed women, because of their high risk of HPV infection. There is however no data of efficacy in this group $^{35}$.

Clinical trails are ongoing to define the duration of efficacy $^{35}$. The prohibitively high cost of the vaccine is a problem $^5$. With negotiation, the price may be dropped for use in the public sector.

Other obstacles are cultural and religious objections against immunising young girls against a sexually transmitted disease $^{5,60}$. Ethical, cultural, social and religious connotations can be addressed by careful education and cooperation of all the role players, including paediatricians, gynaecologists, family medicine practitioners, nursing staff and members of the Health Department $^5$.

There are also questions as to the long term protection against the specific HPV viruses immunised against, the timing of the booster immunisations and whether boys must also be immunised $^{60}$.
**HYPOTHESIS**

My null hypothesis was that patients with HIV infection had the same HR HPV type infections as patients without HIV infection.

**OBJECTIVES OF THE STUDY**

The aim of the study was to test for the prevalence of different HPV types in patients with abnormal cervical cytology.

**METHODS**

The study was a prospective cohort trail. It is a pilot study for bigger studies to test for the effectiveness of using HR HPV screening as a primary screening method. We enrolled 29 HIV positive patients, 12 HIV negative patients and 10 patients who opted out of HIV testing with abnormal Pap smears. The study was done from March 2007 to September 2007.

The patients were recruited from the Tshepang Clinic of Dr. George Mukhari Hospital, the Gynaecological Outpatient Clinic of Dr. George Mukhari Hospital, the Gynaecological Oncology Clinic of the George Mukhari Hospital and the Setshaba Research Centre of the University of Limpopo Medunsa Branch in Soshanguve.
The Tshepang Clinic is the clinic at Dr. George Mukhari Hospital where HIV positive patients are followed up and where they receive HIV related Medical care.

The Setshaba Research Centre is a centre where, amongst other research, research on the use of vaginal microbicides for the possible prevention of HIV infection during sexual intercourse, is done. The centre is sponsored by the Population Council in New York, USA and is under management of the Microbiology Department of the University of Limpopo-Medunsa Branch. It is situated in Soshanguve.

Cervical smears were done with cytobrushes of each patient’s cervix at entry to the study. The cervical smears were done by dr T.L Msibi, Consultant in charge of the Gynaecological Oncology Clinic at Dr George Mukhari Hospital, Dr D.S. Beltchev, Consultant in charge of the Gynaecological Clinic at Dr George Mukhari Hospital, Dr E. Freislich, the chief researcher and registrars working in the Gynaecological Oncology Clinic.

Patients with abnormal pap smears (specifically Cin II and Cin III lesions) underwent Colposcopy. Any suspicious area on the cervix and specifically of the Transformation Zone of the cervix was biopsied under colposcopically direction.
The Colposcopies were done by the Consultant working in the Gynaecological Oncology Clinic, Dr E Freislich and Registrars working in the Gynaecological Oncology Clinic of Dr George Mukhari Hospital.

All the colposcopies done by Registrars were done under supervision of the Consultant working in the Gynaecological Oncology Clinic.

The cervixes were cleaned with a 3% Acetic acid solution to remove excess mucus and cellular debris. The Acetic acid also accentuates the difference between normal and abnormal colposcopic patterns.

An Excision punch biopsy was then performed from any acetowhite areas on the cervix and specifically from acetowhite areas on the Transformation Zone. If there were no uptake of the Acetic acid by the cells of the Transformation zone, multiple excision Punch Biopsies were done from both the anterior and posterior lip of the cervix in the Transformation zone.

For patients with Cin I lesions on Pap smear, a repeat pap smear was done as per protocol of the Gynaecological Oncology Unit.

Several patients of whom the Histology of the excision Punch biopsy indicated High Grade SIL (Cin II, Cin III), were counselled and received either a Total Abdominal Hysterectomy with a bilateral Salpingo-
ooverectomy or a Vaginal Hysterectomy. Some patients were unfortunately lost to follow-up. The patients who desired future fertility, were counselled and they were offered Cone biopsies.

The Linear Array Human Papillomavirus (HPV) Genotyping Test from Roche was used to identify the specific HPV DNA Genotypes in DNA material collected from the cytobrushes. The tests were done by the Special Biochemistry Laboratory at AMPATH National Laboratory Services and were validated by their standard quality control methods.

The Linear Array HPV Genotyping Test is a qualitative in vitro test. It utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization. It is a highly reproducible genotyping assay. Van Hamont et al compare the SPF10 LiPA version 1 and the Linear Array HPV Genotyping Test in order to assess the reproducibility of the two tests for a performance assessment. Of the 160 samples used for comparison analysis, 80.6% showed absolute concordant results, 11.2% showed compatible results and 8.2% showed discordant results. The genotyping assays were found to be highly comparable and reproducible.\(^{61}\)
The test detects 37 (thirty seven) anogenital HPV DNA genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108).

The patients were also assessed for:

1. Age.
2. Parity.
3. Interval between first delivery and enrolment in the study.
4. Age at menarche and coitarche.
5. Interval between menarche and coitarche.
6. Marital status.
7. Number of Sexual partners during the patients’ lifetime.
8. Socio-economic status.
9. Use of contraception including barrier contraception.

The data collection was done by Dr T. L Msibi, Consultant in charge of the Gynaecological Oncology clinic at Dr George Mukhari Hospital, Dr D.S. Beltchev, Consultant in charge of the Gynaecological Clinic at Dr George Mukhari Hospital, Dr E. Freislich, the chief researcher, and registrars working in the Gynaecological Oncology Clinic, according to the data form attached.
DATA ANALYSIS

The data of both groups was first analysed to ascertain whether it is parametric or non-parametric. To test the reliability of the data, Cronbach’s Alpha was done between the groups. The data was non-parametric, thus Spearman’s correlation coefficients were performed. A Chi-square analysis was done. The data determinants were assessed with appropriate multivariate analysis. Data was recoded to indicate low, medium and high risk HPV genotypes. Backward stepwise regression was done to determine the relationship between HPV, HIV and the interval between menarche and coitarche. SPSS and SAS software were used in analyses of the data. Significance was taken as p < 0.05.

ETHICAL CONSIDERATIONS

Informed consent was obtained from all the participants of the prospective study. All personal information of patients in the trail remained confidential. The patients’ names were deleted and codes were used to identify participants. The trail was performed with the approval of the Research, Ethics and Publications Committee of the University of Limpopo (Project number: MP 14/2007).
RESULTS:

Demographics:

Fifty one patients, aged between the ages of 18 and 67 years, (with a mean age of 38.43) who had abnormal pap smears, participated in the study. The parity of the majority of the patients (79%) was 1 – 4 (with a mean of 2.29) and 66.5 % of the patients were unmarried, as shown in table 1 and 2.

Table 1: Parity.

<table>
<thead>
<tr>
<th>Parity</th>
<th>Nr of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>5.8</td>
</tr>
<tr>
<td>1 – 2</td>
<td>29</td>
<td>56.9</td>
</tr>
<tr>
<td>3 - 4</td>
<td>16</td>
<td>31.5</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>3</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Table 2: Marital Status.

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>Nr of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>16</td>
<td>31.5</td>
</tr>
<tr>
<td>Single</td>
<td>34</td>
<td>66.5</td>
</tr>
<tr>
<td>Widowed</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Characteristics:

80.2 % of the patients had a family support structure.

Socio – economic Status:

Table 3: Socio-economic Status.

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Employed</td>
<td>9</td>
<td>17.7</td>
</tr>
<tr>
<td>Pensioner</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Family Support Structure</td>
<td>39</td>
<td>76.5</td>
</tr>
<tr>
<td>No Support</td>
<td>2</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Examination:

Both a general and a gynaecological examination were performed on all patients. 82.3% of patients had no abnormalities on general or gynaecological examination. 4% of patients had either lymphadenopathy, myomatous uteri or multiple condylomata. 6% of patients had vaginitis/cervitis.

Table 4: Findings on Examination.

<table>
<thead>
<tr>
<th>Examination</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>42</td>
<td>82.3</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>2</td>
<td>3.9</td>
</tr>
<tr>
<td>Myomatous Uterus</td>
<td>2</td>
<td>3.9</td>
</tr>
<tr>
<td>Vaginitis / Cervicitis</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>Giant/ Multiple Condylomata</td>
<td>2</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Co-morbidities:

Fifty-nine percent of the patients had no co-morbidities. The other forty-one percent of the patients had quite a number of co-morbidities, as illustrated in Table 5.

Table 5: Co-Morbidities.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Hypertension</td>
<td>10</td>
<td>19.6</td>
</tr>
<tr>
<td>Chronic Hypertension and NIDDM</td>
<td>4</td>
<td>7.8</td>
</tr>
<tr>
<td>PTB (treated)</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td>Obesity</td>
<td>2</td>
<td>3.9</td>
</tr>
<tr>
<td>AIDS related illnesses</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Menometroragha with secondary anaemia</td>
<td>1</td>
<td>2.0</td>
</tr>
</tbody>
</table>
**Menarche, Coitarche and Interval between Menarche and Coitarche.**

The patients’ menarche ranged between 12 and 18 years with a mean of 15.1 years. The patients’ coitarche ranged from 13 to 21 years with a mean of 17.04 years. The interval between menarche and coitarche ranged from 0 – 8 years with a mean of 1.98 years.

**Use of Contraceptives:**

The majority of patients (25 patients or 49% of the total) were not using any contraception. Four patients (7.8%) were postmenopausal. 43.2 % of patients were using contraception, of which the majority (15.6%) were using injectable depot Progestogen contraception. The breakdown of contraceptive useage is given in table 6.

Out of 25 HIV positive patients who were potentially fertile, only 8 were using condoms. Only 1 patient was using condoms in combination with another form of contraceptive method (a Combined Oral contraceptive pill).

Only 4 HIV positive patients are sexually inactive.
Table 6: Use of Contraceptives.

<table>
<thead>
<tr>
<th>Contraception</th>
<th>Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>25</td>
<td>49%</td>
</tr>
<tr>
<td>Injectable depot Progestogen</td>
<td>8</td>
<td>15.6%</td>
</tr>
<tr>
<td>Condoms (only)</td>
<td>7</td>
<td>14%</td>
</tr>
<tr>
<td>Sterilization</td>
<td>5</td>
<td>9.8%</td>
</tr>
<tr>
<td>Combined oral contraceptive pill</td>
<td>2</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

**HIV Status:**

The majority of the patients consented to HIV testing. 41 patients tested (80.4%) and 10 patients opted out (19.6%).

Of the 41 patients who tested, 12 were HIV negative (23.5%) and 29 were HIV positive (56.9%).

Of the 29 HIV positive patients, 11 were on ARV’s. Three patients were being counselled for ARV treatment (CD4 count of 97, 175 and 194 x 10^6 / l).

Three patients were newly diagnosed HIV positive patients and their CD4 counts were unknown.
The remaining 11 HIV positive patients have CD4 counts > 200 \times 10^6/l.

Table 7: HIV Status.

<table>
<thead>
<tr>
<th>Number of patients who tested</th>
<th>HIV positive</th>
<th>HIV negative</th>
<th>HIV unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>41 (80.4%)</td>
<td>29 (56.9%)</td>
<td>12 (23.5%)</td>
<td>10 (19.6%)</td>
</tr>
</tbody>
</table>

Table 8: HIV Positive Patients with CD4 counts < 200 or > 200

<table>
<thead>
<tr>
<th>CD4 counts:</th>
<th>On ARV’s</th>
<th>Qualifying for ARV’s, but in process of Counselling</th>
<th>Not on ARV’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200</td>
<td>8 (27.5%)</td>
<td>3 (10.3%)</td>
<td>12 (41.5%)</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>2 (6.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td>1 (3.5%)</td>
<td></td>
<td>3 (10.3%)</td>
</tr>
</tbody>
</table>
Co – Infection with HIV and HPV:

HIV Positive patients:

HPV genotypes in order of prevalence were:

HPV 52 (15 pts), 62 (13 pts), 16 (11 pts), 53 and 58 (9 pts), 18 and 33 (8 pts).

HIV negative patients:

HPV genotypes in order of prevalence were:

HPV 16 (4 pts), 33 and 52 (3 pts), 45 (2 pts), 39, 42, 51, 53, 62, 67, 68 and 72 (1 pt).

In two HIV negative patients, there were no HPV genotypes present.

Patients with unknown HIV status:

HPV genotypes in order of prevalence were:

HPV 16 (5 pts), CP 6108 (3 pts), 39, 52, 54, 62, 69 and 70 (2 pts), 31, 33, 35, 53, 58, 61, 66, 67, 73, IS 39 and 83 (1 pt).

Table 9: Co-Infection with HIV and HPV.

<table>
<thead>
<tr>
<th>HIV Status</th>
<th>On ARV’s (+)/ not on ARV’s (-)</th>
<th>HPV Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Positive</td>
<td>+ (14 patients)</td>
<td>52, 62, 16, 58, 53, 61, 18, 66, 69, 84, CP 6108</td>
</tr>
<tr>
<td></td>
<td>- (15 patients)</td>
<td>52, 62, 16, 18, 33, 53</td>
</tr>
<tr>
<td>HIV negative</td>
<td>12 patients</td>
<td>16, 52, 33, 45</td>
</tr>
<tr>
<td>HIV unknown</td>
<td>10 patients</td>
<td>16, CP 6108, 39, 52, 54, 62, 69, 70</td>
</tr>
</tbody>
</table>
Pattern of HIV and HPV Infections in relation to patients’ ages

The results are listed in table 10.

Table 10: HIV and HPV Infection according to Age Groups.

<table>
<thead>
<tr>
<th>Age Range</th>
<th>HIV infection (number of patients)</th>
<th>HPV infection (in order of prevalence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 years</td>
<td>Positive (1)</td>
<td>18, 62</td>
</tr>
<tr>
<td></td>
<td>Negative (1)</td>
<td>42, 45, 52</td>
</tr>
<tr>
<td></td>
<td>Unknown (0)</td>
<td></td>
</tr>
<tr>
<td>21 – 29 years</td>
<td>Positive (10)</td>
<td>52, 53, 16, 18, 62, 33, 51, 56, CP 6108, 35, 42, 58, 59, 66, 68, 69, 83, 11, 26, 31, 39, 40, 45, 61, 71, 73, 81, 82</td>
</tr>
<tr>
<td></td>
<td>Negative (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unknown (2)</td>
<td>16, 39, 53, 69, 70, CP 6108</td>
</tr>
<tr>
<td>30 – 39 years</td>
<td>Positive (11)</td>
<td>16, 52, 62, 84, 33, 58, 61, CP 6108, 53, 66, 68, 69, 81, 6, 18, 31, 35, 56, 67, 71, IS 39</td>
</tr>
<tr>
<td></td>
<td>Negative (3)</td>
<td>16, 45, 51, 68, 71</td>
</tr>
<tr>
<td></td>
<td>Unknown (2)</td>
<td>16</td>
</tr>
<tr>
<td>≥ 40 years</td>
<td>Positive (7)</td>
<td>62, 18, 33, 53, 55, 58, 59, 66, 72, 84, 26, 35, 39, 56, 61, 67, 68, 70, 73, 82, CP 6108</td>
</tr>
<tr>
<td></td>
<td>Negative (8)</td>
<td>33, 52, 16, 39, 62, 67, 69</td>
</tr>
<tr>
<td></td>
<td>Unknown (6)</td>
<td>16, 52, 54, 62, CP 6108, 31, 35, 39, 58, 61, 66, 67, 70, 73, 83, IS 39</td>
</tr>
</tbody>
</table>
Results of Colposcopy and Punch biopsies:

The results are listed in table 11, 12 and 13. The presence of Acetowhite areas (AWA) from which the Punch biopsy was taken on the cervix, is indicated in brackets after the histological lesion. This indicates the accuracy of the Acetowhite test to correctly identify pathology areas on the cervix in this series.

Table 11: CIN I.

<table>
<thead>
<tr>
<th>Pap smear</th>
<th>Repeat Pap smear</th>
<th>Colposcopy</th>
<th>Punch Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cin I = 3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 12 : CIN II.

<table>
<thead>
<tr>
<th>Pap smear</th>
<th>Repeat Pap smear</th>
<th>Colposcopy</th>
<th>Punch Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cin II = 24</td>
<td></td>
<td>19 AWA</td>
<td>Cin I = 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 No AWA</td>
<td>(1 AWA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 cervicitis</td>
<td></td>
</tr>
<tr>
<td>Cin II = 4</td>
<td></td>
<td></td>
<td>Cin II = 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3 AWA)</td>
<td>(7 AWA)</td>
</tr>
<tr>
<td>Cin III = 9</td>
<td></td>
<td></td>
<td>Invasive Ca :</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WDSCCa = 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1 AWA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MDSCCa = 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1 AWA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chronic cervicitis = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2 AWA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Invasive Ca :</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N D = 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4 AWA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Condylomata acuminata = 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1 AWA)</td>
</tr>
</tbody>
</table>

AWA = Acetowhite areas WDSCCa = Well differentiated squamous cell carcinoma.
PDSCCA = Poorly differentiated squamous cell ca.
MDSCCa = Moderately squamous cell carcinoma. N D = Non Diagnostic
As can be seen from the above table, there is poor correlation between Pap smear results and histology in the CIN II group.

**Table 13: CIN III**

<table>
<thead>
<tr>
<th>Pap smear</th>
<th>Repeat Pap smear</th>
<th>Colposcopy</th>
<th>Punch Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cin III = 24</td>
<td></td>
<td>20 AWA</td>
<td>Cin II = 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 No AWA</td>
<td>( 1 AWA )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Unsatisfactory Colposcopy</td>
<td></td>
</tr>
<tr>
<td>Cin III = 15</td>
<td></td>
<td></td>
<td>( 14 AWA )</td>
</tr>
<tr>
<td>Invvasive Ca: PDSCCa = 1</td>
<td></td>
<td></td>
<td>( 1 AWA )</td>
</tr>
<tr>
<td>MDSCCa = 3</td>
<td></td>
<td></td>
<td>( 2 AWA )</td>
</tr>
<tr>
<td>Chronic Cervicitis = 2</td>
<td></td>
<td></td>
<td>( 1 AWA )</td>
</tr>
<tr>
<td>Koilocytosis = 1</td>
<td></td>
<td></td>
<td>( 1 AWA )</td>
</tr>
<tr>
<td>N D = 1</td>
<td></td>
<td></td>
<td>( no AWA )</td>
</tr>
</tbody>
</table>

AWA = Acetowhite areas WDSCCa = Well differentiated squamous cell carcinoma. PDSCCa = Poorly differentiated squamous cell ca. MDSCCa = Moderately squamous cell carcinoma. N D = Non Diagnostic.

**Similiar to the CIN II group, there is poor correlation between Pap smear results and histology.**

**The relationship between Histology on Punch Biopsy and HPV genotypes.**
The results are listed in table 14.

**Table 14: Histology and HPV Genotypes.**

<table>
<thead>
<tr>
<th>Histology of Punch biopsy</th>
<th>No of patients</th>
<th>High risk HPV Genotypes in order of prevalence.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WDSCCa</td>
<td>1</td>
<td>33, 52</td>
</tr>
<tr>
<td>MDSCCa</td>
<td>4</td>
<td>16 (3pt); 6, 52, 58, 62, 67, 70, 81, 82 (once)</td>
</tr>
<tr>
<td>PDSCCa</td>
<td>1</td>
<td>33, 52</td>
</tr>
<tr>
<td>CIN III</td>
<td>24</td>
<td>16, 52(8pt); 62, 33, (5pt); 18, 53,(4pt); 58(3pt); 35, 39, 51, 61, 68, 69, CP 6108, 31, 45, 59, 67, 70, 66, 73, 82(2pt); 33, 54, 55, 56, 58, 71, 72, 81, 82, 83, 84, IS 39(once)</td>
</tr>
<tr>
<td>CIN II</td>
<td>5</td>
<td>58(3pt); 16(2pt), 52, 62, 66, 69, 84(2pt); 26,33,42,45,53,55,56,61,71, CP 6108 (once)</td>
</tr>
<tr>
<td>CIN I</td>
<td>4</td>
<td>52(4pt); 58, 62 (3pt); 18, 56, (2pt); 11, 26, 31, 33, 35, 42, 53, 59, 68, 72, 81, 83, 84 (once)</td>
</tr>
<tr>
<td>Condylomata Accuminata / Koilocytosis</td>
<td>2</td>
<td>53(2pt); 16, 18, 33, 40, 52, 56, 61, 62, 66, 68, 69, CP 6108 (once)</td>
</tr>
<tr>
<td>Chronic Cervicitis.</td>
<td>5</td>
<td>16(3pt); 39, 53(2 pt); 18, 26, 33, 35, 42, 51, 52, 62, 66, 71, 73 (once)</td>
</tr>
<tr>
<td>N D</td>
<td>5</td>
<td>16, 62(3pt); 52, CP 6108(2pt); 33, 53, 54, 61, 67, 72, IS 39(once)</td>
</tr>
</tbody>
</table>

WDSCCa = Well differentiated squamous cell carcinoma. PDSCCa = Poorly differentiated squamous cell ca. MDSCCa = Moderately squamous cell carcinoma. N D = Non Diagnostic.
**Discussion:**

High risk HPV genotypes and HIV co-infection.

In this study, patients with HIV co-infection had a greater number of high risk HPV genotypes present (OR 3.2; 95% CI = 1.6-4.8) compared with patients who were HIV negative. 86.2% of the 29 HIV positive patients had multiple HPV genotypes.

The **HIV positive patients** had, in order of prevalence: HPV 52, 62, 16, 58, 53, 18 and 33. This is different to the results of the Zimbabwean study, where HPV 11, 39, 43, 51 and 59 were more prevalent.\(^{55}\)

The **HIV negative** patients had, in order of prevalence: HPV 16, 33, 52, 62 and 53. HPV 18 and 58 were not present in any of the HIV negative patients.

Of the patients whose **HIV status was unknown**, the most prevalent HPV genotypes were: HPV 16, CP 6108, 39, 52, 54, 62, 69, 70, 33, 53 and 58.
Table 15: Most Common HR HPV Genotypes.

The Most common HPV Genotypes in this study:

The most common HPV genotypes in all the patients in the study, including HIV positive, HIV negative and patients with an unknown HIV status were, in order of prevalence:

HPV 16 (in 41% of patients), 52 (in 39% of patients), 62 (in 31% of the patients), 33 (in 24% of patients), 53 (in 20% of patients), 58 (in 20% of patients) and 18 (16% of patients).

Clifford et al found that in patients in Sub-Saharan Africa:

8% had HPV 16 and 35, 7% had HPV 31 and 33 and 4% had HPV 18.9.

The study used for the pooled analysis, was done in Nigeria.
In this study, HPV 52, 62, 53 and 58 had a high prevalence amongst the patients, in contrast with the Nigerian study, where this genotypes were not found to be prevalent.

**Comparison of HPV Genotypes found in Low grade SIL lesions:**

Amongst the 16 patients in this study with CIN I (LGSIL), the HPV genotypes in order of prevalence were:

HPV 52 and 62 (in 50% of patients with LGSIL), 16 (in 44% of patients), 53 (in 38% of patients), 18 and 33 (in 25% of patients) and 58 (in 19% of patients).

The ALTS study was a multicentre randomized controlled trial done in 4 centres in the USA. Patients with ASCUS, or a Low grade SIL lesion on cytology, were enrolled in the study. Wheeler et al found that in women who participated in the ALTS study, the most common HPV genotypes were, in order of prevalence:

HPV 16 (in 16.8% of patients), 52 (in 9.4% of patients), 51 (in 8.1% of patients), 31 (in 7.1% of patients) and 18 (in 6.6% of patients) 53 (in 6.1% of patients), 39 (in 5.9% of patients),56 (in 5.9% of patients), 62 (in 5.7%
of patients), 59 (in 5.6% of patients) and 58 (in 5.5% of patients)\textsuperscript{24}.

HPV 52 was the HPV genotypes most prevalent in both studies. HPV 62, 16, 18, 53 and 58 also was amongst the more prevalent HPV genotypes, but not in the same order of prevalence. HPV 33 was not prevalent in the patients of the ALTS study, but was prevalent in the patients of this study.

Table 16: Prevalence of HPV Genotypes in this study.
Table 17: HPV type under and over 30 years of age.

**Histology and multiple HPV genotypes:**

In this study, patients with multiple HPV genotypes were more likely to have High grade SIL (CIN II and CIN III) lesions. 45% of the patients with CIN II and CIN III on histology, had ≥ 2 HPV genotypes and 23.5% of the patients had ≥ 5 HPV genotypes.

Trottier found that HSIL risk increased with the number of types (OR 41.5; 95% CI=5.3-323.2), for 2 to 3 types (OR 91.7; 95% CI=11.6-728.1) and for 4-6 types (OR 424; 95% CI = 31.8 – 5651.8) relative to women who were HPV negative.23
HPV as a screening tool:

In this study, in the age group < 20 years, 1 HIV positive patient had HPV 18 and 62. She had CIN II on Pap smear.

In the age group 20 – 29 years, 2 HIV positive patients had CIN I lesions on Pap smear. They both had HPV 18, 52, 56, 58, 59, 68 and 83. Neither had HPV 16.

Eight HIV positive patients had CIN II on Pap smear. 50 % of the patients had HPV 33, 52, 53. Thirty-eight percent of the patients had HPV 16, 18 and 62. They also had HPV 40, 51, 56, 58, 61, 66, 68, 71,73 and CP 6108.

One HIV positive patient had CIN III on Pap smear. She had HPV 16, 53 and 69.

Seventy-five percent of the HIV positive patients < 30 years had either HPV 16 or 18 and 83% of them had CIN II or CIN III on Pap smear.

Viscindi et al \(^6^2\) found that HPV 16 is significantly more prevalent in HIV positive than HIV negative women. However, only 5% of the HIV positive women had HPV 16 DNA in the cervicovaginal cells, which indicate active infection.
The HIV positive patients in the age group < 30 years in this study would have been missed according to the HPV testing protocol as suggested by the Women’s Health Advisory Board and the South African National Screening Policy.

At the moment, the Women’s Health Advisory Board and ACOG have suggested that women < 30 years of age should not have a HPV test ab initio. According to the protocol, only if the Pap smear result is abnormal, a HPV test should be done. According to the South African National Screening Policy, the recommended first screening is at age 30.

In the UK the recommended first screening is at age 20. The American Cancer Society recommends first cytology at age 18 or when first sexually active.

HPV persistence in HIV positive patients has been linked to a reduction in HLA class II molecules and a greater number of immature Langerhans cells within the cervix. Data suggest that in adults, HPV infections and squamous intraepithelial lesions occur more commonly among HIV positive women, because of the HIV-associated CD4 T-cell immunosuppression.
Moscicki et al found that 77.4% of HIV positive adolescents in their study were positive for HPV, with a risk for HR HPV types (RR 1.8; 95% CI 1.2-2.7). 29.9% of the HIV positive girls had normal cytology compared to 70% of the HIV negative girls (P< 0.001). HIV positive status was a significant risk for SIL (OR 4.7; 95% CI 1.8-14.8) 64.

With the reality of a large percentage of patients getting infected with HIV as teenagers, the onset of HPV Screening at age 30 in South Africa, may be too late 1. Lomalisa et al found that HIV positive patients presented with invasive cervical carcinoma almost 10 years earlier than HIV negative patients 57.

**HPV Vaccine.**

In this study, the most prevalent HPV genotypes were HPV 16, 52, 62, 33, 53, 58 and 18. The patients with invasive squamous cell carcinoma, all had either HPV 16 or 52.

The quadrivalent vaccine covers HPV 16, 18 and to some extent 52. The bivalent vaccine also covers HPV 16, 18 and to some extent 52, at a fraction of the cost of the quadrivalent vaccine.

Unfortunately HPV 33, 53, 58 and 62 would not be covered by either
vaccine.

Demographics:

Fifty-one patients, aged between 18 and 67 years, with a mean age of 38.4 years, who had abnormal Pap smears, participated in the study.

Table 18: Age Profiles.

<table>
<thead>
<tr>
<th>Age cohorts</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19</td>
<td>7</td>
</tr>
<tr>
<td>20-24</td>
<td>2</td>
</tr>
<tr>
<td>25-29</td>
<td>4</td>
</tr>
<tr>
<td>30-34</td>
<td>8</td>
</tr>
<tr>
<td>35-39</td>
<td>9</td>
</tr>
<tr>
<td>40-44</td>
<td>6</td>
</tr>
<tr>
<td>45-49</td>
<td>7</td>
</tr>
<tr>
<td>50+</td>
<td>8</td>
</tr>
</tbody>
</table>

The majority of the patients were Parida 1-4, with a mean Parity of 2.29. 66.5% of the patients were unmarried, with 80.2% having a family support structure. There is a significant correlation between being HIV positive and being unmarried. (p = 0.002)
17.7% of patients is employed, with 3.9% having no social support structure. These last patients were dependant on State grants.

The mean age of the interval between menarche and coitarche was 1.98 years.

**Table 19: Interval between Menarche and Coitarche.**

![Bar chart showing intervals between menarche and coitarche](chart.png)

All the women in the study have had more than one sexual partner in their lifetimes. This puts them in a higher risk category for all Sexual transmitted diseases, including HPV and HIV.
Twenty-five (49%) potentially fertile patients, were using no contraception at all. Only 8 Patients (14%), all of them HIV positive, were using condoms. Only 1 of the HIV positive patients uses condoms plus another form of contraception (Injectable contraception). Of the 29 HIV positive patients in the study, only 4 is sexually inactive. The campaign to promote condom use, is obviously failing amongst the patients in this study and there is a need for better education on contraception.

51% of the patients in the study, had co-morbidities. 19.6% of the patients had chronic hypertension, 7.8% had chronic hypertension and NIDDM and 5.9% had pulmonary TB.

**Conclusion:**

The study’s limitation is that it is very small and underpowered to prove the hypothesis. There was a trend toward different HR HPV types in HIV negative and positive patients.

The most prevalent HPV genotypes in this study were HPV 16, 52, 62, 33, 53, 58 and 18. The HIV positive patients had, in order of prevalence: HPV 52, 62, 16, 58, 53, 18 and 33.
This may be an indication that the quadrivalent vaccine which covers only HPV 16, 18 and to some extent 52, may not be cost-effective to prevent cervical neoplasia in South African patients. The other prevalent HR HPV types in this study, such as HPV 33, 53, 58 and 62 are not covered by either the quadrivalent or the bivalent vaccines.

HPV 16 and 18 together cause around 70% of all cervical cancer.\(^6,7\). The cheaper bivalent vaccine that also covers HPV 16, 18 and to some extent 52 may be more cost-effective in South Africa to prevent cervical neoplasia.

Seventy-five % of the HIV positive patients < 30 years had either HPV 16 or 18 and 83% of them had CIN II or CIN III on Pap smear.

Thirty-eight % of the HIV positive patients were in the age group 20-29 years. This raises the question whether primary cytology screening in HIV positive patients in South Africa shouldn’t begin at age 20.

A much bigger, multi-centre study under the directorship of Professor Lynn Denny of UCT is currently underway. This study will be powered to make recommendations to change the protocol of primary screening for cervical cancer in South Africa and to make recommendations about
the National initiation of HPV vaccination in South Africa.

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# DR GEORGE MUKHARI HOSPITAL – DATA SHEET

## GENERAL CONDITION

### ABDOMEN

### MEDICAL HISTORY:

### SURGICAL HISTORY:

### SOCIAL HISTORY:

## CONTRACEPTION:

### ADENOPATHY

### VAGINAL EXAMINATION:
- VULVA
- VAGINA
- CERVIX
- UTERUS
- ADNEXAE

## NAME

## AGE

## PARITY:

## FIRST DELIVERY

## LAST DELIVERY

## EXAMINED BY

## HOSPITAL NO.

## MENARCHE:

## COITARCHE:

## LNMP:

## MENOPAUSE:

## SEXUAL ACTIVITY:

## NO. OF PARTNERS:

## MARITAL STATUS:

## HIV STATUS:

## CD4 COUNT:
Statement concerning participation in a Clinical Trial

Name of Clinical Trail:

SCREENING AND TYPING OF HUMAN PAPILLOMA VIRUS IN PATIENTS WITH ATYPICAL PAP SMEARS WHO ARE HIV POSITIVE AND HIV NEGATIVE.

I have read the information on *heard the aims and objectives of* the proposed Clinical Trail and was provided the opportunity to ask questions and given adequate time to rethink the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way.

I understand that participation in this Clinical trail is completely voluntary and that I may withdraw from it at any time and without supplying reasons. This will have no influence on the regular treatment that holds for my condition neither will it influence the care that I receive from my regular doctor.

I know that this Trial has been approved by the Research, Ethics and Publications Committee of University of Limpopo-Medunsa Branch / Dr George Mukhari Hospital.

I am fully aware that the results of this Trial will be used for scientific purposes and may be published.

I agree to this, provided my privacy is guaranteed.

I hereby give consent to participate in this Trial

…………………………………………………………………

Name of patient/volunteer Signature of patient or guardian.

…………………………………………………………………

Place. Date. Witness

Statement by the Researcher

I provided verbal and/or written* information regarding this Trial. I agree to answer any future questions concerning the Trial as best as I am able. I will adhere to the approved protocol.

DR E. FREISLICH [Dept. Obstetrics & Gynaecology] Signature Date Place

Delete whatever is not applicable.