COMPARISON OF THINPREP™ MONOLAYER CYTOLOGY VERSUS CONVENTIONAL PAP SMEAR AND THE CORRELATION WITH HUMAN PAPILLOMAVIRUS DETECTION USING HYBRID CAPTURE® 2 SYSTEM

By

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Dissertation Submitted In Partial Fulfillment of the Requirements for the Degree of Master of Pathology (Anatomic Pathology)

UNIVERSITI SAINS MALAYSIA
2007

ACKNOWLEDGEMENTS

In the name of ALLAH, the Most Beneficient, and the Most Merciful

Praise ALLAH almighty, despite the obstacles, I finally able to complete this study.

My sincere thanks and deepest appreciation to my supervisor, Professor (Dr.) Nor Hayati Othman, for her guidance and support throughout the study and as a master student. A special thanks and greatest appreciation to the Head of Department of Obstetric and Gynecology, Pusat Pengajian Sains Perubatan, Universiti Sains Malaysia; Head of Department of Obstetric and Gynecology, Hospital Kota Bharu, Kelantan and Head of Department of Obstetric and Gynecology, Hospital Kuala Terengganu, Terengganu, for without their help the cervical specimens would not have been made available.

I also wish to express my appreciation to En. Mazlan Muda and Pn. Ng Poh Pheng in Cytology Laboratory, for their guidance in cytology techniques. Many thanks to Mr. Tae Han Shen and Ms Nordiah Othman in the Molecular Laboratory for their molecular technical assistance.

Finally, I would like to dedicate this hard work to my beloved parents, Tn. Hj. Md. Shah Buang and Hjh Siti Faridah Md Noor, for their endless love and supports throughout this study.

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ABBREVIATIONS

AGC	Atypical glandular cells
AHCPR	the Agency for Health Care Policy and Research
ASC-H	Atypical squamous cells, cannot excluded HSIL
ASCUS	Atypical squamous cells of undetermined significant
CIN	cervical intraepithelial neoplasia
DES	diethylstilbestrol
DNA	Deoxyribonucleic acid
EBV	Ebstein-Barr virus
FDA	United States Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
HC II	Hybrid Capture® 2 system
HKB	Hospital Kota Bharu
HKT	Hospital Kuala Terengganu
HLA	Human Leucocyte Antigen
HPV	Human Papillomavirus
HSIL	High-grade squamous intraepithelial lesions
HSV	Herpes Simplex Virus
HUSM	Hospital Universiti Sains Malaysia
IARC	the International Agency for Research on Cancer
ISH	in-situ hybridization
LSIL	Low-grade squamous intraepithelial lesions
NOS	Not otherwise specified

ABSTRAK

Smer servikal papanicolaou adalah berguna sebagai cara penyaringan lesi awal servik dan kanser servik. Teknik ini menyebabkan penurunan insiden kanser servik di seluruh dunia. Walau bagaimanapun, smer pap konvensional mempunyai banyak kelemahan, termasuklah kadar kepalsuan negatif dan kepalsuan positif yang signifikan, dan tidak sesuai kerana ketebalan smer itu sendiri. Sitologi berasaskan cecair seperti teknik ThinPrep™ merupakan alternatif terbaik bagi smer pap konvensional. Ia memberikan teknik satu lapisan sel untuk mempermudahkan pemeriksaan sitologi. Kanser servik disebabkan oleh HPV yang berisiko tinggi. Identifikasi virus menggunakan teknik Hybrid Capture® 2 berguna di dalam penyaringan kanser servik. Hybrid Capture® 2 merupakan salah satu contoh ujian HPV untuk sampel yang banyak.

Kami melakukan kajian ini untuk menentukan teknik sitologi satu lapisan mampu memberi keputusan yang lebih baik daripada smer konvensional. Enam ratus lapan puluh lapan wanita yang memberikan akuan kebenaran telah dipilih. Kami mengumpul sampel servik eksofoliatif daripada semua subjek. Setiap sampel akan dismer untuk dijadikan smer pap konvensional sebagaimana yang dilakukan secara rutin dan dibilas dengan cecair bufer (PreservCyt) untuk diproses menjadi smer ThinPrep™ and untuk ujian HPV. Kesemua maklumat ujian dirahsiakan. Perbandingan hasil ujian smer konvensional dan smer ThinPrep™ telah dijalankan. Analisis smer serviks adalah berdasarkan Pengkelasan Bethesda 2001. Kesemua kes yang positif lesi awal servik dan kanser seterusnya menjalani ujian Hybrid Capture® 2.

94.3% smer konvensional dan 89.4% smer ThinPrep™ adalah smer yang memuaskan. Dalam kedua-dua teknik, Candida spp adalah organisme yang kerap dijumpai (4.1% smer konvensional, 3.9% ThinPrep™) dan persetujuan kedua-dua teknik di dalam diagnosis tersebut boleh diterima (k=0.27). Diagnosis ASCUS, LSIL, HSIL dan Karsinoma sel skuamus adalah rendah dalam kedua-dua teknik. Kes sel kelenjar abnormal juga rendah. Kajian persetujuan untuk LSIL adalah boleh diterima (k=0.40). Sementara itu, kajian persetujuan untuk ASCUS, HSIL, Karsinoma sel skuamus, AGC (NOS) dan Adenocarcinoma (NOS) tidak dilakukan kerana bilangan yang terlalu sedikit. Ujian HPV untuk sitologi abnormal menunjukkan positiviti yang rendah (38.5%).

Hasil keseluruhan ThinPrep™ adalah tidak sebaik smer pap konvensional. Penggunaan ThinPrep™ tidak menunjukkan peningkatan penemuan organisme infeksi dan sel epitelium abnormal. Kadar positiviti teknik Hybrid Capture® 2 adalah diragui. Pengesahan dengan teknik lain seperti PCR adalah perlu.

Kesimpulannya, teknik sampel terpisah mampu memberikan smer yang memuaskan. Cara ini boleh dilaksanakan oleh makmal-makmal yang ingin menukar dari satu teknik kepada yang lain.

ABSTRACT

Pap smear is a useful screening tool for cancer of the cervix. Screening has resulted in the tremendous fall in cervical cancer incidence worldwide. However, the conventional pap smear has many limitations due to significant false negative and false positive rate, and unsuitability due to thick smears. Liquid-based cytology, such as ThinPrep™ technique is an alternative to conventional pap smear. This method provides monolayer cells which make cytological examination easier. HPV is the cause of cervical cancer. Identification of the virus is helpful in cervical cancer screening. Hybrid Capture® 2 systems is one of the examples of HPV testing that is useful to detect high-risk HPV in a large number of samples.

We embarked on pap smear split-sampling study to determine if monolayer cytology would give better results than conventional cytology. Six hundred eighty eight women who gave consent were recruited. We collected the exfoliative cervical samples from all the subjects. Each sample was smeared as for the conventional pap smears as done routinely and then rinsed into a liquid buffer (PreservCyt) for monolayer cytology and HPV testing. The cytological examination was performed in blinded fashion. The performance of conventional and ThinPrep™ monolayer cytology pap smears was compared. Smears were classified based on The 2001 Bethesda classification. All cases which had epithelial abnormalities were further tested for HPV by Hybrid Capture® 2 technique.

We found that adequacy of sampling was 94.3% in conventional pap smear and 89.4% in ThinPrep™ monolayer cytology. The commonest organism identified was Candida spp (4.1% in conventional smears, 3.9% by ThinPrep™) and the agreement study is fair

(k=0.27). Diagnosis of ASCUS, LSIL, HSIL and squamous cell carcinoma, were low in both techniques. The glandular cells abnormalities were also low in numbers. The agreement studies for LSIL showed k=0.40. The agreement studies for ASCUS, HSIL, squamous cell carcinoma, AGC (NOS) and adenocarcinoma (NOS) were not done as the number was too few. The HPV testing of cases with abnormal cytology showed low positivity (38.5%).

The overall performance of ThinPrep™ is no better than conventional pap smears. The usage of the ThinPrep™ pap smear does not improve in detecting certain infective organisms nor abnormal epithelial cells. The low positivity of Hybrid Capture® 2 technique is questionable. Confirmation with other techniques like PCR is essential.

In conclusion, split sampling gives adequate smears for cytological assessment. It can be carried out in laboratories working to change from one technique to another.

INTRODUCTION

Invasive cervical cancer is the second most common cancer in women worldwide. It is the most common cause of death among women in developing countries. It account for about 6% of all cancers found in women. It is estimated that near half a million new cases of cervical cancer are diagnosed each year. In Malaysia, it is the second most common cancer in women. The association of persistent precursor lesions term intraepithelial lesion, is well established. The higher the grade of the lesion, the more likely is the progression to cancer. However, the progression into invasive cancer takes several years. With the basis of this knowledge, many cervical cancer screening methods have been developed. In view of the ability to detect these precancerous lesions using papaniculaou smear (pap smear), there is a remarkable decrease in the cervical cancer incidence and mortality rates. Many countries such as Canada and the United States have started a mass screening program since the early fifties.

The conventional method of screening has been shown to be an effective method of preventing cervical cancer. After a while, its value as a screening method for cervical cancer has been questioned. It shows marked variability in its performance characteristics. Many studies were done to re-evaluate the conventional method that has been introduced since the Papanicolaou era.

Due to limitation of the conventional pap smear, new methods of cervical screening have been devised. The liquid based cytology has emerged as an alternative to the conventional pap smear. It was introduced in the 1990s and since then many cytology laboratories were have used this technique in their service.

An infection of the uterine cervix with the high-risk Human Papillomavirus (HPV) is accepted to be causally associated with the development of invasive cervical cancer. It is important that not all HPV types are associated with cervical precursor lesions or cervical cancer. Therefore, HPV testing strategies should focus on the detection of high-risk HPV types only. Hybrid Capture II system is a promising method to achieve this goal. Today, the ancillary testing for HPV detection has been one of the main approaches to reduce the number of cervical cancer cases.

The present study aims to assess the ThinPrep™ technique as an example of the liquid based cytology technology. The comparison with the current conventional pap smear is necessary to evaluate the overall effectiveness as a screening method in cervical cancer, especially in the Malaysia population of the east coast of peninsular Malaysia. The assessment of HPV testing using Hybrid Capture II system as a complimentary test, to the abnormal cervical cytology is a step to take the full advantage of molecular biology in the preventing invasive cervical cancer.

LITERATURE REVIEW

1. Uterine cervix

1.1. Anatomy

The adult cervix is cylindrical in shape and divided into two portions. The area that projects into the vagina is called the ectocervix or previously known as portio vaginalis. The ectocervix consisting anterior and posterior lips, contains an external os in the center. In nulliparous women, the cervix measures 2.5cm to 3.0cm in length and 2.5cm in diameter. The os has a slit-like horizontal space. The surface of ectocervix has a smooth, opaque, white mucosa contiguous with that of the vagina. The vaginal mucosa is reflected around the cervix forming the fornices. On gross examination, the anterior lip is shorter and thicker, and projects lower than the posterior lip. The posterior fornix is deeper than the anterior fornix due to slight downward and backward position of the cervix. The endocervix is a fusiform cavity and bounded by external os and the internal os.

The cervix is supported by uterosacral and cardinal ligaments. The uterosacral ligaments maintain the relationship between the cervix and rectum. The cardinal ligament suspended the cervix in the pelvis. The parametrium is the loose connective tissue located between the supravaginal portion of the cervix and the urinary bladder.

The ectocervix acts as a barrier between the sterile endocervical cavity and the vagina. It also facilitates the sperm transport distally. The cervical ithmus acts as a sphincter during childbirth.

The cervix is supplied by cervicovaginal branch of the uterine artery which enters at the level of the isthmus. The venous drainage is parallel to that of the arterial system and drains into the Internal Iliac vein. The cervical lymphatic drainage has three sets, the lateral, in the broad ligament to the external iliac nodes; the posterolateral, along the uterine vessels to the internal iliac nodes; and the posterior, along the recto-uterine folds to the sacral nodes (Ellis, 1997).

1.2. Histology

The mucosa of the ectocervix is composed of stratified squamous epithelium. It is divided into five layers: basement membrane, basal cells, parabasal cells, intermediate cells and superficial cells, from the base to the surface (Figure 1).The normal superficial squamous cells are shown in Figure 2. The endocervical or the cervical canal mucosa is covered by a single layer of mucin-secreting columnar epithelium with underlying glandular structures.

Figure 1: Normal histology of the ectocervix

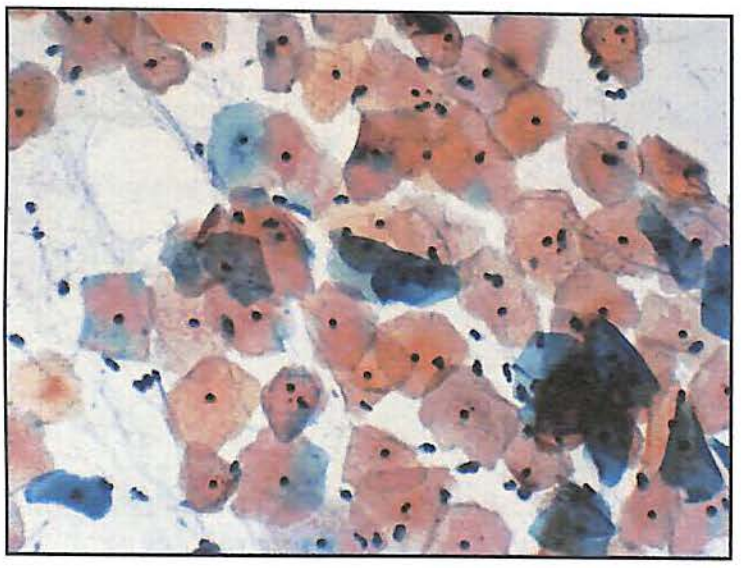

Figure 2: Normal superficial squamous cells of ectocervix

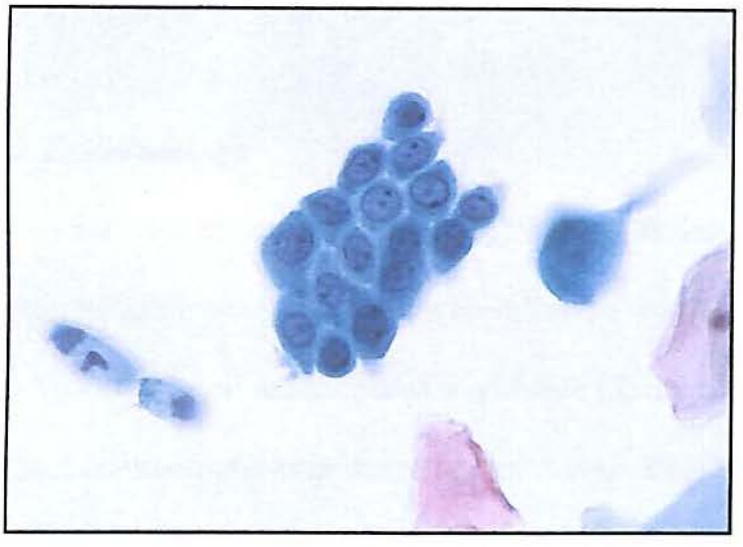

Figure 3: Normal endocervicals cell of the uterine cervix

The transformation zone or the squamo-columnar junction is a transition between the squamous epithelium of the ectocervix and the mucus secreting columnar epithelium of the endocervix (Figure 3). At birth, the transformation zone is situated at the ectocervix. In adolescence, the transformation zone appears at or near the external os.

In child bearing age, the endocervical epithelium extends to the ectocervix and is often called ectropion or eversion. Squamous metaplasia of the cervix refers to a process by which columnar epithelium is replaced by stratified squamous epithelium. It is a physiologic response to the hormones. This zone is the common site of the development of most precancerous and cancerous lesion of the cervix (Ross, 2003)

1.3. Embryology

The uterus including the cervix is derived from a pair of paramesonephric ducts, which are formed by invagination of coelomic epithelium during the 6^{th} week of gestation (Larseen, 2001).

2. Epidemiology

Cervical cancer remains a major global health issue and every year approximately more than 400,000 new cases of invasive cancer are diagnosed (Richard et al, 2004). Almost 250,000 reported deaths occur worldwide (Richard et al, 2004). Currently, it is the second most common cancer in women (Brinck et al, 2004).

In Malaysia, cervical carcinoma is the second most common cancer in women after breast cancer estimating about 12.9% of overall cancer in female population (National Cancer Registry, 2003). The incidence is 16.5 per 100,000 populations, which accounting for 1557 cases (National Cancer Registry, 2003). The Chinese have the highest number of cases, 782 (56.4%), compared to the Malays, 454 cases (32.8%) and Indians, 150 cases (10.8%) respectively. The highest age group of these cases falls into the seventh decade group.

Due to good screening practice, the prevalence shows a declining trends. In Belgium, cervical cancer ranks fifth after breast, colon, ovarian and rectal cancer respectively (Beeren et al, 2005). In the United States for example, a study showed a declining incidence of squamous cell carcinoma of the cervix from 1979 to 1999, which are 0.94% and 1.1% respectively. However, there is an increase in adenocarcinoma of the cervix to 2.9% (Chan et al, 2003).

3. Clinical Features

The clinical features of cervical cancer sometimes depend on the neoplasm growth character. In exophytic growth, post coital bleed, intermenstrual bleed and bloody discharge are common. The frequent complaints are per vaginal bleed in more than 90% of patients (Maalej et al, 2004). Besides that, pelvic pain and malodorous discharge are also not uncommon (Mangili et al, 2004). In endophytic growth, most are asymptomatic (Ben, 1983).

On physical examination, the cervix is barrel shaped with occasional contact bleeding. Ulceration is present with the endophytic type.

In Adenocarcinoma, endophytic type is the commonest feature while some of the cases presented with papillary lesion and ulcer. Up to 50% of patients are asymptomatic (Burton et al, 1999). Majority of this tumour occur in post menopausal women. Other symptoms include per vaginal bleed or discharge and dyspareunia (Chargui et al, 2006). In 15% to 30% of patients, the tumour arises high in the endocervical canal and 85% are found to be

confined to the cervix or invading the parametrium (Burton et al, 1999). Other rare presentation, like uterine prolapse are also been reported (da Silva et al, 2002).

4. Etiology

It is widely accepted that viral etiology is known to cause uterine cervical cancer (Bosch et al, 2003). Environmental factors also play some role in the development of cervical cancer (Reick et al, 2006). This includes various factors like lifestyle, infections, and genetic factors. Few other studies mention that smoking, alcohol consumption, oral contraception, reproductive and sexual history contribute to the precursor lesion of cervical cancer or cervical carcinoma.

4.1. HPV

In the 1970s, zur Hausen suggested that there might be an association between HPV and cervical cancer (Figure 4). Since then, many studies had been directed to this entity (Kurman, 2001).

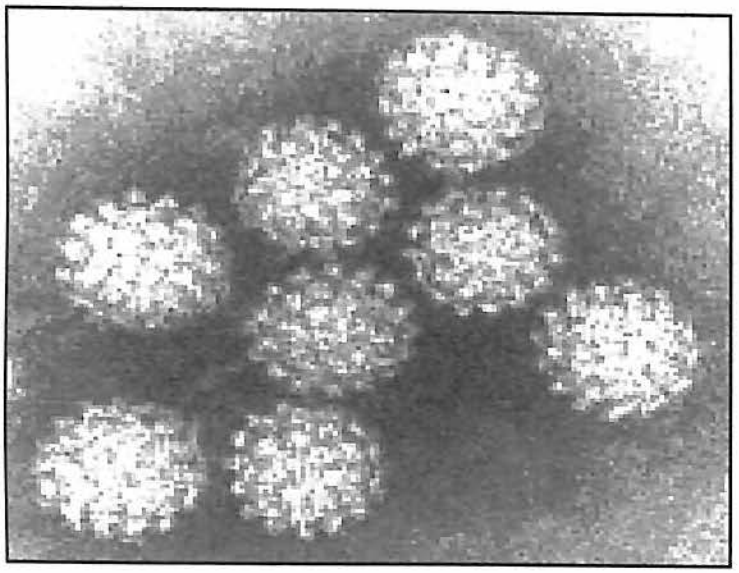

Figure 4: HPV seen in the electron microscope

More than hundred types of HPV have been isolated and studied (Trottier, H and Franco EL, 2005). The virus is classified into low-risk and high-risk subtypes (Soliman et al, 2004). The low-risk subtypes are associated with benign lesion and the high-risk subtypes are associated with high grade lesions and cancers.

HPV have a double stranded DNA genome of approximately 8000 bps encoding for 10 viral proteins, including early and late products (Bernard, 2005; Anderson, 2002). Expression of the early gene products determines whether the HPV infection is active and can lead to malignant transformation (Anderson, 2002). E6 and E7 are the early gene products, which can bind to and inactivate and cause degradation of cellular tumour suppressor gene products, p53 and Rb. Current evidence shows that their combined effects in deregulating cell cycle control mechanism are the major factors in the development of cervical cancer, especially in a HPV subtype, HPV 16 (Rose et al, 1995).

Pathogenesis of cervical cancer due to HPV infections is a complex process but is well recognised. The early gene products (E6 and E7) from the HPV encode the cellular main transforming proteins capable of immortalization and oncogenic transformation. These products will interfere with normal function of protein products of the tumour suppressor genes (P53 and Rb) and this can lead to cancer (Modley, 2005; Dimitrakakis et al, 2000). HPV DNA is rarely integrated in the cervical intraepithelial neoplasia but occur in a majority of invasive cancers. However, in absent of regression, the lesion may persist and may progress to cancer (Doorbar, 2005).

HPV preferentially infect the transitional zone of the cervix, mainly the basal cells and can remain dormant (Kurman, et al 1992). The infectious virus is released and manifested by a mild degree of proliferation of the basal cells and koilocytosis. At this stage, viral DNA and capsid protein can be detected by immunochemistry and viral particles can be detected by electron microscope.

Testing of HPV relies on the detection of viral DNA (Denny and Wright, 2005). The most widely used HPV testing methods include the FDA-approved Hybrid Capture® 2 (HC II) methods and polymerase chain reaction (PCR) based method (Brink et al, 2005; Desai and Cubie, 2005). Other tests include DNA in-situ hybridization of the cytological slides or by the detection of its transcripts.

4.2. Smoking

Since early 1970s, smoking has been associated with cervical neoplasia but regarded as a confounding variable secondary to sexual habits. A study has proved that smoking is an independent risk factor (Nunez et al, 2002). Tobacco carcinogens NNk (4-methylnitrosamino-1-3-pyridyl-1-butanone) and benzo-(a) pyrenes are present in high concentration in the cervix of females who smoke (Rieck and Fiander, 2006). Number of cigarettes per day and number of years as a smoker have been identified as risk factors for preinvasive and invasive cervical neoplasm (Nunez et al, 2002).

4.3. Alcohol consumption

A study in a Swedish population-based cohort study of patients with alcoholism noted that there was an increased risk of cervical intraepithelial lesion (CIN) and invasive cervical neoplasm. They suggested that it was related to lifestyle factors, however, the exact mechanism is unknown (Rieck and Fiander, 2006).

4.4. Oral contraceptive pill

Usage of oral contraception (OCP) could lead to cervical cancer. The OCP duration-dependent studies by the International Agency for Research on Cancer (IARC) reported that more usage of more than 5 years of OCP may increase the risk of developing cervical cancer compared to less than 5 years usage of OCP (Rieck and Fiander, 2006). Todd and Shafi (2004), also reported that prolonged use of OCP has been linked with up to a four-fold increase in the risk for cervical cancer in HPV-DNA positive women.

4.5. Parity

The risk of cervical cancer is higher in parous women and increases with the number of births (Rieck and Fiander, 2006). The IARC studies demonstrated that seven or more full term pregnancies in women who are HPV positive has higher risk of cervical cancer as compared with women who are HPV positive women but nulliparous (Bosch and Quinn, 2002). Women who have children with two or more partners are also said to have higher risk (Rieck and Fiander, 2006).

5. Genetics and Cervical Cancer

It is well documented that high-risk subtype HPV infection are the causative agent for developing cervical cancer. It is also associated with many environmental susceptibility factors which have been mentioned before. However, many researchers feel that these factors alone are not enough. Other susceptibility factors that have high possibility for development of cervical cancer are genetic factors. This includes inheritance of polymorphisms in chemical metabolizing genes and DNA repair genes (Au, 2004). Au et al (2003) did a study on the genetic analysis from the United States population. They found out that GSTMI null allele was significantly associated with cervical cancer.

Human Leucocyte Antigen (HLA) molecules are responsible for antigen presentation. As in case of cervical cancer, HPV is the main antigen associated with HLA. Variation at HLA genes might be important in determining the reaction to HPV. They are evidence of existence of predisposing and protective HLA alleles (Magnusson and Gyllensten, 2000). Other authors had demonstrated that HLA play a role in cervical cancer. They found out

that HLA class II DRB1*13/DBQ1*0603 has a protective effects against cervical cancer (Hildesheim and Wang, 2002).

Microsatellite instability is well documented in colorectal cancer; however its role in cervical cancer is still debatable. A few studies were done in cervical cancer cells and the results are variable, as low as 5.3% to 35%. Nashimura et al (2000) reported 35% of microsatellite instability incidence, suggesting that microsatellite instability is a late event in the pathogenesis of cervical cancer.

There is limited data showing that cervical cancer has the tendency to occur in the family. Horn et al (2002) reported that 21.6% of his patients, 159 out of 737 have malignant disease in first degree relatives. Only 6.9% of these patients have cervical cancer in first degree relatives. The author also pointed out that HPV infection and environmental factors still play an important role in the development of cervical cancer.

6. The Bethesda System

Previously, the cervical cytology has no standardized system in classifying the cervical cells observed on the smears. Papanicolaou classification and the dysplasia nomenclature were used in most laboratories. Unfortunately, these classifications are confusing and had poor interobserver reproducibility in practice (Solomon and Nayar, 2004). There were also problems for clinicians to interpret the pap smear results, affecting patient management.

In December 1988, a small group of individuals consisting of cytology experts, pathologist and clinicians participated a meeting in Bethesda, Maryland. The main objective is to design a system for reporting pap smears. The result of this first meeting was The 1988 Bethesda System (TBS).

This system was developed mainly for cervical cytology specimens. The principles of TBS are to relay relevant information from the laboratory to the patient's health-care provider; standardized among cytotechnologist and cytopathologist, and flexibly adapted in wide laboratory settings; and it must reflect the most current concept of cervical neoplasia (Solomon and Nayar, 2004). The most recent Bethesda Workshop was held in 2001 and the new TBS 2001 system was introduced. The component in TBS 2001 is summarized in Table 1.

Table 1: The 2001 Bethesda System classification

The 2001 Bethesda System Classifications
Specimen type
Specimen adequacy
General categorization (optional)
Interpretation/Result
Negative for intraepithelial lesion or malignancy
Organism
Other non-neoplastic findings
Other
Epithelial cell abnormalities
Squamous cell
Atypical squamous cells
- of undetermined significant (ASCUS)
- cannot exclude HSIL (ASC-H)
Low-grade squamous intraepithelial lesion (LSIL)
High-grade squamous intraepithelial lesion (HSIL)
Squamous cell carcinoma
Glandular cell
Atypical
- endocervical cells
- endometrial cells
- glandular cells not otherwise specified
Atypical
- endocervical cells, favour neoplastic
- endometrial cells, favour neoplastic
Endocervical adenocarcinoma in-situ
Adenocarcinoma
- endocervical
- endometrial
- extrauterine
- not otherwise specified (NOS)
Other malignant neoplasm
Ancillary testing
Automated review
Educational notes and suggestions (optional)

7. Tumour and Precursor Lesions of the Cervix

7.1. Precursor lesion

The precursor lesions of the cervix are classified into squamous and glandular component (Tiltman, 2005). Squamous intraepithelial lesion is morphologically and biologically

diverse group of abnormalities and can progress into invasive cancers, which is associated with HPV infection (Joste et al, 1996).

The term cervical intraepithelial neoplasia (CIN) was described as a spectrum of intraepithelial abnormalities ranging from mild dysplasia to carcinoma in-situ (Todd and Shafi, 2004). The Bethesda System (TBS) has introduced a new term of these spectrums into low-grade squamous intraepithelial lesion (LSIL) and high-grade intraepithelial lesion (HSIL) (Solomon and Nayar, 2004). LSIL include the cellular changes due to 'HPV cytopathic effect' or koilocytosis and mild dysplasia or CIN 1 (Solomon, 2004). High-grade lesions include moderate dysplasia up to carcinoma in-situ or CIN 2 and CIN 3. Kurman (2001) has nicely illustrated the WHO and TBS grading in Table 2.

Table 2: Terminologies for cervical cancer Precursor lesions

WHO/ISGYP classification	**The Bethesda System terminology**
Mild dysplasia (CIN 1)	Low-grade squamous intraepithelial lesion (LSIL)
Moderate dysplasia (CIN 2) Severe dysplasia (CIN 3)	High-grade squamous intraepithelial lesion (HSIL)

*World Health Organization and International Society of Gynecological Pathologies
(Kurman, 2001)

In low-grade lesion, koilocytes is one of the early cytophatic changes due to HPV infection (Figure 5). These HPV-associated abnormalities can regress over period of time. However, persistence of this lesion may progress into cancers (Huang et al, 2005; Heldesheim and Wang, 2002). Histologically, the cells exhibit abnormality of the lower third of the epithelium. Cytologically, the infected cells exhibit increment in size with slight increase in

nuclear-cytoplasmic ratio. The nucleus shows variable degree of hyperchromasia with inconspicuous nucleolus with irregular nuclear membrane.

In HSIL, the histological features play an important role to identify the entity (Figure 6). The immature basal-type cells should occupy more than the lower third of the cervical squamous epithelium. The nuclei are much larger with higher nuclear cytoplasmic ratio. The cytoplasm is scanty. The nuclei show hyperchromatic to coarse granular chromatin with prominent nucleoli (Figure 7). Koilocytes might be encountered. In CIN classification, HSIL is divided into CIN 2and CIN 3 based on the epithelial thickness involvement.

Few studies had shown that high-risk HPV subtype infection can progress into invasive cancer. Park et al (1998) reported that the lesion containing CIN 1 and CIN 2 most likely represent morphologic progression in a single infection. However, lesions having CIN 1 and CIN 3 may be due to both lesion progression and two coincident infections, probably a different type of HPV. Not all, CIN progress into cancers. Only 1% of CIN 1 and 12% of CIN 3 progressed into invasive lesion (Tiltman, 2005).

Previously, the precursor lesion of cervical Adenocarcinoma was referred as cervical glandular intraepithelial neoplasia (CGIN) (Kurman, 2001; Burton et al, 1999). It includes Adenocarcinoma in-situ (AIS). AIS is now regarded as a precursor lesion to Adenocarcinoma and is recognized in TBS 2001 (Tiltman, 2005; Solomon and Nayar, 2004). Terms such as 'endocervical glandular dysplasia' or 'low grade glandular intraepithelial lesion' are not included in TBS 2001 (Solomon and Nayar, 2004).

AIS of the cervix was first described by Helper in 1952. It is a rare premaglinant lesion occurring 1 in 25,000 pap smears (West et al, 2002). This lesion is usually confined to one gland with no stromal invasion.

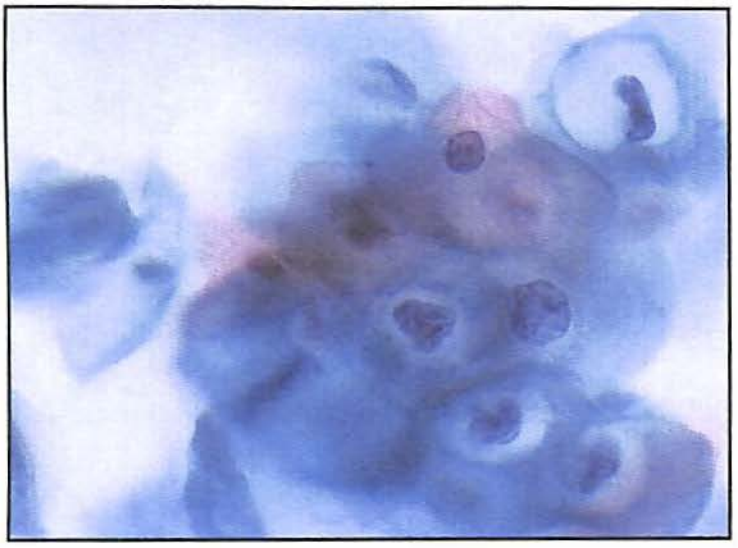

Figure 5: Cytological features of Koilocytes in pap smear

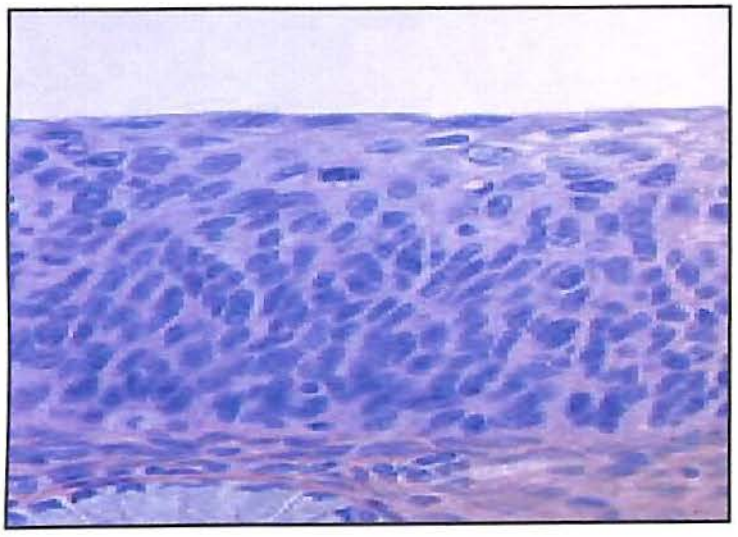

Figure 6: Histological features of HSIL

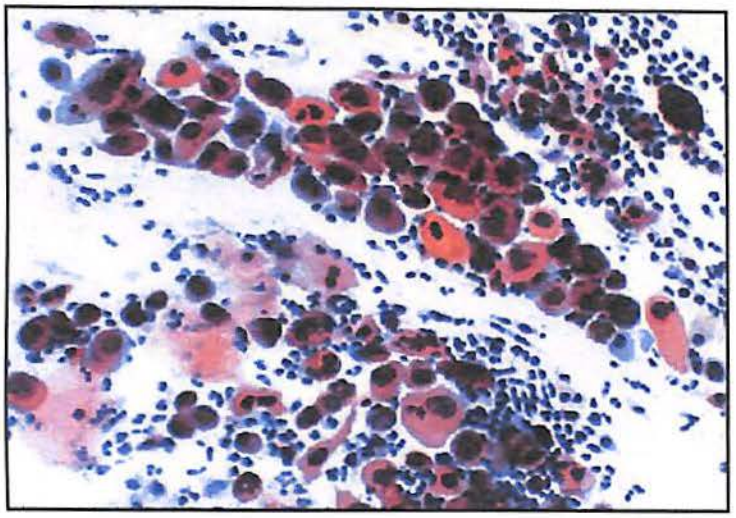

Figure 7: Cytological features of HSIL in pap smear

7.2. Histologic Type of Epithelial Tumours of the Uterine Cervix

The WHO has categorized epithelial invasive carcinoma of the cervix into different categories (Table 3).

Table 3: WHO histological classification of epithelial tumours of uterine cervix

Epithelial Tumours
Squamous tumours and precursors
Squamous cell carcinoma, not otherwise specified
Keratinizing
Non-keratinizing
Basaloid
Verrucous
Warty
Papillary
Lymphoepithelioma-like
Squamotransitional
Early invasive (microinvasive Squamous cell carcinoma)
Squamous intraepithelial neoplasia
Cervical intraepithelial neoplasia (CIN 3)
Squamous cell carcinoma in situ
Benign Squamous cell lesions
Condyloma acuminatum
Squamous papilloma
Fibroepithelial polyp
Glandular tumours and precursors
Adenocarcinoma
Mucinous adenocarcinoma
Endocervical
Intestinal
Signet-ring cell
Minimal deviation
Villoglandular
Endometrioid adenocarcinoma
Clear cell carcinoma
Serous adenocarcinoma
Mesonephric adenocarcinoma
Early invasive adenocarcinoma
Adenocarcinoma in situ
Glandular dysplasia
Benign glandular lesions
Müllerian papilloma
Endocervical polyp
Other epithelial tumours
Adenosquamous carcinoma
Glassy cell carcinoma variant
Adenoid cystic carcinoma
Adenoid basal carcinoma
Neuroendocrine tumours
Carcinoid
Atypical carcinoid
Small cell carcinoma
Large cell neuroendocrine carcinoma
Undifferentiated carcinoma

7.2.1 Squamous cell carcinoma (SCC)

a. Microinvasive Squamous cell carcinoma

Microinvasive SCC is considered a preclinical stage in the progressive spectrum of SIL or CIN and frank clinical invasive carcinoma of the cervix (Kurman, 2001). The tumour is defined based on the depth of invasion, horizontal tumour spread, tumour volume, lymph-vascular involvement and invasive growth pattern (Raspagliesi et al, 2003). International Federation of Gynecology and Obstetrics (FIGO) divides it into stage Ia1 or invasion of stroma not greater than 3.0mm in depth and no wider than 7mm, and stage Ia2 or 3-5mm invasion in depth and no wider than 7.0mm (Raspagliesi et al, 2003). Micrscopically, the lesion shows penetration of tongues of malignant cells through the basement membrane of the squamous epithelium. The cells exhibit abundant eosinophilic cytoplasm and prominent nucleoli. Foci of keratinizations and chronic inflammatory cells infiltrate are present. Five year survival rate for FIGO stage Ia1 is 94.6% (Kohlberger et al, 2002).

b. Invasive Squamous Cell Carcinoma

Microscopically, the tumour cells are arranged in tongues and cords invading the underlying desmoplastic stroma. The cells in the center of the tumour nests may become necrotic. The tumour can be classified into large cell keratinizing, large cell non keratinizing and small cell non keratinizing. Abundant keratin pearls are the characteristic of the former. Currently, WHO classifies this tumour into keratinizing or non keratinizing

c. Verrucous Carcinoma

It is a rare variant of SCC of the cervix (Kurman, 2001; Yorganci et al, 2003). Besides cervix it has also been reported in the vulva, vagina, endometrium, bladder and aerodigestive tracts (Kurman, 2001; Yorganci et al, 2003). It is a well differentiated, slow growing and verrucous appearing lesion. It also show endophytic growth pattern with mild atypia. In certain circumstances, the tumour might show a benign-like appearance (Kashimura et al, 1984).

d. Warty (condylomatous) Carcinoma

This is another rare variant of SCC of the cervix. Kurman (2001) claimed that the tumour showing condylomatous changes is identical to the warty carcinoma of the vulva. It also contains cells with vacuolated cytoplasm feature similar to koilocytes (Tiltman, 2004). It is not a variant of verrucous carcinoma. It appears to be less aggressive than typical SCC.

e. Papillary (Transitional) Squamous Cell Carcinoma

Papillary SCC of the cervix is a rare variant of SCC. It has been reported that HPV 16 has a strong association with this tumour (Ollayos et al, 1996). Other high-risk HPV involved include HPV 18, 31, 33 and 35 (Mirhashemi et al, 2003). The tumour shows poorly organized squamous epithelium with cytologic atypia. Numerous papillary structures with fibrovascular core are present. Mitoses are numerous.

f. Lymphoepithelioma-like Carcinoma

The tumour rarely occurs in the cervix. It only represents 0.7% and 5.5% of all primary cervical malignancy in the western countries and Asia respectively (Bais et al, 2005). Epstein-Barr virus (EBV) is the common etiological factor in the nasopharynx, but is not identified in the cervix (Noel et al, 2001). HPV 16 and 18 are isolated by PCR from this tumour. Therefore, there is evidence that HPV plays a role in the pathogenesis of this tumour. Histologically, the tumour cells are poorly differentiated, arranged in groups and nests having abundant cytoplasm and have ill-defined cell borders. The cells are surrounded by marked chronic inflammatory cells.

7.2.2. Adenocarcinoma

a. Mucinous Adenocarcinoma

It is the commonest type of invasive adenocarcinoma of the cervix (Kurman, 2001; Tiltman, 2005). Mucinous material can be identified in the cytoplasm. It has three histological variants. The endocervical type shows features of endocervical epithelium and arranged in a spectrum from diffuse to well differentiated acini and tubules. The tubules or acini that are lined by goblet cells similar to that of intestine are classified into intestinal variant. The signet ring type has a signet ring cells with peripheral nuclei. It is difficult however, to differentiate from mucinous tumour of the ovary. Even mucin stain and immunohistochemical studies are difficult to distinguish the type of the mucinous neoplasm (Matsecane et al, 1991).

b. Endometrioid Adenocarcinoma

The tumour accounts up to 30% of endocervical carcinoma (Kurman, 2001). The morphology is similar to endometrial endometrioid adenocarcinoma, having mixed papillary and nodular pattern, and focal squamous metaplasia (Burton et al, 1999). Mucin is absent in the tumour.

c. Well Differentiated Villoglandular Adenocarcinoma

This tumour occurs commonly in younger age group, with mean of 33 year old and has a good prognosis (Burton et al, 1999). Oral contraceptives have been suggested as a causative factor for this tumour (Jones et al, 1993). There is also association with HPV infection especially HPV 18 (Yamazawa et al, 2000). Microscopically, the tumour is composed of papillae lined by endocervical, endometrioid or intestinal epithelium. The tumour has mild cytologic atypia. It has a favourable diagnosis (Stanley-Christian et al, 1997)

d. Clear Cell Adenocarcinoma

It is a rare variant encompassing 4% of Adenocarcinoma of the cervix (Kurman, 2001). History of exposure to diethylstilbestrol (DES) has been reported (Horwitz et al, 1988). HPV 31 has been suggested as a cofactor in the development of this tumour (Waggener et al, 1994). Other causative factors include, genetic factors, microsatellite instability, Bcl-2 over expression, and p53 gene mutations (Reich et al, 2000). In DES exposed women, the tumour occurs in the ectocervix and endocervix. There are three microscopic patterns which are solid, tubulocystic and papillary. Due to its rarity, the prognosis is inconsistent (Reich et al, 2000).