THE PREVALENCE OF CERVICO-VAGINAL HUMAN PAPILLOMA VIRUS
IN WOMEN VISITING THE GYNAECOLOGICAL DEPARTMENT OF THE
KORLE-BU TEACHING HOSPITAL.

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
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JUNE, 2003
DEDICATION

I DEDICATE THIS WORK TO THE ALMIGHTY GOD
FOR MAKING THIS ALL POSSIBLE.

I ALSO DEDICATE IT TO THE THREE LOVES OF MY
LIFE: AGBEKO, SENA AND SEDINA.
DECLARATION

The author carried out the work in this thesis alone unless otherwise indicated. Whenever the work of others is included, references are made to the source of information. This thesis has not in its present form or otherwise been submitted to this University for a degree, diploma or other qualification.

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Finally to all the patients who consented to take part in the project, I render my heartfelt thanks.
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<th>Description</th>
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<tr>
<td>CIN</td>
<td>Cervical Intraepithelial Neoplasia</td>
</tr>
<tr>
<td>CVLPs</td>
<td>Chimeric virus-like particles</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal Growth Factor</td>
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<tr>
<td>HSV-2</td>
<td>Herpes Simplex Virus Type-2</td>
</tr>
<tr>
<td>HSIL</td>
<td>High Grade Squamous Intraepithelial Lesion</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HPV</td>
<td>Human Papillomavirus</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>JHPIEGO</td>
<td>John Hopkins Programme for International Education on Gynaecology and Obstetrics</td>
</tr>
<tr>
<td>ORF</td>
<td>Open Reading Frame</td>
</tr>
<tr>
<td>PAP smear</td>
<td>Papanicolaou Smear</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet Derived Growth Factor</td>
</tr>
<tr>
<td>STD</td>
<td>Sexually Transmitted Disease</td>
</tr>
<tr>
<td>TBE</td>
<td>Trisborate- EDTA</td>
</tr>
<tr>
<td>URR</td>
<td>Upstream regulatory Region</td>
</tr>
<tr>
<td>VIA</td>
<td>Visual Inspection with Acetic Acid</td>
</tr>
<tr>
<td>VIAM</td>
<td>Visual Inspection with Magnification</td>
</tr>
<tr>
<td>VLPs</td>
<td>Virus Like Particles</td>
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ABSTRACT

Human Papilloma virus (HPV) is the primary cause of cervical cancer and it has been implicated in the pathogenesis of various tumours and clinical entities. HPV infections are acquired through skin abrasions, sexual intercourse and during passage through an infected birth canal. It is clearly known that cervical HPV infection is sexually transmitted. HPV prevalence is high in young, sexually active women with a peak incidence in the age group 15-25 years. In elderly women, HPV prevalence shows a decline, and this is probably due to fewer sexual partners as well as immunity acquired as a result of previous infections. The prevalence is also directly related to the number of sexual partners, and any recent change in sexual partners. Cervical cancer is the second most common cancer worldwide and an estimated 371,000 cases are diagnosed annually worldwide. It has an estimated mortality of 231,000 cases annually with more than 80% occurring in developing countries particularly tropical Africa. To our knowledge, in Ghana, there is no current available data on the prevalence of HPV infection. The current research into vaccine development gives an urgent need to determine the prevalence of HPV infections and the types that infect Ghanaian women. The aim of this study was to determine the prevalence of HPV infection in Ghanaian women visiting the Gynaecological department of the Korle-Bu teaching hospital. The study was conducted at the Departments of Pathology, Obstetrics and Gynaecology and Microbiology of the Korle-Bu Teaching Hospital, Accra, Ghana between April and December 2002. The first 75 patients who visited the clinic and met the inclusion criteria of the study were recruited into the study. Following informed consent, a structured questionnaire on personal information, sexual behavior, lifestyle and knowledge about cervical cancer
were completed by the patients. On completion of the questionnaire, a gynaecological examination was performed by a consultant gynaecologist and cervical scrapes obtained from the patients studied. These samples were analysed in the Microbiology Department by PCR using the GP5+/GP6+ consensus primers for HPV DNA. Eight (10.7%) of patients studied were HPV positive. Women between the ages of 20-39 years who belong to the most sexually active age groups were found to have an HPV prevalence rate of 75%. Fifty (66.7%) of all patients studied had had their first sexual intercourse by the age 19 years and 5 of the HPV positive patients had their sexual debut by this age. Twenty-eight (37.3%) had had 2 sexual partners in their lifetime and 5 of them were HPV positive. Contraceptive use was low in this study. Fifty-six (74.7%) used no form of contraception and 6 of them were HPV positive. Forty-two (56%) had heard of cervical cancer whilst 33 (44%) had never heard about it. Sixty-two (82.7%) had never heard of a cervical smear and only 6 (8%) had had one done before. Even though majority of study patients had heard of cervical cancer, knowledge on its’ causes, prevention and the Pap smear test were poor. Education on the risks of early sexual intercourse and multiple sexual partners will go a long way to reduce risks in acquiring HPV infection. Also, the knowledge of cervical cancer causes and prevention leaves much to be desired, and as such there is a need for well-organized cervical screening programmes.
CHAPTER ONE
INTRODUCTION

1.1 GENERAL INTRODUCTION

Human Papilloma viruses (HPV) are small non-enveloped DNA viruses that belong to the Papovaviridae group of viruses. They are known to infect squamous epithelium causing hyperproliferation especially in the skin. HPV is the primary underlying cause of cervical cancer. They have also been implicated in the pathogenesis of various tumors and clinical entities. These include plantar warts, anogenital warts, laryngeal papillomas, Bowenoid papulosis of the penis, vulvar cancer, penile cancer, conjunctival and nasal papillomas.

HPV infections are acquired through abrasions of the skin (skin warts), by sexual intercourse (genital warts) and during passage through an infected birth canal (juvenile-onset laryngeal papilloma). It is clearly known that cervical HPV infection is usually sexually transmitted. About 90 HPV types have been identified by molecular cloning and polymerase chain reactions and up to 150 types are assumed to exist making this an extremely common and diverse viral infection. Based on current data, 25 HPV types have been found to infect cervical epithelia. Of these, type 16 accounts for about half the cases in the United States and Europe. In Latin America however, types 39 and 59 are the most prevalent, while in West Africa, type 45 is the most common. HPV types 16, 18, 45 and 31 have been detected frequently in cervical cancers.

HPV prevalence is high in young sexually active women with a peak incidence in the age group 15 to 25 years. In elderly women, HPV prevalence shows a decline and
this is probably due to fewer recent sexual partners as well as some immunity acquired as a result of previous infections (7). The prevalence is also directly related to the number of lifetime sexual partners, and to any recent change in sexual partners (8). Oral contraceptive use has also been associated with the increase in the prevalence of HPV infections (8). Also implicated is the effect of smoking on HPV infections, which shows smoking to increase HPV associated lesions (9). Immunosuppression for example, organ transplantation and Human Immunodeficiency Virus (HIV) infection is associated with increased prevalence of HPV infections and HPV associated abnormalities (10-11). Increased prevalence of HPV during pregnancy and decreased prevalence postpartum have been reported in some studies (12-13).

Early detection of pre-neoplastic and neoplastic lesions of the cervix through the use of Pap smears has led to a reduction in the number of deaths due to cervical cancer in countries where women have good access to screening. With the knowledge of the role of HPV in cervical cancer, there is a growing appreciation of the potential value of routine screening of female patients not only for cytological abnormalities in Pap smears but also for the presence and type of HPV.

This can be done by the use of polymerase chain reaction (PCR) with consensus primers on DNA extracted from cervical scrapes followed by typing using specific HPV probes on the PCR products. An estimated 371,000 new cases of invasive cervical cancer are diagnosed worldwide each year, representing nearly 10% of all cancers in women. In frequency, it is the seventh cancer site overall and the second among women, after breast and colorectal cancer (14). Eighty percent of newly diagnosed cervical cancer cases are seen in developing countries. Worldwide, cervical cancer takes the lives of 231,000
women annually with over 80% of these deaths occurring in developing countries \(^{(14)}\).

Studies in Ivory Coast and Gambia have revealed cervical cancer incidences of 24% and 34% respectively \(^{(15-16)}\).

The recognition that HPV infections cause cervical cancer has led to efforts to devise a strategy to prevent cervical cancer by immunization against HPV. Currently work on “prophylactic” (to prevent infection) and “therapeutic” (to destroy HPV-infected neoplastic cells) vaccines are being carried out. \(^{(17-18)}\).

In Ghana the prevalence of HPV infections has not been studied partly because of financial constraints and lack of interest by health authorities. However, the causal relationship of cervical cancer to the screenable HPV infection as well as current research into the development of vaccines gives an urgent need to determine the prevalence of HPV infections and the types that infect Ghanaian women.
1.2 **OVERALL OBJECTIVE OF THE STUDY**

The overall objective of the study is to determine the prevalence of cervico-vaginal HPV in Ghanaian women.

1.3 **SPECIFIC OBJECTIVES**

1.3.1 To analyze cervical scrapes for HPV DNA using the polymerase chain reaction.

1.3.2 To determine possible association between risk factors on the prevalence of HPV infection by questionnaire.

1.4 **JUSTIFICATION OF THE STUDY**

This study will constitute the much-needed data on the prevalence of cervico-vaginal HPV infections in Ghanaian women. This knowledge as well as studies into the types that infect Ghanaian women will help when HPV vaccines become available.
CHAPTER TWO
LITERATURE REVIEW

2.1 HISTORICAL REVIEW – HUMAN PAPILLOMA VIRUS

The first papilloma virus was described in 1933 when Richard Shope recognized the cottontail rabbit papilloma virus as the aetiology agent responsible for cutaneous papillomatosis in the cottontail rabbit \(^{(19)}\). However, it was not until the late 1970s when the first papillomavirus genome was successfully cloned in bacteria, that investigators used reagents that were sufficiently standardized to begin a detailed analysis of the molecular biology of this group of viruses.

2.2 VIRION STRUCTURE OF HUMAN PAPILLOMA VIRUS

The HPVs belong to the papova virus family. They are small, non-enveloped, double stranded DNA viruses with a circular genome of eight kilobases. The HPVs measure 52 to 55 nm in diameter and have an icosahedral nucleocapsid composed of 72 capsomers \(^{(20)}\). The capsid consists of two structural proteins; a major and a minor protein. The major capsid protein (L1) has a molecular weight of approximately 55 KD and represents 80% of the total viral protein whereas the minor protein (L2) has a molecular size of approximately 70 KD. Both of these proteins are virally encoded \(^{(21)}\).
2.3 GENOMIC STRUCTURE OF HUMAN PAPILLOMAVIRUS

The genome is made up of three major regions, two protein-encoding regions, (the early and late gene regions) and the non-coding upstream regulatory region (URR)(22).

The early gene region has six translational open reading frames (ORF) designated E1, E2, E4, E5, E6 and E7. The late gene region has two open reading frames designated L1 and L2. The open reading frames are DNA segments that are transcriptional units and are capable of encoding for protein (22). E1 and E2 ORFs are critical for viral replication. E1 gene is involved in the initiation of DNA replication while E2 gene stimulates the ability of E1 to initiate DNA replication (23).

The E4 gene is involved in the destabilization of the cytokeratin network causing the characteristic perinuclear halos also known as koilocytosis observed histologically and cytologically in HPV infected cells (24). HPV E5 gene has been shown in some studies to increase the proliferative capacity of human keratinocytes as well as stimulate cellular DNA synthesis in these cells through the interaction with cell membrane receptors such as epidermal growth factor (EGF) and platelet derived growth Factor (PDGF) (25).

E6 and E7 genes are potent oncogenes involved in cellular transformation. HPV E6 protein is made up of 150 amino acids and it inactivates the p53 gene by inducing its’ rapid degradation through the ubiquitin-dependent proteolytic system. p53 gene normally promotes growth arrest and cell death by apoptosis in response to genotoxic insults. This allows transformed cells to proliferate in the absence of p53 gene (26).

HPV E7 is a small nuclear protein of about 100 amino acids. It binds to the hypophosphorylated form of the retinoblastoma gene (Rb) thereby causing its’
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HPV E7 is a small nuclear protein of about 100 amino acids. It binds to the hypophosphorylated form of the retinoblastoma gene (Rb) thereby causing its'
inactivation. The hypophosphorylated Rb gene is involved in the inhibition of cell cycle progression. Thus by HPV E7 forming a complex with it, transformed cells are able to progress through the cell cycle. The viral genomic DNA is surrounded by a protein coat known as the viral capsid that consists of the late ORF regions L1 and L2. The L1 and L2 ORFs together encode for the viral capsid proteins. They both are approximately 500 amino acids in size each and are involved in major humoral and cell-mediated responses to viral infection.

The URR is an approximately 400 base-pair DNA segment adjacent to the origin of viral replication. The URR does not encode for proteins, but, instead, contains a complex array of overlapping binding sites for many different transcriptional repressors and transcriptional activators. These include activator protein 1 (AP1), keratinocytic-specific transcription factor 1 (KRF1), and nuclear factor (NF-1/CTF) as well as virally derived transcriptional factors encoded by the early region. The URR regulates transcription from the early and late regions and controls the production of viral proteins and infectious particles.

2.4 REPLICATION OF HUMAN PAPILLOMA VIRUS

HPV replication occurs in the cells of the lower portion of the epidermis including the basal cells. The viral genome replicates on the average, once per cell cycle during the S-phase in synchrony with the host cell chromosome. This type of DNA replication ensures a persistent and latent infection in the stem cells of the epidermis. Genome and pregenome replication of HPV occurs in the cell nucleus. In order to reproduce, the infecting virion enters the cell and traverses the cytoplasm towards the nucleus. Using the
cell’s own nuclear import machinery, the viral genome then enters the nucleus through the nuclear pore complex.

Targeting of the virion or viral genome to the multiplication site is therefore an essential process in productive viral infection as well as latent infection and transformation \(^{(30)}\). In benign cervical precursor lesions, viral DNA is maintained in a free, extrachromosomal, circular form termed an “episome” \(^{(22)}\). However, in many cervical cancers as well as in cell lines derived from cervical cancers and human keratinocytes transformed \textit{in vitro} with HPV, the HPV DNA is integrated in the host genome \(^{(31-32)}\). Integration of HPV DNA occurs early in cancer development and forms an important event in the malignant transformation of cervical cancer \(^{(33)}\).

A key protein within the papilloma virus life cycle is known as the E2 protein and is responsible for regulating viral transcription from all viral promoters as well as replication of the papillomavirus genome in tandem with the E1 protein \(^{(34)}\). However, integration of the viral genome into the human chromosome in the cells usually disrupts or deletes the E1 and E2 ORF, which results in loss of expression of these genes, but this causes high levels of E6 and E7 to be maintained \(^{(32),34-35}\).

It is widely known that E6 and E7 oncoproteins, by binding to the p53 and retinoblastoma tumor suppressor proteins cause cellular proliferation to go uninhibited \(^{(27)}\). By binding of HPV E6 to p53 cellular growth is altered through stimulation of cellular p53 by the ubiquitin-dependent proteolytic pathway \(^{(26)}\). A cellular protein referred to as “E6-AP” (E6-associated protein) mediates the ubiquitination and degradation of p53 \(^{(36)}\). Therefore the cellular levels of p53 are low in cells expressing HPV 16 or 18 E6 \(^{(37)}\). The reduced p53 levels detected in cells containing high oncogenic-
risk HPV types leads to unregulated cell cycle progression and allow the accumulation of genetic mutations (37).

In normal cells not infected with high oncogenic-risk HPV, the hypophosphorylated forms of retinoblastoma protein, p107 and p130 form complexes with transcription factors of the E2F family (38). Binding of E2F transcription factors with Rb and its related proteins is regulated through the action of cyclin-dependent kinases at specific stages of the cell cycle (39-40). E2F forms complexes with these proteins in the G0 and G1 phases of the cell cycle. These complexes negatively regulate cell growth by repressing transcription of E2F-dependent genes. The HPV E7 alters this precisely regulated cellular growth control mechanism by binding to retinoblastoma and its related proteins and dissociating the E2F-Rb complex (41). This causes the release of transcriptionally active E2F, which can stimulate the transcription of the E2F-dependent genes necessary for DNA replication.

2.5 MODE OF TRANSMISSION AND PATHOGENESIS OF HPV

Human papillomavirus infections are usually transmitted by person to person contact. It is clear that cervical HPV is usually sexually transmitted (3) (42-43). HPV infection of the cervix is rare among virgins (44). Although sexual transmission is the most important route, fomite transmission of HPV to the cervix appears theoretically possible based on findings of HPV DNA on underclothes and gynaecological equipment (45).

HPV has a strict tropism for epithelial cells. The target tissue for all HPVs is stratified squamous epithelia (both keratinized and non-keratinized). The life cycle is tied tightly to the state of differentiation of the epithelial cell. They infect the cells of the basal
epithelium. Infection occurs as a result of exposure of the basal cells to infectious viral particles after minor trauma to the epithelium for example, during sexual intercourse or after minor skin abrasions (29). The virus stimulates proliferation of basal epithelium and as epithelial cells are pushed to the surface through continued proliferation of deeper cell layers, HPV virions are assembled and exit the apical cell layer of the epithelium. Viral DNA replication in papillomas, as well as some transcription and translation takes place in the stratum spinosum (46).

Late gene expression for example, capsid proteins and viral assembly takes place in the most superficial layers, the stratum granulosum and corneum (46). The epithelial cells infected with HPV often demonstrate koilocytosis, in which affected cells display a large perinuclear cavitation with irregular edges, and a dense cytoplasm in the area surrounding the cavity. The process of infection and cellular proliferation in cutaneous and mucosal infection produce lesions with exophytic appearance being the commonest (29).

Oncogenic HPV types can integrate their genomes into chromosomal DNA (31-33). These lesions, when they progress to more serious disease show increasing degrees of cellular dysplasia with the basal layer being affected first. Evidence from HIV and renal transplant patients’ shows that HPV tends to be more aggressive and progresses more rapidly to cancer in immunosuppressed patients (47-49). The type of virus, immune competency of the host as well as a host of factors termed “risk factors” influence the pathogenesis of HPV infection.
2.6 **RISK FACTORS ASSOCIATED WITH ACQUIRING HPV INFECTION**

2.6.1 **SOCIO-ECONOMIC FACTORS**

Descriptive and analytical studies have demonstrated that HPV and cervical cancer predominantly affect women in lower socio-economic classes \(^{(50-51)}\) and of lower education and income levels \(^{(43)}\). In a study done in Rabat, Morocco, one of the major factors found among women with HPV infection was a high incidence of low socio-economic status \(^{(52)}\). A similar study done in Kenya also found low educational attainment as one of the main predisposing factors associated with HPV infection and cervical cancer \(^{(53)}\).

2.6.2 **MARITAL AND SEXUAL FACTORS**

Early epidemiological studies have shown that the risk of HPV infection and cervical cancer is particularly high among women having sexual intercourse at young ages \(^{(54)}\). Early age of first sexual intercourse has been consistently been found to increase the risk of HPV infection. In a study done in Kenya, sexual intercourse before the age of 16 years was associated with an increased risk of HPV infection \(^{(53)}\).

Peak HPV prevalence is found in sexually active women 15 to 25 years old and thereafter HPV prevalence declines with age \(^{(7)}\). Burk *et al.* \(^{(55)}\) have also found in a study that older women had a lower risk of acquiring HPV infection than younger women and is perhaps due to acquired immunity to HPV from past exposure \(^{(55)}\). The risk of HPV infection is also strongly influenced by the lifetime number of sexual partners a woman has had \(^{(52)}\) \(^{(56)}\). This risk is higher for women with more than five sexual partners as
compared to those with one sexual partner. The role of the male partner in HPV infection as well as cervical cancer has been studied and husbands of patients with cervical cancer were found to report histories of various genital conditions including venereal warts, gonorrhea and herpes. It was also found that there was a low risk of cervical cancer among patients whose husbands frequently used condoms\(^{57}\).

Circumstantial evidence for sexual transmission of HPV infection shows that wives of patients with penile cancer are at increased risk of cervical cancer later in life\(^{58}\).

Such findings are further supported by results from correlation studies in which strong associations were found between cervical and penile cancer mortality and incidence\(^{59-60}\). In a study done in Mali, which has a polygamous society, HPV infection was high in women whose husbands had more than two wives\(^{61}\).

2.6.3 \textit{SMOKING}

Smoking is strongly associated with the risk of cervical HPV infection because of the correlation of smoking with sexual behavior\(^{62}\). A direct carcinogenic action of cigarette smoking on the cervix has been upheld on the grounds that nicotine metabolites can be found in the cervical mucus of women who smoke\(^{63}\). A study was done in which 82 women volunteers with minor grade cervical lesions on copolscopy attempted to give up smoking for 6 months. Of the 82 women, 17 stopped smoking for at least 6 months and 11 others reduced their cigarette consumption by 75%. Of these 28 women, 23 (82%) showed a reduction in lesion size of at least 20% or 4 mm\(^2\) compared with 13 (28%) of the 47 non-quitters.
The remaining seven women had unconfirmed smoking histories. There was a significant correlation between extent of smoking reduction and the change in lesion size. This showed that there is a link between smoking and cervical cancer and suggests that smoking cessation could have a beneficial effect on early cervical abnormalities \(^{64}\). Smoking has also been found to be associated with both squamous cell and adenocarcinoma of the cervix \(^{51}\).

2.6.4 **ORAL CONTRACEPTIVES**

In a recent large study by the WHO, a risk of 2.2 was associated with oral contraceptive use for more than 8 years \(^{65}\). Moreno et al. showed that long term use of oral contraceptives, 10 years or more could be a co-factor that increases risk of cervical carcinoma four-fold in women who are positive for cervical HPV DNA \(^{66}\).

2.6.5 **OTHER CONTRACEPTIVE METHODS**

In a number of studies, users of barrier methods of contraception (diaphragm and condoms) were found to have a low risk of cervical cancer. It has been suggested that part of the protection with diaphragm use may reflect concurrent use of spermicides, which have antiviral properties \(^8\). However it has been found that the most commonly used spermicide nonoxyl has no appreciable anti-HPV activity *in vitro* \(^{67}\).

2.6.6 **PARITY**

The number of live births is a consistent risk factor for HPV and cervical cancer. There is a linear trend in the association between parity and risk as seen in studies in
North, Central and South America \(^{(68)}\). High parity of greater than 10 children have been found to be associated with increased risk of HPV and cervical cancer in various studies\(^{(51-53)(61)}\).

2.6.7 **Dietary Factors**

The evidence on the effect of diet on risk of cervical cancer indicates that a high intake of certain foods containing beta-carotene and to a lesser extent, vitamin A may reduce the risk of cervical cancer \(^{(69-70)}\). The results from studies using diet recall methods have generally been corroborated by laboratory surveys assaying dietary constituents in plasma \(^{(71)}\). As with reproductive factors, it is likely that diet may influence between-country differences in cervical cancer incidence rates.

2.6.8 **Immunosuppression**

The increased diagnosis of HPV in HIV infected individuals is real and related to immunosuppression. \(^{(4)(28)(72-74)}\). HIV infection is associated with a high prevalence of HPV DNA detection especially in immunocompromised women with low CD4 counts \(^{(75)}\). The risk of cervical HPV infection increases with both increased HIV-1 and 2 related immunosuppression \(^{(76)}\).

2.6.9 **Other Infective Agents**

Multiple serological studies have observed higher prevalence of antibodies to Herpes simplex virus type-2 (HSV-2) among patients with cervical neoplasia than among control subjects \(^{(77)}\). Chlamydia cervicitis has been suspected to be a risk factor for
cervical cancer on the basis of case-control comparisons of serologic tests and Chlamydia associated changes seen on stored cervical smears. 

2.6.10 **POOR GENITAL HYGIENE**

A study done in Mali showed that poor genital hygiene increases the persistence of HPV infection especially in women who have not practiced vaginal douching before and those who re-use home-made feminine napkins.

2.7 **EPIDEMIOLOGY OF HPV INFECTION AND CERVICAL CANCER**

Human papilloma viruses are the causative agents of cervical cancer in women as well as some other genital, mucosal and skin lesions. Cervical HPV infection is acquired through sexual intercourse thus making it fall into the category of sexually transmitted diseases. Genital HPV has been found to be a common worldwide sexually transmitted infection, affecting women of all age groups. In the USA it has been found to affect 20-46% of sexually active young women. In Ibadan, a prevalence study in a Sexually Transmitted Disease (STD) clinic found 8.01% of attendees to have HPV infection. This study was done by examining the attendees for genital warts and taking a history on their sexual behaviour. HPV prevalence of 5% and 22% respectively have been found in studies in Bangkok and Sweden respectively. Peak HPV prevalence is found in sexually active women 15 to 25 years old and prevalence declines with increasing age. However lower HPV prevalence has been observed in a survey of women in a rural province of Costa Rica and a suburb of Amsterdam.
Recent molecular and epidemiological studies offer strong evidence that certain types of HPVs cause over 95% of cancers of the uterine cervix \(^{(85)}\). Over twenty of these "oncogenic" or cancer associated types of HPV have been identified; the most common are types 16 and 18 \(^{(2)5(86-89)}\). These viruses are able to integrate into the human genome, and by a variety of mechanisms including viral protein production and binding of human suppressor p53 and retinoblastoma proteins, lead to unregulated cell growth, accumulation of chromosomal mutations, and finally to malignant transformation \(^{(88)(90-91)}\).

There is consistent evidence of a causal association between certain types of HPV and cervical intraepithelial neoplasia, a precursor of cervical cancer \(^{(5)(92)}\). Oncogenic HPV sequences have been identified in the immediate cervical cancer precursors known as high-grade squamous intraepithelial lesions (HSIL), or cervical intraepithelial neoplasia (CIN) 2 and 3 \(^{(92-95)}\). The so-called low-grade cervical lesions, that are CIN 1 and flat condyloma (soft, wart-like growths), also carry HPV sequences. However, many of the types identified have been the "low risk" viruses, most commonly 6 and 11 \(^{(94)}\).

Most warts on the cervix are termed "flat" condylomata and are best visualized with a colposcope after the application of 3-5% acetic acid. Some low-grade cervical lesions may be caused by oncogenic HPV, and only these appear to have the potential of progressing through CIN 2 and 3 and to invasive cancer \(^{(96-100)}\).

The attributable fraction of cervical cancers due to HPV has been estimated at 82% in developed countries and 91% in developing countries \(^{(101)}\). Studies on the natural history of HPV infection have shown that the probability of young women acquiring it is high and that they usually remain positive for a period less than a year \(^{(102-103)}\). Several
studies have concluded that HPV is mainly a transient infection \(^{104-106}\). Infections with high-risk types of HPV and older age have been shown to be associated with persistence of HPV infection \(^{106}\).

In a study done by Ho et al., it was found that persistence of HPV infection greater than 6 months was associated with older age, infection with multiple types and an infection with a high-risk type at the previous visit \(^{107}\). Persistent infection may increase the risk for the development and persistence of cervical intraepithelial lesions \(^{107}\). The WHO International Agency for Research on Cancer (IARC) classified HPV infection as carcinogenic to humans (HPV types 16 and 18), probably carcinogenic (HPV types 31 and 33) and possibly carcinogenic (other HPV types except 6 and 11)\(^{108}\).

A study done on more than 1000 specimens from cervical cancer patients in 22 countries showed 93% of tumors had HPV DNA detected. HPV 16 was present in 50% of specimens and the second predominant type, HPV 18 was present in 14% of all specimens, whilst HPV 31 and 45 were found in 8% and 5% of tumors respectively. A clustering of HPV 45 was apparent in Western Africa while HPV 39 and 59 were almost entirely confined to Central and South America \(^2\).

An estimated 371,000 new cases of invasive cervical cancer are diagnosed worldwide each year, representing nearly 10% of all cancers in women. In frequency, it is the seventh cancer site overall and the third among women, after breast and colorectal cancer \(^{14}\). Eighty percent of newly diagnosed cervical cancer cases are seen in developing countries. Worldwide, cervical cancer takes the lives of 231,000 women annually with over 80% of these deaths occurring in developing countries \(^{14}\).
The highest age-standardized incidence rates of cervical cancer have been reported in Melanesia, Southern Africa, Central America, Eastern Africa, and South America. In all these regions rates were over 40 per 100,000 women. In Northern Africa cervical cancer has been found to be the second most common cancer among women (52). The incidence rate of cervical cancer was 54 per 100,000 in the cancer registry of Zimbabwe (1993-1995) whilst it was 46% in the cancer registry of Guinea (1992-1995) (109-110).

In Ghana, a study done on the pattern of gynaecological cancers in its main tertiary hospital Korle-Bu over a period of 52 months showed cervical cancer having a relative frequency of 58.3% (111). In Rwanda a population-based cancer registry from May 1991 and two months before the civil war broke out in April 1994, showed 12% of cancers to be due to cervical cancer (112). Studies in Ivory Coast and Gambia have revealed cervical cancer incidences of 24% and 34% respectively (15-16).

An important reason for the sharply higher incidence of cervical cancer in developing countries in comparison to developed countries is the lack of effective screening programmes aimed at detecting precancerous conditions and treating them before they progress to invasive cancer. A 1985 estimate indicated that only about five percent of women in developing countries had been screened for cervical neoplasia in the previous five years, compared with some 40 to 50 percent of women in developed countries (113).

It is clear that cervical cancer is taking the heaviest toll on women in developing countries. The age-standardized mortality rate from cervical cancer in these countries is 9.6 per 100,000 women, twice the rate in developed countries (114). Mortality rates
associated with cervical cancer are the most telling indicator on its impact on women, their families, and their communities. Thus there is the need for proper screening facilities as well as proper follow-up in cases of mild dysplasia. Naisell et al. (115) highlighted the importance of regular follow-up of women diagnosed with mild dysplasia, given the potential of progression in more than a sixth of cases (115).

2.8 CLINICAL MANIFESTATIONS

Sexually transmitted HPV are implicated in the pathogenesis of a number of anogenital diseases ranging from external genital warts to cancers of the uterine cervix and anus (2,86-87,90). Genital papilloma viruses have been linked, as well, to recurrent respiratory papillomatosis through vertical transmission during delivery (116). HPV is implicated in the pathogenesis of cancer of the uterine cervix, which is the second most common cause of cancer and cancer death in women worldwide, with 80% of cases occurring in the developing world (98)(117).

Invasive squamous cervical cancer is preceded by preneoplastic lesions that can be identified on Papanicolaou (Pap) smear and subsequently treated. The strategy of screening for these precursor lesions has been associated with a 90% reduction of cervical cancer incidence in some Western countries (98) Recent molecular and epidemiologic studies offer strong evidence that over 95% of cancers of the uterine cervix are caused by certain types of HPV (85). Over twenty of these "oncogenic" or cancer associated types of HPV have been identified; the most common are types 16 and 18 (2,5)(86-88).
These viruses are able to integrate into the human genome, and by a variety of mechanisms including viral protein production and binding of human suppressor p53 and retinoblastoma proteins, lead to unregulated cell growth, accumulation of chromosomal mutations, and finally to malignant transformation (88) (97). Oncogenic HPV sequences have been identified in the immediate cervical cancer precursors known as high-grade squamous intraepithelial lesions (HSIL), or cervical intraepithelial neoplasia (CIN) 2 and 3 (92-94).

The so-called low-grade cervical lesions, that is CIN 1 and flat condyloma (soft, wart-like growths), also carry HPV sequences, however, many of the types identified have been the "low risk" viruses, most commonly 6 and 11 (94). Most warts on the cervix are termed "flat" condylomata and are best visualized with a colposcope after the application of 3-5% acetic acid. Some low-grade cervical lesions may be caused by oncogenic HPV, and only these appear to have the potential of progressing through CIN 2 and 3 and to invasive cancer (96-99)(118).

Adenocarcinoma of the cervix, although less common than squamous cervical cancer, is also associated with oncogenic HPV, most notably type 18 (5). HPV-DNA has also been identified in other anogenital invasive cancers and their precursors; these include squamous cancers originating in the vulva, vagina, anus and penis (119-120).

External genital warts of the vulva, penis and anal canal are caused by infection with low risk HPV (96-100)(121-122). These are not malignant lesions; however, they constitute an important medical problem because they are common, may be difficult to treat, and tend to recur. The typical appearance of external genital warts (condyloma acuminatum) is of flesh-coloured gray hyperkeratotic, exophytic papules, either sessile or
attached to a broad peduncle. They may range from a few millimeters to several centimeters. In women external lesions are an important clue to the possibility of internal genital warts. Though most warts are painless, itching and burning may be experienced. Dyspareunia is sometimes seen in women with external or internal infection.

Recurrent respiratory papillomatosis is primarily a disease of children, in whom numerous papillomas occur in the upper aerodigestive tract, including the larynx and vocal cords. The papillomas commonly cause hoarseness, and obstructing papillomas may result in respiratory distress. High and low risk HPV-DNA sequences have been identified in the papillomas. While the viruses associated with respiratory papillomatosis are not acquired through sexual activity, they are nevertheless likely genital in origin, acquired during delivery (116).

Cervical carcinoma displays a wide range of gross appearances. Early lesions may be focally indurated, ulcerated, or present as a slightly elevated and granular area that bleeds readily on touch. Most advanced tumors have two major types of gross appearance: endophytic and exophytic. Endophytic carcinomas are either nodular or ulcerated. They tend to develop within the endocervical canal and frequently invade the cervical stroma to produce an enlarged, hard, barrel-shaped cervix. The exophytic types have a polypoid or papillary appearance (123).

Presentation of carcinoma of the uterine cervix depends on the size and stage of the lesion (125). Most patients complain of abnormal vaginal bleeding. Post-coital and post-douching bleeding is a most significant and important feature found in a recent study (124). Intermittent spotting, serosanguinous discharge and frank hemorrhage are
frequently encountered. Locally advanced or metastatic disease presents with weakness, pallor, and weight loss, oedema of the extremities, rectal pain and hematuria.

2.9 SCREENING

2.9.1 THE PAP SMEAR

The need for early detection and treatment of cervical cancer resulted in the first screening test pioneered by a physician named George Papanicolaou in the 1930's. It was formally introduced as a screening test after the Second World War and called the Pap smear. Pap smear programmes, also known as cytologic screening programmes, have achieved impressive results in reducing cervical cancer incidence and mortality in some developed countries. Cervical cancer incidence can be reduced by as much as 90 percent where screening quality and coverage is high (125). Health care practitioners in low-resource settings frequently report a lack of access to a well-organized infrastructure and health care system.

A Pap smear is a cytological test designed to detect abnormal cervical cells. The procedure involves scraping of cells from the cervix and then fixing them on a glass slide. The slides are then sent to a cytology laboratory and evaluated by a trained cytologist or cytotechnician. Results of this evaluation are generally available within several weeks. In most developed countries, women are advised to have their first Pap smear soon after becoming sexually active and subsequently every one to three years.

Although Pap smear-based screening efforts have been introduced in many developing countries, in general they have achieved limited success. For example, in Mexico, where national Pap smear screening programmes have been in place since 1974,
the mortality rate over 15 years held steady at 16 per 100,000 women (126). This is largely because younger women in urban areas were repeatedly being screened while older “at risk” women were not being reached. However, the Pap smear is recognised as an important but imperfect screening methodology.

2.9.2 **VISUAL APPROACHES**

Considerable research has been undertaken recently to explore the accuracy and acceptability of visual inspection as a means of detecting precursor cervical disease and/or cancer. There are several types of visual screening.

2.9.3 **VISUAL INSPECTION WITH ACETIC ACID (VIA)**

This screening involves swabbing the cervix with a 4% acetic acid solution prior to examination. Differences in precancerous cell structure and absorption rates make abnormal cells temporarily turn white when exposed to this solution. Many aspects of VIA make it a promising approach for use in low-resource settings. Costs associated with running a VIA screening programmes are relatively lower than those associated with other screening methods.

VIA is also a relatively simple, low-tech approach that is minimally reliant upon infrastructure for its’ adequate performance, assuming treatment services are available. Non-physicians can perform the procedure, provided that they receive adequate and ongoing training. Furthermore, results of the procedure are available immediately, making it possible, in principle, to provide treatment during the same visit (127). This method also allows screening of large numbers of women in a highly cost-effective
manner (128-129). Studies done in Zimbabwe in the John Hopkins Programme for International Education on Gynaecology and Obstetrics (JHPIEGO) cervical cancer project using this screening method comparing it to cytology in the same patients showed a sensitivity of 77% and a specificity of 64% (130).

The specificity of VIA remains a cause for concern due to the potential of the over treatment of women with false-positive test results. Over treatment may result in additional health risks to women as well as overburdening the health care system and increasing costs. VIA is also less effective for screening women in their fifties because of the tendency of the squamo-columnar-junction to recede into the cervical os, making observation of lesions difficult. Despite these drawbacks, VIA shows promise as an option for identifying pre-cancerous lesions in many settings, either in conjunction with or as an alternative to other screening methods.

2.9.4 VISUAL INSPECTION WITH MAGNIFICATION

VIA with magnification (VIAM) uses a telescopic device with an in built light source to examine the cervix after 4% acetic acid has been applied.

Aviscope: The Aviscope, which is a low power hand-held visual inspection device with a built in light source, is used to examine the cervix after acetic acid application. A small Indonesian evaluation of an earlier version of the device indicated that VIAM might achieve sensitivity and specificity of over 90% in identifying pre-invasive cervical lesions (132).
Gynescope: This is a monocular telescope used for cervical examination after acetic acid application.

Colposcopy: The colposcope is a large binocular microscope used to visualize the cervix for aceto-white areas. A biopsy of these areas is taken by the Gynaecologist for evaluation by Pathologists.

Speculoscopy: This utilizes a chemoilluminiscent light source for visualization of the cervix for aceto-white areas.

2.9.5 CERVICOGRAPHY

This involves photographing of the cervix after acetic acid application. The developed photographs, called cervigrams are projected as slides and interpreted by specially trained colposcopists. The sensitivity of cervicography is comparable to cytology and its' specificity is slightly lower. Cervicography, is however expensive and requires a reliable logistics infrastructure (133).

2.9.6 THE POLARPROBE

The Polarprobe is a computerized diagnostic instrument consisting of a pen-sized probe that is inserted into the vagina and moved across the cervix. The probe is attached to a portable computer that processes electrical and optical properties of cervical tissue and compares information with data from normal or abnormal tissue. Coppleson found in a study that Polarprobe diagnosis with concurrently obtained histologic-colposcopic
diagnosis had an 85% concordance between the two for low-grade intraepithelial abnormalities, 90% for high-grade abnormalities and 99% for cancer (133).

2.10 LABORATORY DIAGNOSIS OF HPV INFECTION

In developed countries, more sophisticated tests have been developed. Using the newest technology and appropriately constructed probes, major laboratories have been able to detect HPV DNA in up to 100% of invasive cancer (134). The initial molecular detection of HPV DNA was performed using nucleic acid hybridization techniques such as Southern blot. This was the original method of detection because of its' high sensitivity and specificity. It allows for accurate differentiation between HPV types and it is the only method by which clinicians can identify unknown types.

There are a variety of laboratory-based approaches for detecting HPV in cervical samples. There is currently only one company, the Digene Corporation which provides a United States Food and Drug-approved commercial kit that detects high-risk HPV types. This is the Hybrid Capture II kit (HC II), which indicates whether a person is infected with one or more high-risk HPV viral types (types 16, 18,31,33,35,39,45,51,52,56,58,59 and 68). For detection of high-grade dysplasia, the sensitivity range of HC II test is 80-90%; and specificity from 57-89% (135).

2.10.1 POLYMERASE CHAIN REACTION IN HPV DIAGNOSTICS

The application of a newer powerful molecular biologic technique called the polymerase chain reaction (PCR) has enabled investigators to search for HPV DNA with ease. With PCR, specific DNA segments can be amplified up to a million fold in
quantities that are easily detectable. PCR has the added benefit of unparalleled sensitivity for HPV detection with an early similar specificity.

PCR techniques can detect a single specific HPV type or up to 25 different types in one reaction. HPV DNA testing using PCR techniques utilizes both fresh and paraffin embedded specimens. HPV DNA detection is being considered as a primary screening method and an adjunctive screening method to both developing and developed countries. The PCR is an in vitro method of amplifying a target sequence of DNA specificity, based on the use of two oligonucleotides primers that hybridize to complementary sequences on opposite strands of DNA and flank target sequences. The PCR has been shown to be more sensitive than the Southern blot in detection of HPV in cervical scrapes (136). The DNA sample is first heated to separate the two strands; the primers are allowed to bind to the DNA. A DNA polymerase known, as Taq polymerase, is a heat stable DNA polymerase derived from the organism Thermophilus aquaticus that is able to withstand the heat denaturation process by virtue of the organism living and replicating at temperatures of 70-95°C.

This polymerase copies each strand starting at the primer site. The two DNA strands each serve as a template for the synthesis of new DNA from the two primers. Repeated cycles of heat denaturation, annealing of the primers to the complementary strands and extension of the annealed primers with DNA polymerase results in exponential amplification of the DNA segments. The combination of Pap smear and HPV DNA screening offers the potential for detecting almost all prevalent cases of HPV infections and cervical neoplasia (137).
2.11 PREVENTION AND CONTROL

A WHO consulting group met in 1985 to consider possible approaches for primary prevention of cervical cancer. Given that cervical cancer is strongly linked to early onset of sexual activity and multiple sexual partners, the WHO group recommended sex education and studies in sexual behavior while acknowledging that behavior is difficult to change. They also recommended that development of vaccines for HPV be made high priority, together with further study of the possible contribution of smoking to cervical cancer. They also recommended the use of barrier methods of contraception, particularly condoms, should be encouraged \(^{113}\).

Cervical cancer prevention can be obtained through screening programmes aimed at detecting pre-neoplastic lesions. Screening programmes that address women’s concerns in a respectful and culturally appropriate manner have been recognized to be successful.

In many countries, women’s lack of awareness of cervical cancer and its prevention is a major barrier to seeking screening services. One Nigerian study of women aged 20 to 65 found that only 15% had heard of the disease \(^{138}\). Another small study in Kenya found that 80% of women in the study did not know how cervical cancer is prevented \(^{139}\). This has therefore led to the increase in educational awareness programmes on the prevention of cervical cancer.

Since the WHO meeting in 1985 a lot of research has gone into providing a vaccine that will provide substantial protection to humans against HPV infection. Such a vaccine if developed will prevent hundreds of thousands of deaths a year as well as reduce the huge amounts of money spent on diagnosing and treating HPV-related lesions.
Studies done so far have focused on the induction of effective humoral responses against subsequent HPV infections \(^{(140)}\). In this respect immunoprophylactic effects have been demonstrated in animals using papilloma virus-like particles (VLPs). VLPs are antigenic and protective but are devoid of any viral DNA that may be carcinogenic to the host \(^{(141)}\). Another study has generated chimeric virus-like particles (CVLPs) by expression of an HPV 16L1E7-fusion protein. Immunization of mice with CVLPs induces neutralizing antibodies directed against L1 virus-like particles. Vaccinated animals have been found to be protected against tumor growth following inoculation of syngeneic HPV-16 transformed cells. Currently a vaccine based on HPV16L1E7 CVLPs is under development \(^{(141)}\).

In a recent double blind study, 2,392 young women (defined as females 16 to 23 years of age) were randomly assigned to receive three doses of placebo or HPV-16 virus-like-particle vaccine (40 microg per dose), given at day 0, month 2 and month 6. Genital samples to test for HPV-16 DNA were obtained at enrollment, one month after the third vaccination, and every six months thereafter. Women were referred for colposcopy according to a protocol. Biopsy tissue was evaluated for CIN and analyzed for HPV-16 DNA with the use of PCR.

The primary endpoint was persistent HPV-16 infection, defined as the detection of HPV-16 DNA in samples obtained at two or more visits. The primary analysis was limited to women who were negative for HPV-16 DNA and HPV-16 antibodies at enrollment as well HPV-16 DNA at month 7. The women were followed for a median of 17.4 months after completion of the vaccination regimen. The incidence of HPV-16 infection was 3.8 per 100 woman-years at risk in the placebo group and 0 per 100
woman-years at risk in the vaccine group. All nine cases of HPV-16 related CIN occurred amongst placebo recipients. Administration of this HPV-16 vaccine reduced the incidence of both HPV-16 infection and HPV-16 related CIN. Immunizing HPV-16 negative women may eventually reduce the incidence of cervical cancer. (142)
CHAPTER THREE

MATERIAL AND METHODS

3.1 STUDY SITES AND AREA

The study was conducted at the Departments of Pathology, Obstetrics and Gynaecology and Microbiology of the Korle-Bu Teaching Hospital, Accra Ghana between April and December 2002.

Korle-Bu Teaching Hospital opened by Sir Gordon Guggisberg in October 1923 is the most important medical institution in Ghana. It is the largest hospital and the major referral centre in Ghana. It is situated in the nation’s capital, Accra (Figures 3.1 and 3.2) in the Ablekuma district and serves as the teaching hospital of the University of Ghana Medical School (143). The Korle-Bu Teaching Hospital was an instant success and became the ‘general’ and model hospital for the entire nation, to which serious cases needing skilled specialist attention were referred. It brought so much relief to the sick that for many years, the people expressed their appreciation in a Ga improvised song: “Korle-Bu, Korle-Bu, Korle-Bu Oyiwala don” meaning Korle-Bu, Korle-Bu, Korle-Bu how grateful we are to you. (144).

It has since its establishment been accredited with huge success in the treatment of diseases as well as the training of health personnel. Its success is not only derived from the treatment of diseases alone but it serves also as a teaching centre for clinicians, nurses, laboratory technologists, midwives, dispensers, sanitary inspectors and other para-clinical personnel. When the hospital was opened it accommodated 96 patients initially, today as a result of periodic extensions, it accommodates over 2000 patients. It
became and remains the focal point of medical activity in the country. This shows that the hospital has a strong patient base good for clinical research. \(1^{43}\)

Figure 3.1

Map of Ghana showing the various regions and Accra
3.2 **SAMPLE SIZE AND STUDY POPULATION**

The first 75 patients who visited the Gynaecological clinic between September 2002 to March 2003 and who met the inclusion criteria of the study were recruited into the study. The inclusion criteria required a patient to consent to take part in the study and also should not have any form of vaginal bleeding at the time of the study. The first 75 patients who were approached consented to take part in the study. Only 75 patients were recruited due to financial constraints.
The protocol for the study was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School and written informed consent were obtained from the patients. Following informed consent a detailed demographic questionnaire was completed.

3.3 DEVELOPMENT OF QUESTIONNAIRE

A structured-interviewed administered questionnaire (Appendix 1) was designed to determine the risk factors associated with HPV infections, knowledge about cervical cancer screening and any previous histories of sexually transmitted diseases. This involved questions on personal information, sexual behavior, lifestyle and knowledge about cervical cancer.

3.4 SAMPLE ACQUISITION AND CLINICAL EXAMINATION

At the Obstetrics and Gynaecology clinic, following completion of the questionnaire, a consultant gynaecologist performed a gynaecological examination. A sample was obtained by inserting a sterile vaginal speculum into the vagina of a patient and, using a sterile Ayre’s spatula exfoliated cells were obtained from the transformation zone of the cervix. The spatula was then rinsed in 15 mls of sterile phosphate buffered saline (PBS). The samples were then transported to the Microbiology Department of the University of Ghana Medical School for analysis in the Parasitology Diagnostic and Research Laboratory.
3.5 **LABORATORY HPV TESTING**

3.5.1 **SAMPLE PREPARATION**

The samples for HPV testing were centrifuged at 2000 x g for 5 minutes to pellet the cells. The supernatant was removed and discarded using a plugged Pasteur pipette. The pellets were suspended in 50 μl of lysis buffer containing 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris HCl (pH 8.3) and 0.5% Tween 20. Two microlitres of proteinase K (QIAGEN, GmbH, Germany) was also added and the sample digested for two hours at 56°C. The samples were spun briefly for 2 minutes at 2000 x g in an Eppendorf micocentrifuge to remove all condensation from the cap of the Eppendorf tubes. These were then heated for 10 minutes at 95°C to denature the residual protease. The digested samples were centrifuged again briefly and 5 μl of the supernatant of each sample was used for PCR assay.

3.5.2 **PCR PRIMERS**

Two sets of primers were used for the PCR reactions. For the PCR amplification, primers GP5+ with a nucleotide sequence of 5’ TTT GTT GTG GTA GAT ACT AC 3’ and GP6+ with a nucleotide sequence 5’GAA AAA TAA ACT GTA AAT CAT ATT C 3’ were used. Invitrogen Life Technologies (Frederick, MD, USA) synthesized all primers.

3.5.3 **PCR AMPLIFICATION**

Amplification reactions were done using 5 μl of DNA (0.5 to 2% of the total extractable DNA) as DNA template, in a 50 μl reaction mixture containing 50 mM KCl,
5.0 μl of 10x PCR Buffer, 10 mM Tris-HCl (pH 8.8), 3.6 mM MgCl₂, 0.1 mg bovine serum albumin (BSA)/ml (New England Biolabs), 2.5 μl deoxynucleoside triphosphates, and 1μL of *Taq* Polymerase (QIAGEN, GmbH, Germany), 1.0 μl of enhanceser solution and 32 μl of autoclaved water. Fifty pico moles of each primer were used for ultrasensitive amplification. (Table 3.1)

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<tr>
<th>REAGENT</th>
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<tr>
<td>10x PCR Buffer</td>
<td>5.0μl</td>
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<tr>
<td>DNA</td>
<td>5.0μl</td>
</tr>
<tr>
<td>Deoxynucleosidetriphosphates</td>
<td>2.5μl</td>
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<tr>
<td><em>Taq</em> Polymerase</td>
<td>0.5μl</td>
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<tr>
<td>3.6mM MgCl₂</td>
<td>1.0μl</td>
</tr>
<tr>
<td>0.1mg BSA/ml</td>
<td>1.0μl</td>
</tr>
<tr>
<td>GP5+primer(50pmol)</td>
<td>1.0μl</td>
</tr>
<tr>
<td>GP6+primer(50pmol)</td>
<td>1.0μl</td>
</tr>
<tr>
<td>Enhancer solution</td>
<td>1.0μl</td>
</tr>
<tr>
<td>Autoclaved water</td>
<td>32.0μl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>50.0μl</td>
</tr>
</tbody>
</table>
In all 40 cycles were performed, initially 5 cycles of amplification was performed with each cycle having denaturation at 95°C for 1 minute, annealing at 50°C for 1.5 minutes and extension at 72°C for 2 minutes. This was followed subsequently by 35 cycles performed with denaturation at 95°C for 1 minute, annealing for 1 minute at 55°C and extension for 2 minutes at 72°C. (Table 3.2)

For every PCR assay, a negative control (water only with no DNA) was included. The controls were processed in the same way as the tissue specimens throughout the DNA preparation and steps of PCR amplification.

Table 3.2 AMPLIFICATION CONDITIONS FOR PCR REACTION

<table>
<thead>
<tr>
<th>TAQ POLYMERASE ACTIVATION</th>
<th>TEMPERATURE</th>
<th>TIME</th>
<th>NUMBER OF CYCLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>1.0min</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>55°C</td>
<td>1.0min</td>
<td>35</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>2.0min</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>1.0min</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>50°C</td>
<td>1.5min</td>
<td>5</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>2.0min</td>
<td></td>
</tr>
</tbody>
</table>
3.5.4 SEQUENCE ANALYSIS

Amplified DNA fragments were separated in 2% agarose gel by electrophoresis using 0.5x Tris borate-EDTA (TBE) as buffer medium at 40 mV. The gel was immersed in electrophoresis buffer containing 0.5 mg/ml ethidium bromide for one hour and examined under ultraviolet light. Photographs were taken using a Kodak DC265 digital camera.

3.5.5 DATA ANALYSIS

Data from the questionnaires were analyzed using the EPI 2000 software programme.
CHAPTER FOUR

RESULTS

Majority of the patients studied were in the 30-39 age group forming 48%. (Table 4.1).

<table>
<thead>
<tr>
<th>AGE (YEARS)</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>20-29</td>
<td>22</td>
<td>29.3</td>
</tr>
<tr>
<td>30-39</td>
<td>36</td>
<td>48.0</td>
</tr>
<tr>
<td>40-49</td>
<td>11</td>
<td>14.7</td>
</tr>
<tr>
<td>50-59</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>

These 75 patients had various educational backgrounds with 38 forming 50.6% having had at least some form of basic education. (Table 4.2) Majority of the patients were traders (29; 38.7%) and married, (55; 73.3%) (Tables 4.3 and 4.4) and were in the clinic for various reasons (Appendix 3).
### Table 4.2 EDUCATIONAL STATUSES OF PATIENTS

<table>
<thead>
<tr>
<th>TYPE OF EDUCATION</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMARY</td>
<td>13</td>
<td>17.3</td>
</tr>
<tr>
<td>JUNIOR SECONDARY SCHOOL</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>MIDDLE SCHOOL</td>
<td>21</td>
<td>28.0</td>
</tr>
<tr>
<td>SECONDARY SCHOOL</td>
<td>11</td>
<td>14.7</td>
</tr>
<tr>
<td>SENIOR SECONDARY SCHOOL</td>
<td>6</td>
<td>8.0</td>
</tr>
<tr>
<td>UNIVERSITY</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>NO SCHOOLING</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>OTHER</td>
<td>10</td>
<td>13.3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
<tr>
<td>OCCUPATION</td>
<td>FREQUENCY</td>
<td>PERCENTAGE %</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------</td>
<td>--------------</td>
</tr>
<tr>
<td>ACCOUNTANT</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>BAKER</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>CATERER</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>CATECHIST</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>CLEANER</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>FACTORY HAND</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>HAIR DRESSER</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>HOUSE WIFE</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>MESSENGER</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>NURSE</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>SALES GIRL</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>SEAMSTRESS</td>
<td>10</td>
<td>13.4</td>
</tr>
<tr>
<td>SECRETARY</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>SOCIAL WORKER</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>STUDENT</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>TAX OFFICER</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>TEACHER</td>
<td>5</td>
<td>6.7</td>
</tr>
<tr>
<td>TELEPHONIST</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>TRADER</td>
<td>29</td>
<td>38.7</td>
</tr>
<tr>
<td>UNEMPLOYED</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 4.4  MARITAL STATUS OF PATIENTS

<table>
<thead>
<tr>
<th>MARITAL STATUS</th>
<th>FREQUENCY</th>
<th>PERCENTAGE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SINGLE</td>
<td>8</td>
<td>10.7</td>
</tr>
<tr>
<td>WIDOWED</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>DIVORCED</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>MARRIED</td>
<td>55</td>
<td>73.3</td>
</tr>
<tr>
<td>IN PARTNERSHIP</td>
<td>9</td>
<td>12.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.1  AGE AT FIRST SEXUAL INTERCOURSE

Age at first sexual intercourse has been found to be a prominent risk factor associated with acquiring HPV infection. In particular early age at first sexual intercourse between the ages of 15-25 years or less has been found to be associated with a peak prevalence of HPV infection (7). Young women who initiate sexual intercourse at an early age are more likely than those with later sexual debuts to become infected with HPV. The factors and characteristics that influence the association between age at first sex and HPV include number of sexual partners, sexual transmitted infection (STI), and partner's number of sexual partners (146).

Of the 75 patients studied, 50 forming 66.7% had had their first sexual intercourse in the age group 10-19 years with the youngest age in this group being 14 years (Table 4.5).
Table 4.5  Age at first sexual intercourse

<table>
<thead>
<tr>
<th>AGE</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19</td>
<td>50</td>
<td>66.7</td>
</tr>
<tr>
<td>20-29</td>
<td>23</td>
<td>30.7</td>
</tr>
<tr>
<td>30-39</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.2 SEXUAL BEHAVIOR OF PATIENTS STUDIED

The role of the sexual behavior of women as well as that of their male sexual partners in the risk of acquiring HPV infection has been studied. It is known that having more than 2 male sexual partners increases the risk of infection (147). Of the 75 women studied, 61 forming 81.3% had had 2 or more sexual partners in their lifetime (Table 4.6).

Table 4.6  Number of male sexual partners a patient has had

<table>
<thead>
<tr>
<th>SEXUAL PARTNERS</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>18.7</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>37.3</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>28.0</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>12.0</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>&gt;5</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.3 SEXUAL BEHAVIOR OF PATIENTS’ MALE SEXUAL PARTNERS

The number of other women a male sexual partner has also increases the risk of infection in their female sexual partners¹⁴⁸. Most patients, 44 out of the 65 (67.7%) who said they had male sexual partners didn’t know if their male sexual partners had had any other female sexual partners apart from them (Table 4.7).

Ten of the patients had no sexual partners (8 single and 2 divorced). Of the 55 patients who were married, 9 (16.4%) had their husbands having one more wife and only one patient’s husband had 2 more wives (Table 4.8).

Table 4.7 Number of female sexual partners patients’ partner has had.

<table>
<thead>
<tr>
<th>FEMALE PARTNERS</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>10.8</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>9.2</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>7.7</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>&gt;5</td>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>DON’T KNOW</td>
<td>44</td>
<td>67.7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>65</td>
<td>100.0</td>
</tr>
</tbody>
</table>

¹⁴⁸ University of Ghana  http://ugspace.ug.edu.gh
Table 4.8  Number of other wives married to husbands of patients

<table>
<thead>
<tr>
<th>NUMBER OF OTHER WIVES</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45</td>
<td>81.8</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>16.4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>55</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.4 CONTRACEPTIVES USED BY PATIENTS STUDIED

Contraceptive use among the 75 patients studied found that majority 56 (74.7%) used no form of contraceptive (Table 4.9). Only 8 (10.7%) used the barrier method which is known to be protective against HPV.

Table 4.9  Type of contraceptive used by patients

<table>
<thead>
<tr>
<th>CONTRACEPTIVE</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BARRIER</td>
<td>8</td>
<td>10.7</td>
</tr>
<tr>
<td>INJECTABLE</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>IUCD</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>ORAL</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>NONE</td>
<td>56</td>
<td>74.7</td>
</tr>
<tr>
<td>RHYTHM</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.5 PAST HISTORY OF SEXUALLY TRANSMITTED DISEASE AMONG PATIENTS

Thirty-four (45.3%) had had pelvic pain in the past whilst 41 (54.7%) had not had any form of pelvic pain before. Twenty-four (32.0%) had had an offensive vaginal discharge in the past whilst 51 (68.0%) had not had any form of vaginal discharge. Four (5.3%) said they had had genital warts before whilst 71 (94.7%) had never had genital warts before.

4.6 CLINICAL MANIFESTATIONS OF CERVICAL CANCER

Cervical cancer presents in most patients with abnormal vaginal, post-coital and post-menopausal bleeding. Four forming 5.3% had had post-coital bleeding in the past. (Table 4.10).

Table 4.10 Post coital bleeding among patients

<table>
<thead>
<tr>
<th>POST COITAL BLEEDING</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>NO</td>
<td>71</td>
<td>94.7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.7 KNOWLEDGE ABOUT CERVICAL CANCER

Knowledge about cervical cancer among these patients studied was found to be rather poor. Out of the 75 patients studied, 42 (56.0%) said they had heard of cervical cancer whilst 33 (44%) had never heard about it. (Figure 4.1)
Figure 4.1  Knowledge of having heard of cervical cancer
Of the 42 patients who said they had heard about cervical cancer only 1 (2.3%) knew it could be caused through early sexual intercourse. Thirty-nine (92.9%) had no idea what the causes of cervical cancer were (Figure 4.2).

Figure 4.2 Knowledge of the causes of cervical cancer
Of the 42 patients that had heard of cervical cancer, 20 (47.6%) said it is preventable and 18 (42.9%) didn’t know it could be prevented (Table 4.11).

On knowledge of how cervical cancer could be prevented 28 of the 42 who said they had heard of cervical cancer had no idea how it could be prevented. This formed 66.7% out of a total of 42. Only 1 (2.3%) said it could be prevented by doing the Pap smear test (Figure 4.3).

<table>
<thead>
<tr>
<th>PREVENTABLE</th>
<th>FREQUENCY</th>
<th>PERCENTAGE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>20</td>
<td>47.6</td>
</tr>
<tr>
<td>NO</td>
<td>4</td>
<td>9.5</td>
</tr>
<tr>
<td>DON’T KNOW</td>
<td>18</td>
<td>42.9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>42</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Figure 4.3 Knowledge on how cervical cancer can be prevented
The Pap smear is the major form of screening worldwide and after its' introduction after the second world war has led to a decline in the incidence of cervical cancer by 70 to 80%. However from this study it was found that most of the patients had never heard of the cervical or Pap smear as well as having had it done before. Sixty-two (82.7%) had never heard of a cervical smear before whilst 13 (17.3%) had heard of it (Figure 4.4). However, of the 75 patients studied only 6 (8.0%) had ever had a Pap smear done in their lifetime (Figure 4.5).

![Knowledge of Cervical smear](Figure 4.4) Knowledge of cervical smear
Figure 4.5  Distribution of having had a cervical smear done
4.8 **PCR AMPLIFICATION AND GEL ELECTROPHORESIS**

HPV specific PCR amplification of DNA was done using the GP5+/GP6+ primers that synthesizes the L1 consensus region of the HPV DNA. This synthesizes a 140 bp in this region. A total of 75 cervical scrapings were evaluated using simple PCR protocol already described. PCR amplification was achieved in 8 of the specimens. DNA from these patients banded around the 140 bp showing that the correct region of the HPV DNA had been amplified (Figure 4.6). This confirmed the presence of HPV infection in these patients.
Plate 4.1

Gel photograph of simple Human Papilloma virus PCR products. PCR products were separated electrophoretically in a 2% agarose gel. M is the 100 bp DNA marker. NC is the negative control, PC positive control and S1 and S2 are samples one and two respectively.
4.9 **AGE OF HPV POSITIVE PATIENTS**

HPV positivity among patients studied was compared to age of patients seen at the Gynaecology Department. Six out of the 8 positive patients were in the age group 20-39 forming 75% (Table 4.12).

<table>
<thead>
<tr>
<th>AGE</th>
<th>FREQUENCY</th>
<th>PERCENTAGE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>20-29</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>30-39</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>40-49</td>
<td>2</td>
<td>25.0</td>
</tr>
<tr>
<td>50-59</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.10 **HPV POSITIVE PATIENTS AND AGE AT FIRST SEXUAL INTERCOURSE**

Age of first sexual intercourse and HPV positivity showed 5 (62.5%) having had their first sexual intercourse between the ages of 10-19 (Table 4.13).
### Table 4.13  HPV positivity and age at sexual first sexual intercourse

<table>
<thead>
<tr>
<th>AGE AT FIRST INTERCOURSE</th>
<th>FREQUENCY</th>
<th>PERCENTAGE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19</td>
<td>5</td>
<td>62.5</td>
</tr>
<tr>
<td>20-29</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td>30-39</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### 4.11  HPV POSITIVE PATIENTS AND SEXUAL BEHAVIOR

5 out of the 8 HPV positive patients had had 2 male sexual partners in their lifetime forming 62.5% of the total (Table 4.14).

### Table 4.14  Distribution of HPV positivity and number of male sexual partners

<table>
<thead>
<tr>
<th>MALE SEXUAL PARTNERS</th>
<th>FREQUENCY</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>62.5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>&gt;5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8</td>
<td>100.0</td>
</tr>
</tbody>
</table>
4.12 **TYPE OF CONTRACEPTIVE USED BY HPV POSITIVE PATIENTS**

Six (75%) of the HPV positive patients did not use any form of contraceptive at all and 1 (12.5%) used the rhythm method, which can be also included as no form of contraception since the rhythm method doesn’t use any physical device to prevent pregnancy but rather relies on the menstrual period as a guide to determine the safe periods. (Table 4.15).

<table>
<thead>
<tr>
<th>CONTRACEPTIVE</th>
<th>FREQUENCY</th>
<th>PERCENTAGE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BARRIER</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>INJECTABLE</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>IUCD</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>ORAL</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>NONE</td>
<td>6</td>
<td>75.0</td>
</tr>
<tr>
<td>RHYTHM</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

4.13 **PREVALENCE OF HPV INFECTION IN THIS STUDY**

Eight out of the 75 patients studied were determined to be HPV positive by PCR amplification of HPV DNA. Prevalence was calculated as a percentage of the number of patients studied. This therefore gave a prevalence of 10.7%.
CHAPTER FIVE

DISCUSSION

HPV infection as an STD particularly affects women of early reproductive age\(^{(54)}\). In the developed countries it has been reported as being among the commonest sexually transmitted pathogens. In these countries large numbers of cases are reported annually. In the USA it is estimated that 20-46% of women are infected with HPV\(^{(3)}\). Studies done in Sweden and Norway have found HPV prevalence rates of 22% and 15% respectively\(^{(82)}(149)\). However in the developing countries, there is very little information on the prevalence of HPV infection. Available data show rates of 5% in women with normal cytological findings in Bangkok, 8.01% in STD attendees in Ibadan, Nigeria, and 28.7% in Jamaica\(^{(80-81)}(150)\). In view of the lack of information on HPV prevalence in developing countries including tropical Africa, this study was designed to establish the prevalence of HPV infections in Ghanaian women attending a Gynaecological clinic of its main tertiary hospital.

This study is the only available work done on HPV prevalence in Ghana and has demonstrated a high rate of HPV infection (10.7%) among women visiting the Gynaecological department of the Korle-Bu Teaching hospital, Accra, Ghana. Other studies have shown rates ranging from 5-46.0% depending on the mode of diagnosis\(^{(3)(80-81)(150)}\).

5.1 AGE AND HPV INFECTION

Women in the age group 20-39 years who belong to the most sexually active age group were found to have an HPV prevalence rate of 75%. Fifty (66.7%) of all the patients
studied had had their first sexual intercourse by the age of 19 years and 5 of the HPV positive patients were among these. This finding is in agreement with other studies, which have also shown that risk of acquiring HPV infection is increased by early age of first sexual intercourse (53-54). Bauer et al. have reported the inverse association of HPV infection with age (151). However in this study, patients in the age group 40-49 years had 2 (25.5%) HPV positive patients. There were no HPV positive cases in the age group 50-59 years.

A Jamaican study found HPV prevalence rate of 38% among women between the ages of 15 to 19 years, whilst that of women older than 30 years had a prevalence rate of 17% (150). This indicates that the prevalence rate of HPV declines with age. This is believed to be due to HPV acquired immunity in older women and the transient nature of this infection (7,55). In a Swedish study, 276 women were studied for 2 years and the prevalence rate of HPV infection was found to decrease from 21% to 8.3% (103). A similar study in England found 93% of sexually active women initially infected with HPV shed their infection after been followed for four months (100).

5.2 SEXUAL BEHAVIOR

This is a very sensitive area to discuss in the Ghanaian society, given the socio-cultural norms and an almost forbidden subject to discuss openly. Sex outside marriage is considered a taboo, and as such it is a very difficult exposure to measure in our conservative society. Responses on sexual behavior of women and their sex partners were elicited from the women studied. A study done in eighteen countries of Asia and Africa on sexual behavior by face-to-face interviews found that extra-marital contacts of men
ranged from 4-47%. Women were less likely to report extramarital sex than men. The study therefore suggests that broad generalizations about sexual behavior especially multiple sex partners could be misleading (152).

A high number of sexual partners a woman has had increases her risk of acquiring HPV infection (52,56). Figueroa et al. reported that sexual behavior especially having more than one sexual partner per month was a significant determinant of HPV infection in STD clinic attendees in Jamaica (150). It has been found that women who have had 6 or more lifetime sexual partners have a fourfold risk of acquiring HPV infection (153). Studies done in Sweden and Italy respectively identified multiple lifetime sexual partners as the most relevant and major risk factor for HPV infection (82,154). The sexual pattern of women's male partners also is a contributory factor to acquiring HPV infection. A history of male sexual partners having multiple sexual partners increases a woman's risk of being infected with HPV (61,148). In a Finnish study it was shown that 16.5% of men had HPV infection demonstrated from penile swabs. Multiple sexual partners, previous history of sexually transmitted diseases and the practice of not using condoms were significant risk factors for the infection (155).

In this study quite a number of women admitted to having had more than one sexual partner. Twenty-eight (37.3%) out of 75 patients studied, had had 2 sexual partners in their lifetime and 5 of these were HPV positive. Most of the women in this study had no idea if their male sexual partners had had any other female sexual partners. Forty-four (67.7%) out of the 65 study patients who admitted to having male sexual partners, had no idea if their male sexual partners had any other female sexual partners.
It is possible that sexual behavior was under reported in this study but the high risk in those who reported multiple sexual partners suggests they have come out with the truth.

5.3 CONTRACEPTIVE USE

It has been hypothesized that oestrogen or other hormones are capable of either reactivating latent HPV infection or of increasing viral gene expression\(^{(156)}\). In support of this hypothesis some studies suggest that use of oral contraceptives increased HPV detection rates\(^{(44,65-66)}\). However one study done in New Mexico on cytologically normal women found that oral contraceptives tended to lower the risk\(^{(157)}\). Four (5.3\%) of the 75 study patients admitted to use of oral contraceptives but none of them was HPV positive. This finding is in agreement with the study done in New Mexico.

The barrier method of contraception is believed to protect against acquiring HPV infection. One patient who admitted to using the barrier method of contraception was HPV positive. However, this could be due to the fact that the patient was not using the barrier method frequently and also she could have acquired the infection prior to her initiation of this mode of contraception.

Most women (56; 74.7\%) in this study used no form of contraception. Six out of this number were HPV positive forming 75\% of the 8 HPV positive cases. It could also be argued that since most of these women presented with cases of infertility, (28; 37.4\%) (Appendix 3) and were anxious to get pregnant, hence their not using any form of contraception. The only patient who admitted to using the rhythm method as a form of contraception was HPV positive. This mode of contraception does not utilize any form of barrier methods since the patients’ menstrual periods are used as a marker of safety. In
actual fact she can be included among the 6 who used no contraception at all since no physical contraceptive device was used.

5.4 EDUCATIONAL BACKGROUND

Low socioeconomic status and educational status of women has been implicated as risk for acquiring HPV infection (44)(50-51). In the case of educational status, 5 (62.5%) of the HPV positive patients had had only basic education (primary, and middle school respectively). Ironically, all 7 who said they had had no form of education were HPV negative.

5.5 SMOKING

Cigarette smoking has been associated with HPV infections and cervical neoplasia and has been suggested to increase the risk of HPV infection as well as the size of precancerous lesions (62)(158-159). A Swedish study found however that smoking was not related to increase risk of HPV infection (82). Ghana being a conservative society frowns on smoking in women, so all the 75 patients seen, claimed they had never smoked a cigarette in their lifetime. This made it difficult to determine whether smoking affected risk of acquiring HPV infection in this study.

5.6 HISTORY OF SEXUALLY TRANSMITTED DISEASE

Only 4 (5.3%) out of the 75 study patients said they had had vulval warts in the past. Since HPV infection is known to be a transient infection most of the patients may have had the infection without them being aware of it. Moreover, warts occurring on the
cervix will be difficult for a patient to see unless it is identified through a routine
gynaecological examination. Acquiring other STDs puts one at risk of being infected
with HPV infection but this present study did not test the patients for other STDs so this
comparison cannot be made.

5.7 KNOWLEDGE OF CERVICAL CANCER

In many developing countries, women may not know about cervical cancer or that
detecting and treating precancerous lesions can prevent the disease; this lack of
awareness is a major barrier to seeking screening services. A study conducted in Nigeria
investigated Nigerian women’s knowledge about cervical cancer, their source of
information and their general attitude about cancer. A total of 254 women aged between
20 to 65 years were randomly selected from patients and accompanying persons attending
a general outpatient clinic at a tertiary hospital in Ibadan, Nigeria and they were made to
complete a structured questionnaire. Only 15% had heard of cervical cancer, and media
and peers were their main sources of information. More than half of the respondents had
no knowledge of the description of cervical cancer, clinical presentation, or causes. It was
therefore concluded in this study that knowledge of cervical cancer is poor and there is a
need to educate Nigerian women about cervical cancer and its early warning signs\(^{138}\).

In this study, 42 (56%) out of 75 study patients said they had heard of cervical
cancer whilst 33 (44%) had never heard about it. Of the 42 patients who had heard about
cervical cancer only 1 (2.3%) knew it could be caused through early sexual intercourse.
Thirty-nine (92.9%) out of the 42 patients who had heard of cervical cancer had no idea
what the causes of cervical cancer were and 18 (42.9%) out of these 42 patients didn’t
know it could be prevented. Only 1 (2.3%) out of these 42 who had heard about cervical cancer said it could be prevented by Pap smear test and 28 (66.7%) had no idea how it could be prevented. Sixty-two (82.7%) out of the 75 study patients had never heard of a cervical smear before and only 6 (8%) had had a Pap smear done in their lifetime. Knowledge about Pap smear is poor, considering the fact that lots of women in Ghana are escaping detection of premalignant lesions and coming with overt cervical carcinoma\(^{(111)}\).

From this study, one can categorically say that majority of women have no idea about the causes, screening and prevention of cervical cancer even though majority admitted to hearing about the disease. These findings show that knowledge about causes, screening and prevention of cervical cancer leaves much to be desired in most developing countries including Ghana and Nigeria and can explain the high prevalence rates of cervical cancer in these countries.

One hundred and seventy-five well-educated women in Accra, Ghana completed a study on cervical cancer knowledge and screening practices. These women were made up of medical students, non-medical undergraduate students, nurses and senior university workers\(^{(160)}\). Ninety-three percent of respondents had heard about cervical cancer but only 37% had adequate knowledge of the disease. Only 39% had adequate knowledge of Pap smears and of these, only 8.5% had ever had a Pap smear done before. Medical students and nurses had the most knowledge of cervical cancer and Pap smears, while actual Pap smear use was high among university staff. This study showed that the level of knowledge is not necessarily translated into use and that there is a need for an organized cervical screening programme that would include better education of non-medical personnel and increased initiative from doctors to perform routine Pap smears\(^{(160)}\).
In a similar study conducted in Alberta, Canada, which is a developed country, a study on cervical cancer screening knowledge, attitudes and behaviors, showed 53% of participants knowing cervical cancer could be prevented by Pap smear tests. Also 99% had had a Pap smear done in their lifetime and 88% had had it done in the last three years. This study reveals that even though 99% had had Pap smear tests only 53% knew that it was used to prevent cervical cancer and as such majority of women had no idea why they were having the Pap smear tests. However this shows better knowledge of cervical cancer and its prevention when compared to what pertains to a developing country like Ghana. The frequent follow-ups in developed countries may be the reason for low cervical cancer rates in such developed countries.

In many developing countries, women lack information about cervical cancer prevention options. Women may know little about the disease, may not trust medical protocols and use limited programme resources inefficiently. Efforts to improve women’s awareness and provider knowledge of prevention options are essential to a successful cervical cancer prevention programme.

Several barriers to early detection of cervical cancer have been identified in some studies. One study found that barriers to having the Pap smear test included lack of knowledge about cervical cancer aetiology, lack of knowing of the Pap smear test, the perception that cancer is an inevitably fatal disease, problems in client-provider relationships, opposition by male sexual partners, rejection of pelvic examinations, long waits for sample collection and results, and perceived high costs of care. In order for women to seek cervical cancer screening services, they must be informed about the disease and have access to services that are sensitive and responsive to their needs.
Women at risk of cervical cancer need complete and accurate information so they can understand prevention options and utilize screening services. Cervical cancer programmes must address the cultural, emotional, and practical barriers that influence whether or not women will utilize screening services. Women may be reluctant to undergo screening due to embarrassment, fear of screening procedure, or fear of cancer. Women frequently mistrust health care providers and have various family pressures that prevent them from seeking health care, especially gynaecological care. Therefore women require services that will address their concerns in a respectful and culturally appropriate manner (139)(163).

HPV has been considered as the major aetiological agent for cervical cancer and the main risk factor identified is sexual behavior. Prevention strategies are been based on the control of STDs and the possibility of vaccination. Vaccination against HPV is currently under experimentation and might offer a better option for cervical cancer control. HPV -16 vaccine trials have recently been done in the USA and were successful(142). If an efficient and cheap polyvalent vaccine is made available it can be considered for mass vaccination, since different oncogenic types infect women at risk.

Sexual behavior modification is a difficult task in conservative societies like Ghana. However, since it is a major determinant of HPV infection as well as other STDs, health education with special emphasis on sexual behavior is needed. It may be difficult in older generations but certainly; it has to be considered for the younger generations who are exposed to the emerging social and cultural changes.
An organized Pap smear based cytology screening programme has been shown to be the best strategy for the control of cervical cancer, but in a developing country like Ghana this has not been feasible considering the lack of infrastructure, finance and technical expertise. With the introduction of HPV vaccines, HPV prevalence studies to determine the oncogenic types that infect Ghanaian women need to be done, so that development of overt cervical cancer can be prevented through vaccinations and monies spent on treatment of cervical cancer can be utilized for other developments in the country.
CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

This study provides an insight into the sexual behavior and knowledge about cervical cancer among women visiting the Gynaecological Department of Korle-Bu Teaching hospital, Accra, Ghana. It also finds an HPV prevalence of 10.7% among the patients studied, which can be compared with other studies done in West Africa.

In this study, most of the women had their first sexual encounter between the ages of 14 and 19 years (66.7%; 50 out of 75 study patients). Five out of the 8 HPV positive patients (62.5%) belonged to this group. Sixty-one (81.3%) out of the 75 study patients had had 2 or more sexual partners in their lifetime. Five (62.5%) of the 8 HPV positive patients were in this group. Fifty-six (74.4%) did not use any form of contraceptive, and 6 (75%) of the 8 HPV positive cases were in this group.

It can be concluded that most women are having sexual intercourse at early ages when the cervix is immature making it vulnerable to STDs including HPV infection hence increasing their risk. Also majority of women were exposed to the risk of multiple sexual partners as well increasing the possibility of being infected. Contraceptive use especially the barrier form was lacking exposing these women to the infection.

Knowledge about cervical cancer, its' causes and prevention was poor and as such poses a tremendous health problem since most women are not even aware of the disease and the steps to prevent it occurring. This poses an important public health problem in Ghana.
Since cervical cancer is an important public health problem its' prevention must be integrated in all existing primary health care and women’s health programmes in Ghana. Public health interventions and control strategies for improving reproductive health need to be planned for controlling HPV infection in this population. There is an urgent need to train nurses and other medical personnel including cytotechnicians for cervical cancer screening. Emphasis should also be placed on providing facilities for treatment of precancerous lesions in district and regional hospitals.

National policy guidelines on cervical cancer screening should be urgently drawn addressing specific age at risk groups and frequency of screening. For adequate assessment on the impact of cervical cancer screening programmes, cancer registries should be established at district and regional hospitals.

Education of the general public at the community level on the risks of early sexual intercourse, cervical cancer and its screening should be done utilizing the services of the print and electronic media. With the current HPV vaccine trials going on further work on HPV types that infect Ghanaian women needs to be done.
REFERENCES


41. Chellapan S et al. (1992): Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between the transcription factor E2F and the retinoblastoma gene product. *Proc Natl Acad Sci USA*; 89:4442.


APPENDIX 1

THE PREVALENCE OF CERVICO-VAGINAL HUMAN PAPILLOMA VIRUS IN WOMEN VISITING THE GYNAECOLOGICAL CLINIC AT THE KORLE-BU TEACHING HOSPITAL

QUESTIONNAIRE

1. Age of respondent:..................................... Date:.................................

2. Educational level of respondent: No schooling □ Primary □ Middle school □ Secondary school □ Junior Sec. school □ Senior Sec. school □ University □ Other □

3. Occupation of respondent:...............................................................

4. Reason for attending Gynaecological clinic:..............................................

5. Marital Status Single, no partner □ In partnership □ Married □ Divorced □ Widowed □

6. If married, how many other wives does husband have?

   1. □
   2. □
   3. □
   4. □
   None □

7. Can you estimate the number of female sexual partners your partner/spouse has had? .......... /Don’t know

8. Age at first sexual intercourse ......................................................

88
9. Number of male sexual partners respondent has had

1. 
2. 
3. 
4. 
5. 
6. 

10. Do you smoke? Yes
No

11. What contraceptive method do you use?

- Oral contraceptives
- Injectable
- Norplant
- Barrier method
  (condom, female condom, diaphragm)
- IUCD
- None
- Other, please state

12. Have you ever had post coital bleeding Yes
No

13. Have you ever had:

(a) Pelvic pain with discharge
(b) Abnormal offensive discharge
(c) Ulcers/sores
(d) Warts

14. If yes to any of the above, did you see a doctor? Yes
No

15. Has current partner/spouse ever had

Yes
No
Don't know
<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Pain on urination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Abnormal offensive discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Ulcers/sores on penis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) Warts on penis</td>
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</tbody>
</table>

16. Has earlier partner/spouse ever had

| (a) Pain on urination                                                  |     |    |            |
| (b) Abnormal offensive discharge                                      |     |    |            |
| (c) Ulcers/sores on penis                                             |     |    |            |
| (d) Warts on penis                                                    |     |    |            |

17. Have you heard of cervical cancer
   (If no go to Q.23)

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

18. If yes to question 17;
   What causes it?

19. Is it sexually transmitted?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

20. Is cervical cancer preventable

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

21. If yes to question 20;
   How can it be prevented?

22. Mother/sister has had cervical cancer?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

23. Have you heard of a cervical smear?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

24. If yes to question 23,
   Have you had it done before?
   (If no go to Q. 27)

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
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</table>

25. How many times

<p>| | |</p>
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<th></th>
<th></th>
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<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td></td>
</tr>
<tr>
<td>Twice</td>
<td></td>
</tr>
<tr>
<td>Thrice</td>
<td></td>
</tr>
<tr>
<td>&gt;3x</td>
<td></td>
</tr>
</tbody>
</table>
26. When was your last smear done?  <3 years ago  □
                                □
                        >3 years ago  □

27. Would you want a cervical smear done  Yes  □
                                         No  □

28. If no, Why? .................................................................

Thank you for your time.
APPENDIX 2
PREPARATION OF REAGENTS

1. PHOSPHATE-BUFFERD SALINE (PBS)

8g of NaCl, 0.2g of KCl, 1.44g of Na₂HPO₄ and 0.24g of KH₂PO₄ was dissolved in 800mls of distilled water. pH was adjusted to 7.4 with HCl and distilled water was added to make one litre. The solution was dispensed in aliquots and sterilized by autoclaving for 20 minutes at 15lb/sq.in on liquid cycle and stored at room temperature.

2. 5X TRIS-BORATE EDTA (TBE)

54g of Tris base, 27.5g of Boric acid and 20mls of 0.5M EDTA at pH 8.0 was dissolved in 800mls of distilled water and volume was adjusted with distilled water to make one litre. A one in ten dilution of the concentrated stock was made to obtain a 0.5x TBE for the agarose gel electrophoresis.

3. 2% AGAROSE GEL

2g of Agarose was added to 100mls of 0.5x TBE and heated in a microwave oven till the agarose dissolved. The solution was then allowed to cool quickly to 70°C and the gel was poured immediately in a clean, dry glass plate sealed at both ends with autoclave tape. The glass plate had been fitted with a comb 0.5-1.0mm above the plate. The gel was allowed to set for 30-45 minutes at room temperature. After the gel was completely set the comb and the autoclave tapes were carefully removed and the gel placed in the electrophoresis tank containing electrophoresis buffer.
## DISTRIBUTION OF REASON FOR ATTENDING CLINIC

<table>
<thead>
<tr>
<th>REASON FOR ATTENDING</th>
<th>FREQUENCY</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABDOMINAL MASS</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>BLEEDING PER RECTUM</td>
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<td>1.3</td>
</tr>
<tr>
<td>BLEEDING PER VAGINAM</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>DELAYED MENSES</td>
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<td>1.3</td>
</tr>
<tr>
<td>DYSPAREUNIA</td>
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<td>1.3</td>
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<tr>
<td>INFERTILITY</td>
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<td>37.4</td>
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<tr>
<td>IRREGULAR MENSES</td>
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<td>4.0</td>
</tr>
<tr>
<td>LOW BACK ACHE</td>
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<td>1.3</td>
</tr>
<tr>
<td>LOWER ABDOMINAL PAIN</td>
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<td>6.7</td>
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<tr>
<td>MENORRHAGIA</td>
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<td>5.3</td>
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<td>OLIGOMENORRHEA</td>
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<td>1.3</td>
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<td>PAP SMEAR</td>
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<td>2.7</td>
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<td>PELVIC PAIN</td>
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<td>POST MENOPAUSAL BLEEDING</td>
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<td>UTERINE FIBROIDS</td>
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<tr>
<td>VULVAL WARTS</td>
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<td>4.0</td>
</tr>
<tr>
<td>TOTAL</td>
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<td>100.0</td>
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