DIAGNOSTIC ACCURACY OF CYTOLOGY AND COLPOSCOPY IN CERVICAL SQUAMOUS INTRAEPITHELIAL LESION: A FOUR-YEAR EXPERIENCE IN HOSPITAL UNIVERSITY SAINS MALAYSIA.

A cross sectional study conducted in the Obstetrics and Gynaecology Department, Hospital University Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

By

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Dissertation Submitted in **Partial** Fulfilment Of The Requirements For The Degree Of Master Of Medicine (Obstetrics and Gynaecology)

UNIVERSITI SAINS MALAYSIA

November 2001

ACKNOWLEDGEMENT

Praise be to Allah (SWT) and His prophet Mohamed (PBUH) for His guidance in life and for answering all our prayers.

Acknowledgements to all the contributors to my dissertation are beyond names mentioning. I, however, am indebted to Dr. Siti Zaleha, who is now in Kuala Lumpur, who set the ball rolling. I am especially grateful to my supervisor, Dr. Nik Mohd. Zaki bin Nik Mahmood, Senior Lecturer and Specialist in O&G, for his sleepless nights, reading and correcting this paper. His incessant encouragement and criticisms were essential to make this thesis a success. I am thankful to Dr. Syed Hatim and Dr. Wan Zahiruddin for their input on statistics and making intimidating statistics friendly. To Dr. Awang Nila bin Ismail, the Head of Department of O&G, and Assoc. Prof. (Dr.) Mohd. Shukri bin Othman, for their guidance and input.

To my colleagues, I pray for our success fervently. To all patients who underwent the colposcopic examination, I thank you sincerely and I dedicate this paper to thee.

To my wife, Sharifah Suzenna Noraimi bt. Dato' Syed Hassan Nazari Al- Jamalullail, I thank you for 'putting up' with me through the programme. Finally, not forgetting my parents, Dato' Mohd. Tarmizi bin Mohd. Tahir and Datin Rusna bt. Kamaruddin; and my parents in-law, Dato' Syed Hassan Nazari Al-Jamalullail and Datin Famelia Zalfika bt. Md. Noor, thank you for envisaging it and supporting your son through his years of studying.

Mohamed Hatta bin Mohamed Tarmizi.

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ABBREVIATIONS

AGUS - Atypical Glandular of Undetermined Significance.

AIS - Adenocarcinoma in situ.

ASCUS - Atypical Squamous Cells of Undetermined Significance.

CIN - Cervical intraepithelial neoplasia

HPV – Human papilloma Virus.

HRT - hormone replacement therapy.

HSIL - High Grade Squamous Intraepithelial Lesion.

IUCD – intrauterine contraceptive device.

LSIL - Grade Squamous Intraepithelial Lesion.

N/C ratio - nuclei cytoplasmic ratio.

Pap smear - Papanicoloau smear.

SCJ - Squamous columnar junction.

ABSTRAK

OBJEKTIF: Kajian dijalankan untuk menilai ketepatan diagnostik sitologi dan kolposkopi pada wanita yang mempunyai lesion intraepithelium di pangkal rahim dengan mengunakan histologi sebagai 'standad emas'. Kami juga mengira sensitiviti, spesifisiti, nilai-nilai ramalan positif dan negatif sitology dan kolposkopi berbanding dengan histologi dalam memastikan keujudan lesion grad tinggi. Tujuan kedua adalah untuk mengenal pasti faktor-faktor berisiko yang mengaitkan wanita yang dikaji dengan Pap smear yang bergrad tinggi.

METODOLOGI: Satu kajian garis lintang melibatkan seramai seratus dua belas wanita yang mempunyai sitologi pangkal rahim yang tidak normal dikaji selama empat tahun dari Jun 1997 hingga Mei 2001. Mereka semuanya telah menjalani ujian kolposkopi di Hospital Universiti Sains Malaysia, Kelantan. Satu analisa korelasi diantara sitologi dan kolposkopi dengan gambaran kolposkopi dan histologi telah di buat. Kolposkopi telah dilakukan oleh para ginaekologi di jabatan kami.

KEPUTUSAN: Sensitiviti sitologi dalam mengesan lesion bergrad tinggi adalah 60% dan spesifisiti adalah 85.4%. Nilai ramalan positif adalah 76.9% manakala nilai ramalan negatif adalah 72.2%. Sensitiviti untuk kolposkopi dalam mengesan lesion bergrad tiinggi adalah 72.0%, dan spesifisitinya adalah 82.2%. Nilai ramalan positifnya adalah 76.9% manakala nilai ramalan

negatifnya adalah 78.4%. Apabila dicampurkan keduanya sensitiviti meningkat kepada 74.2% dan spesifisitinya adalah 82%. Bagi pesakit yang menpunyai sejarah lebih dari satu teman seks, risiko untuk mendapat lesion bergrad tinggi adalah 4.3 (95%CI [1.7 to 11.1], p= 0.013) dan bagi pesakit yang suami mereka mempunyai sejarah lebih dari satu teman seks, risiko untuk mendapat lesion bergrad tinggi adalah 2.6 (95%CI [1.1 to 6.1], p=0.02). Setelah mengira dan membuang konfounder, pesakit yang mempunyai sejarah lebih dari satu teman seks, risiko untuk mendapat lesion grad tinggi adalah 1.4 (95%CI [0.4 to 8.6], p= 0.003) manakala pesakit yang mempunyai suami bersejarah lebih dari satu teman seks, risiko untuk mendapat lesion grad tinggi adalah 1.2 (95%CI [0.4-2.0], p= 0.02).

Dalam kajian kami, insiden lesions bergrad tinggi bagi CIN 1 yang berturutan adalah tinggi (41.2%) manakala untuk CIN2 (68.4%) dan adalah CIN3 (78.9%).

KESIMPULAN: Kolposkopi adalah alat yang lebih baik dalam mengesan lesion grad tinggi dibandingkan dengan sitologi sahaja. Bila dicantumkan keduanya, sensitivity meningkat tetapi ada juga lesion yang tidak dapat dikesan (25.8%). Kajian ini juga menyakini nilai diagnostik kolposkopi. Oleh itu, wanita yang mempunyai sitologi yang tidak normal sederhana atau kadar teruk mesti dirujuk untuk kolposkopi. Bagi mereka yang mempunyai sitologi radang yang berturutan atau dysplasia bergrad rendah selama enam bulan berturut-turut juga patut dirujuk untuk kolposkopi.

ABSTRACT

OBJECTIVE: To evaluate the diagnostic accuracy of cytology and colposcopy in women with cervical intraepithelial lesion using histology as the 'gold standard'. We aim to determine the sensitivity, specificity, positive and negative predictive values of cytology and/or colposcopy against histology in determining high-grade lesions. Secondly, to identify the possible risk factors for abnormal cytological smear of high-grade lesions in the population studied.

STUDY DESIGN: A four-year cross sectional study from June 1997 to May 2001 was conducted in 112 women with abnormal cervical cytology who had undergone colposcopic examination in Hospital University Sains Malaysia. The correlation between cytology and colposcopic impression to colposcopically directed biopsy were analysed. The correlation was accepted accurate when both reports were either the same as histopathological diagnosis.

RESULTS: The sensitivity of cytology for detecting high-grade lesions was 60% and the specificity was 85.4% while the positive predictive value 76.9% and negative predictive value 72.2%. The sensitivity for colposcopy to detect high-grade was 72.0%, and the specificity 82.2%. The positive predictive value was 76.9% while negative predictive value was 78.4%. When in combination, the sensitivity increased to 74.2% but the specificity was 82%. For patients with history of more than one sexual partner, the risk of

developing high grade lesion was 4.3 (95%CI [1.7 to 11.1], p= 0.013) and those with husbands with more than one sexual partners, there was an increase risk of developing high grade lesion of 2.6 (95%CI [1.1 to 6.1], p=0.02). After eliminating the confounders, in patients with history of more than one sexual partner, the risk of developing high-grade lesion was 1.4 (95%CI [0.4 to 8.6], p= 0.003) and multiple sexual partners of the spouse was 1.2 (95%CI [0.4-2.0], p= 0.02).

In our study, the incidence of high-grade lesions (confirmed by histopathology) in persistent CIN1 smears was relatively high (41.2%) while for CIN 2 (68.4%) and CIN 3 (78.9%) respectively.

CONCLUSION: Colposcopy is a better tool in diagnosing high-grade lesion than cytology. In combination however the sensitivity in detecting HGL is higher, but a large number of lesions were missed (25.8%). The study also confirms the diagnostic value of colposcopy in management of abnormal cervical cytology. All women with moderate and severe dyskaryosis should be investigated colposcopically and histologically. Those with persistent inflammatory changes or persistent low-grade dysplasia more than 6 months should also be referred for colposcopic. The incidence of high-grade squamous intraepithelial lesions is higher in patients with multiple sexual partners. It is also high in patients who have spouses with multiple sexual partners.

INTRODUCTION

A) STATE OF KELANTAN

Kelantan's history dates back to between 8000 to 3000 BC. Chinese historical documents chronicle the existence of a government, which maintains the link with China. Kelantan was subsequently referred to as "Ho-lo-tan","Chih-Tu"and "Tan-tan" in these records. In the 18th century, the province came under a powerful warrior, Long Yunus, whose descendants presently make up the Royal house of Kelantan. Kelantan came under the Thai and later the British influence before becoming part of the federation of Malaya in 1957 and later, Malaysia in 1963.

Kelantan is one of thirteen states in tropical Malaysia. This state covers and area of 14,929 km2, consist mostly of lush rainforests and white beaches fringed by crystal clear waters. With a population of 1,413,354, most of them are Malays (95.3%). Ninety percent of the Kelantanese live in rural areas; many of them are either farmers or fishermen. Yet, these are the people responsible for the rich cultural heritage that abounds in Kelantan. Giant kites, giant tops and giant drums- they all have a special meaning in the lives of the locals. The intricacy of silverware and the artistry of batik

fabrics are important parts of Kelantanese tradition. In fact, everything about this land and its people exudes a warm and laid back ambiance. By 1994, three highways had been built in the name of commerce and tourism to connect various parts of Malaysia to this Land of Culture. Even though thousands of visitors have arrived ever since, the green foliage and pristine beaches are still maintained. Traditional cottage industries have multiplied complimenting the other various tourist centres.

Business in indeed booming and it is the women folk who are actively exploiting this economic opportunity. One has only to stride leisurely in the central wet Market of Kota Baru to appreciate this. There, Kelantanese ladies will be haggling over the prices of fresh seafood and fishes, leafy vegetables and exotic herbs, fruit pickles and salted fishes. The traders, mainly women will be calling out the persistently to the shoppers. The same takes place in the dry market section. Food stall upstairs are never empty. Textiles, ready-made garment, and scarves – all made from the exquisite silk or pure cotton are sold as cheap as RM5 (less than US$2). Some women will be selling silver and copper antiquities; the favourite among tourist is the ever popular and tasteful brooch in the shape of the local kite known as 'Wau Bulan'. Buying a

few of the brooches for loved ones back home always seems to be a brilliant ideas.

The women of this state never the passive partner when it comes to running business. No wonder this Cultural State is also known historically as ' Negeri Cik Siti Wan Kembang', which literally means 'A State Governed by the Queen'.

B) HEALTH CARE IN KELANTAN.

In 1930, the first General Hospital was built to improve health care in Kelantan. By 1956, the Malaysian Government introduced National Rural Heath Development programme. This involves the extensive development of health infrastructure facilities. Rural Health Units were built and organized on a three-tier system referral. The services of these units were mainly concentrated on maternal and child health care. During the midterm review of the second Malaysian Plan (1971-1975), the three-tier system was converted to a two-tier system that was consisted to a Health Centre for the 20,000 populations. There were various maternal health care and gynaecological health care services that were and still are provided free by the Government. These are: -

1) Free Pap's smear test.

2) Colposcopic examinations

3) Family planning

4) Antenatal and postnatal care

From then on, the health services in Kelantan continued to improve. The General Hospital in Kota Baru was the only referral centre until 1984 when Hospital University Sains Malaysia started function. By the 1992, there were a total of eight hospitals, at least one n each district with the exception of two districts. The number of health clinics had increased to forty-eight. On the average, the health clinics. On the average the health clinics to the population ratio was 1:24,000. The increasing number of hospital deliveries and reduction of maternal and prenatal mortalities reflected these developments.

C) UNIVERSITY SAINS MALAYSIA – THE SCHOOL OF MEDICAL SCIENCES IN KELANTAN.

This is the third medical school in Malaysia after University Malaya and University Kebangsaan Malaysia. It is unique in comparison with the other two universities as it was the first to adopt an innovative and community orientated curriculum for its students. Furthermore, it is the first medical school, which was built in a les developed state while the other two were located in the busy capital city of Malaysia, Kuala Lumpur. The School of Medical sciences of University Sains Malaysia actually started in the main campus of the university in Penang. The first batch of medical students was enrolled in 1981. However, these pioneers only came to the medical campus in Kubang Kerian, Kelantan at the beginning of their fourth medical year. Further intakes from the 1990 onwards had the students enrolled straight into the campus in Kubang Kerian. From 1991, the School started its postgraduate programme for the department of Obstetrics and Gynaecology.

D) THE DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY.

This is one of the departments in the School of Medical Sciences. It occupies the first floor of the old hospital building and the first, second and third floor of a part of the new wing. The department staffs consist of 10 lectures, 8 registrars and 20 medical officers/trainee lecturers. There are two obstetrics wards with 84 beds and one gynaecology wards with 36 beds. There is a labour room with an adjoining operating theatre, while the obstetrics and the gynaecology clinics are situated on the ground floor. The schedule for the clinics is as below.

Day	*Morning*	*Afternoon*
Saturday	Booking	Booking
Sunday	Antenatal	Gynaecology
Monday	Combined, Menopause, Outpatient scan clinic	Oncology and molar. **Colposcopy clinic**
Tuesday	Antenatal	Gynaecology
Wednesday	Infertility augmentation	Postnatal clinic.

The doctors are divided equally to four teams to man these clinics as well as the wards and duties during normal working days as well as on call days. This system had been well accepted so far.

E) HOSPITAL UNIVERSITY SAINS MALAYSIA.

This is one of the beautiful teaching hospitals in Malaysia, which caters for the students of the School of Medical Sciences. The same hospital also serves as a health centre to the state as well neighbouring states. Canopies of trees subdue the severity of its green building blocks in every shades of green. Crops of bougainvillea bushes peppered on the grounds of hospital buildings. These hot pink and red fiery red flowers vibrate with energy and beauty all year long. When the fields are smoothly lawn, yellow and white daisy will sprung up everywhere in no time. It is difficult for anyone to ignore such sweet yet wild riot of colours.

The buildings were first constructed in 1976 along the tranquil Kelantan River in a small town of Kubang Kerian, providing adequate medical services for the surrounding population as well as functioning as a referral centre for the state of Kelantan and its neighbouring states. Due to the ever growing population and the hospital's aim to provide the best to all, the hospital has added new blocks to its infrastructure, completely functioning by 1997.

This picturesque hospital with its rustic surroundings beckoned patients, the staff and students, but also the Kubang Kerian community to stroll in its grounds for recreational

purposes. The athletics one will jog, most of the time along the temperamental river, which separates this campus from a dark strip of a dense tropical jungle. The calm emerald green waters will turn to raging muddy brown flows when the grey monsoon storm brings strong and heavy rain. However, during the fine days everyone will be tempted to bask in the gentle rays of the evening sun. Parents will stroll with the small ones along the roads that are flanked by swaying palm leaves of coconut trees. Students are sometimes seen to bring their books out studying under the shades of the many beautiful and glorious green trees. At times, there will be a roaring football match on the main field while the members of the cricket team practice their game on another part of the field. Others, either standing or sitting, in groups or alone, on the grass or benches chose to do nothing but calmly watch all that is going on. In this part of the world, it is so easy to let yourself fall under the spell of this serene and undemanding campus life.

INTRODUCTION

A) History at a Glance

It was at the Third Race Betterment conference in Battle Creek, Michigan that Papanicoloau (1928) first suggested the diagnostic potential for exfoliative cytology of vaginal aspirates. At that time he was using cytology to monitor hormonal changes in the menstrual cycle. Subsequently, working with Herbert Traut in New York, he published a paper describing a diagnostic value of vaginal aspirates in diagnosing cancer of the uterus. The cell changes of dyskaryosis noted in cells from the cervix allowed the identification of malignant conditions and premalignant conditions. It was to become the basis of screening programme introduced to reduce the incidence of and mortality from cervical cancer.

The colposcopy was first developed by Hinselmann in 1925, and colposcopy was used for years in Europe an in the South America mainly as a competitive method to cytology. In the late 1960s and early 1970s several physicians in the United States recognized that the main role of colposcopy is not cervical cancer screening but rather evaluation of patient with abnormal cytology. With the use of colposcopy the

frequency of conization dropped significantly. Colposcopy also changes significantly the management of cervical intraepithelial neoplasia. It was Dr. Louis Burke; one of the pioneers of colposcopy in the United States that stressed that colposcopy cannot be learned only by looking at through the instrument, but the pathologist's bench as well. Every Colposcopic lesson has a pathologic counterpart, and physician of experience in both colposcopy and pathology must do proper clinical interpretation of the significance of these lesions.

B) The background of this study

The proper approach to the patient with mildly abnormal cervical cytologic findings has stirred up some controversies over the years in many literatures. One approach in handling low-grade cytologic abnormalities i.e. inflammation with or without atypia, atypical squamous of undetermined origin (ASCUS), Human Papillomavirus (HPV) changes, or mild dysplasia in the cytological examination is by performing colposcopy and Colposcopic directed biopsy. If a Papanicoloau (Pap) smear shows low-grade dysplasia then the test is to be repeated after 3 to 6 months and if the cytologic pathology persist then a colposcopy and colposcopy directed biopsy is performed.

The British tend to be more conservative than the American authors, typically advocating a repeat smear as follow-up for cytologic atypia or low-grade cytologic abnormalities. Some recommend cytologic follow-up rather than biopsy for patients with dysplasia (dyskaryotic) cytologic findings. Kirby et al 1992 followed 500 women with abnormal smears with mild or moderate dyskaryosis. After a median follow up of 7 years, 60% had smears reverting to normal of inflammation. Thirty-seven percent, of which 19% were CIN 3 or worse, had undergone biopsy, but the authors recommended

conservative management of patients with abnormal smears. Giles et al 1989 followed women with mild dyskaryosis by repeat smears. When colposcopy was performed after a single dyskaryotic smear, 31% had a negative biopsy; if two abnormal smear results preceded biopsy, only 10% were negative. Repeat cytologic examination was associated with a 24% false negative rate, but the authors considered the missed lesions to be of low grade.

Since a patient with negative smears need to be repeated many times (up to three) to consider her of having a truly negative, this not only bear a considerable cost in the repeated investigations and subsequent follow up with multiple returned visits and smears, but also a measurable danger of overlooking and missing a significant lesion. Tabbara et al (1992) found that high-grade abnormalities on cytologic examination were predictive of high-grade lesion histologically, but that of low grade cytologic lesion had poor predictive value for either low-grade histologic findings or low risk human Papillomavirus (HPV) types. Maggi et al (1989) followed patients with CIN 1 with repeat Pap smear and biopsy. The abnormal smear was found to identify a group at increased risk of CIN but could not rely on to determine disease severity. They found that 20% of patients with negative repeat smear had CIN at biopsy, and 18% of those with mildly abnormal

smears had a high-grade lesion. They therefore recommend a colposcopy and biopsy to be one in all patients with a mildly abnormal smear.

Morrison et al (1988) studied patients with atypia without dyskaryosis on smear. Of those with atypia, 25% showed CIN on biopsy. All degrees of atypia were found to have a significant association of CIN, and the degree of cytologic atypia could not be used to exclude Colposcopic evaluation. The routine use of colposcopy was advocated for patients with atypical cells either immediately or after a single repeat smear.

Rieter et al (1986) found that persistent atypia unassociated with inflammation to be a significant risk for development of CIN (79%). Jones et al (1987) investigated patient with atypical but not dysplastic smear with repeat Pap smear, colposcopy and biopsy. 25% had CIN, but repeat Pap smear identified only 17% of patient with CIN, 20% of whom had high-grade lesions. One half of those with CIN 2 or CIN 3 had a negative repeat Pap smear. Davis et al 1987 investigated patient with atypical squamous cells with colposcopy, biopsy and repeat smear. Eighteen percent had CIN on biopsy, 10% of which had negative smears. Bolger and Lewis et al (1988) support colposcopy and biopsy for

dysplastic smears or smears with HPV changes, citing findings in 39% with histologic changes of CIN 2 or worse when biopsy weeks later. Soutter et al 1986a recommended that patient with any degree of dysplasia or persistent atypia, at the time a 3- month repeat smear was performed should be referred for colposcopy. In evaluating the conservative approach to mild dysplasia, Campion et al (1986) found that 26% of patient with CIN 1 progressed to CIN 3 in 19 months.

For the study, we will examine the result of all colposcopy biopsies. The majority of these were performed on basis of a smear showing abnormality. In conducting prospective long term follow up studies of the natural history of cervical abnormalities the strategy of surveillance also varies, because the diagnoses are based on cytology alone, colposcopy and cytology or biopsies.

1. CERVICAL INTRAPEITHELIAL LESION

1.1 CLASSIFICATION

Cervical dysplasia is an intraepithelial neoplasm with a variable potential for progression to invasive cancer. The term encompasses a continuum of morphologic and molecular changes, from earliest identifiable abnormalities arising in the deep layers of the stratified squamous epithelium to the appearance of overt malignant feature that persist through the full thickness of the epithelium. The progression is arbitrary subdivided to three phases termed mild, moderate, and severe intraepithelial neoplasia (dysplasia) or CIN 1, 2 and 3. Historically, the term carcinoma in situ was reserved for the most severe degree of intraepithelial abnormality, but this term is no longer in favour because of the confusion it causes with respect to the diagnosis of cancer. The terminology used to classify squamous cell lesion on the cervix has changed over the years in an attempt to reflect changing views of this nature. (Table1).

Table 1. Different terminologies for squamous cell cervical lesion

Scheme 1	Scheme 2	Bethesda System
Mild Dysplasia	HPV and CIN 1	Low Grade lesion (LSIL)
Moderate Dysplasia	CIN 2	High Grade lesion (HSIL)
Severe dysplasia and Carcinoma in situ	CIN 3	

Scheme 1 represents the original terms in used until Richart et al (1969) suggested **scheme 2**, incorporating the term cervical intraepithelial neoplasm (CIN) to indicate the concept of cervical premalignancy as a continuum of change. Later, milder lesions thought to be due to human Papillomavirus (HPV) infection were identified. The Bethesda terminology attempt simplifications by grouping CIN 1 with HPV as a lesion with low potential for malignant change. The Bethesda terminology initially does not have wide support in the UK but now it is.

Adenocarcinoma-in-situ was recognized more frequently than before. It is now often referred as cervical glandular neoplasia. It has become relatively more important where screening has reduced the numbers of squamous cancers without affecting the incidence of Adenocarcinoma.

1.2 SQUAMOUS INTRAEPITHELIAL LESIONS.

1.2.1. Low Grade Squamous Intraepithelial Lesion (LSIL)

Low-grade squamous intraepithelial lesion (LSIL) category of the Bethesda System encompasses cellular changes due to HPV infection and mild or slight dysplasia (Cervical Intraepithelial Neoplasia- CIN 1).

1.2.2 CIN 1

Classical description of CIN 1 refers to mature superficial or intermediate cells containing enlarged, granular or reticular chromatin. Nucleoli are not visible. The nuclear membrane may be slightly convoluted, but generally appears smooth. Binucleation can be seen. The nuclei/cytoplasmic (N/C) ratio is moderately increased. The cytoplasm often appears normal; sometimes the staining is amphophilic and a perinuclear halos can be observed occasionally (Meisels A 1988, Morin C, 1994).

1.2.3 High Grade Squamous Intraepithelial Lesion (HSIL).

In the Bethesda System, high-grade squamous intraepithelial lesion (HSIL) encompasses the cellular changes

previously known as moderate dysplasia (CIN 2) and severe or marked dysplasia and carcinoma in situ (CIN 3). HSIL is characterized on the smears by the presence of immature cells of the parabasal type, with an increased N/C ratio, containing enlarged, somewhat irregular, hyperchromatic nuclei. The chromatin is either granular or reticular, or displays irregular chromatin bands and chromocentres. Nucleoli are small or absent. The nuclear membrane is often unevenly stained and its outline may be jagged. The cytoplasm is dense and cyanophilic, rarely eosinophilic. These cells are found isolated or more often, forming small groups. The pattern is quite uniform, the degree of anisokaryosis is slight and the background in absence of coexisting inflammation is clean (Meisels A 1988, Morin C 1994).

1.2.4 Atypical Squamous Cells of Undetermined Significance (ASCUS).

It was meant to be used sparingly and expressively excluding reactive inflammatory and reparative changes and including only "puzzling", inconclusive smears for which a distinction could not be made between CIN and the unusual reactive changes (Schiffman et al 1993). Three to ten percent of women screened will present with this type of smears.

1.2.5 Atypical Glandular of Undetermined Significance (AGUS).

AGUS consist mostly an enlargement of the nuclei with some degree of anisokaryosis and presence of nucleoli. The endocervical glandular cells are found mostly in clusters or small groupings, without much overlapping. These changes may be reactive, or in some cases, may be precursors to more advanced lesion like Adenocarcinoma in situ (AIS).

1.3 CLINICAL FEATURE.

The patient with a diagnosis of CIN is predominantly in the reproductive age group. Typically CIN produces no symptoms, although many patient report vaginal spotting or discharge. Cervical dysplasia has no feature visible to the naked eye, with occasional keratinising lesion, which has the appearance of a white plaque. Consequently nearly all cases of cervical dysplasia are detected by screening cervical cytology.

1.4 AETIOLOGY

There is a great body of experimental and epidemiological evidence that CIN and cervical carcinoma are caused by various subtypes of HPV in concert with a co-carcinogen such as might be derived from cigarette smoking. HPV, which is thought to be transmitted primarily by sexual contact, is highly infectious, producing initially an intraepithelial or acuminate condyloma of cervix (vagina or vulva). The histologic and cytologic changes found in these lesion are similar to, if not the same as, those of mild dysplasia. HPV DNA can be detected in virtually all cases of CIN 1,2 and 3. Predisposing factors are essentially the same as those for invasive carcinoma of the cervix: early age of sexual intercourse, multiple sexual partners, cigarette smoking, genital HPV or herpes simplex virus infection, oral contraceptive use, and intercourse with promiscuous male.

1.5 PATHOLOGY OF CERVICAL INTRAEPITHELIAL LESION PREMALIGNANCY.

The diagnosis of cervical intraepithelial neoplasia is based upon the architectural and cytological appearances of cervical epithelium. Architectural features include

differentiation, stratification and maturation, term, which are closely related but not synonymous. The proportion of the thickness of the epithelium showing differentiation is a useful feature to be taken into account when deciding the severity of CIN. It is not the most important criterion, despite the fact that it is one of the easiest to assess. In CIN 1, at least the upper half of the epithelium usually shows good differentiations and stratification, where as in CIN 3, differentiation may be very slight or even be absent.

Nuclei abnormalities are the most important combination of feature to be taken into account when assessing CIN. The nuclei are examine using similar criteria to those employs by the cytologist in assessing a cervical smear: nuclear cytoplasmic ratio, hyperchromasia, nuclear pleomorphism and variation in size of nuclei. Both the overall numbers of mitotic figures and their height in the epithelium are assessed. The more superficially the mitotic figures are found, the more severe the CIN is likely to be. Abnormal configurations (3 groups metaphases and multipolar mitotic figures) are more likely to be found in severe form of CIN.

CIN may affect the gland crypts as well as the surface epithelium. Anderson and Hartly (1980) showed that the mean depth of crypt involvement in women with CIN 3 was 1.23mm

and that the mean plus three standard deviation (taking in 99.7% of the population) was 3.8mm. These figures suggested that treatment in a depth of 5 mm into the stroma would be sufficient to eradicate most CIN; however, practical experience has shown that treatment to 10 mm gives much better results without increasing the morbidity (Soutter et al 1986a).

1.6 PROGRESSION TO CANCER

It is now believe that CIN 1 lesions frequently regress spontaneously, and perhaps revert again to dysplasia. CIN 3 are more likely to progress than CIN 1, progression to cancer can occur more than 15% of the time, where else CIN 1 only progresses only 1% of the time. It does appear that regression and persistence of CIN 1 and CIN 2 are similar. Certainly, not all women with abnormal cervical cells develop cancer of the cervix or even progression of CIN. Therefore patient with any degree of dysplasia should be evaluated further.

2 TISSUE BASIS OF COLPOSCOPY

2.2 A brief history

The cervix and vagina first became accessible for the direct inspections as a result of the first invention of the vaginal speculum by Recamier in 1818. With the access to the cervix, investigators began to elucidate the natural history of cervical cancer. As mention earlier Hinselmann in 1925, made a significant contribution to this study by inventing the colposcope, and instrument based on his concept that the primary focus of cervical cancer was a minute ulceration that could not be appreciated by gross inspection with the naked eye.

Colposcopy, which may defined as the examination of epithelial surface with low- power microscope and a strong light, initially was not widely accepted in English speaking countries and, indeed, was derided. There were several factors for this delay. All of the early report was in German, which introduced a language barrier to the understanding of colposcopy. Terminology was based on visual impression that could not be cleared related to the histologic or pathophysiology of tumour development and growth. A third reason was that colposcopy and cytology, the latter had been developed about the same time by Papanicolaou, were initially

construes as competitive rather than as a complimentary techniques for cervical cancer detection. In the 1960s however, colposcopy became more popular in English speaking countries, based on an improved understanding of the morphologic basis for the colposcopic images and the recognition of the complimentary roles of cytology and colposcopy in the detection of neoplasia.

In modern practice of gynaecology, colposcopy has became an integral part of gynaecological examination. Colposcopy, however, must be performed in concert with cytology and tissue sampling. Because various combination of epithelial and stromal abnormalities produce identifiable colposcopic images, both normal and abnormal epithelia assume colposcopic appearances almost as characteristics as those seen in histologic examination.

2.3 Basic colposcopic images

The five images of leukoplakia, aceto-white epithelium, punctation, mosaic pattern and atypical blood vessels are the descriptive vocabulary of the colposcopic method. Any process that increase keratin production, increase cellular division, increase vascular changes, and produces new blood vessel can cause any of this abnormal images. Thus,

metaplasia, infection, inflammation, regeneration, repairs and most important, neoplasia can produce the changes.

2.4 The colposcope

All colposcope basically consist of optics, eyepiece and filters, light source, stand and ancillary equipment.

2.5 Colposcopy of the cervix

At puberty, during pregnancy or when the combined oral contraceptive pill is taken, the cervix enlarges. As it does so there is a tendency to eversion and to the exposure of columnar epithelium on the ectocervix. The thin columnar epithelium appears red and the results are what are so often erroneously referred to as the cervical erosion. The squamous metaplasia is when the columnar epithelium on the ectocervix is gradually replaced by a process of squamous metaplasia, spreading from the SCJ towards the cervical canal. The normal end result of this replacement is the ectopic columnar epithelium with mature squamous epithelium. If the squamous epithelium now covers the entrance to the cervical crypt and the columnar cells continue to secrete mucus in the crypt, a Nabothian follicle results. When the cervix shrinks in the months and years following pregnancy and after the menopause, it gradually inverts, drawing the new SCJ up the

endocervical canal. The transformation zone is the region of the cervix in which the process of metaplasia occurs. In some women, the transformation zone may extend to the vaginal walls. Squamous cervical neoplasia results from a disruption of the normal metaplastic process. Thus, cervical intraepithelial neoplasia develops as a confluent lesion confined to an area of the cervix continuous with the SCJ.

OBJECTIVES OF THE STUDY

The objectives of this study are:

Primary Objective

1. To evaluate the accuracy of colposcopy in diagnosis of cervical intraepithelial lesions.

2. To determine the concordance between cervical cytology (Pap) smear and histopathological biopsy taken from a colposcopic directed biopsy.

3. To determine the concordance between colposcopic impressions and histopathological biopsy taken from a colposcopic directed biopsy.

4. To determine the sensitivity, specificity and the predictive values of cervical cytology and colposcopic impressions to colposcopically directed biopsy (histology) in both high-grade lesions and non high-grade lesions.

Secondary Objective

To identify the possible risk factors for abnormal cytological (Pap) smear of high-grade lesions in the population studied.

RESEARCH METHODOLOGY AND MATERIALS

1. The population

This is a comparative cross-sectional study, reviewing patients who were referred to the colposcopic clinic in University Sains Malaysia, Kelantan with abnormal smears and whom all underwent colposcopy and colposcopically directed biopsies. It was conducted in a period of 4 years from 1st June 1997 until the 31st May 2001. History of possible related risk factors was obtained from the case notes and where there is deficient information, the women were later interviewed in the clinic to obtain a complete and comprehensive medical and gynaecological history. All women volunteered to give their history and consented for colposcopy to be done on them. One person conducted the interview and patients' confidentiality was respected and kept upon questioning.

2. The Cytological (Pap) smear.

Patients were referred from a variety of sources e.g. Gynaecological Clinic of Hospital University Sains, Malaysia, the Staff clinic of the University Hospital, the outpatient clinic,

the district hospitals in Kelantan, the Kelantan Family Planning Clinic in Kota Bharu. The cytology technologist in the pathology laboratory of Hospital University Sains Malaysia read most of the cytological smears. The remaining smears were read by private cytological laboratory in Kota Bharu or were evaluated by the Kelantan Family Planning Association Pathological Clinic in Kota Bharu. A gynaecological history was taken with a gynaecological examination.

The cytology was classified according to the Bethesda System:

-Inflammation

- **Atypical**: ASCUS and AGUS.

-Low-grade squamous intraepithelial lesions (LSIL): HPV or CIN 1.

- **High-grade squamous intraepithelial lesions (HSIL)**: CIN 2 and CIN 3.

3. The Colposcopic procedure.

Our consultants/specialists in the colposcopic clinic performed the colposcopic procedure. A preliminary examination was done which include inspection of both cervix and upper vagina. This is to achieve 3 major objectives, which are to see for leukoplakia, invasion (as determine d by the vascular changes and surface changes) and condylomata acuminata.

3.1 The Application of Acetic Acid

The cervix is then soaked liberally and gently with Acetic Acid 5% using a small cotton bud to baste the cervix, endocervical canal and vaginal fornices. At the same time the cervix and vaginal fornices were inspected under low power in order to have the widest field of view possible.

3.2 Normal Findings

Normal native squamous and columnar epithelium were identified and normal squamous columnar junction was also identified. Any endocervical polyp was documented and atrophic changes, when visualised were also noted. If the squamous columnar junctions was not visualised then the colposcopy was deemed unsatisfactory.

3.3 Determining the nature of the lesion.

After identifying the limit of the lesion the colposcopist determined the nature of the lesion. The colposcopic findings were very subjective to expect a high level of accuracy, as it was not based on any scoring system in order for it to be more objective. More than one consultant in the colposcopic clinic also did the colposcopic examinations. A huge margin of inter observer variations cannot be avoided due to this practice. There the colposcopist will try to decide through clinical experiences and knowledge whether or not the lesion is CIN and whether there are any signs of invasion. This is base on the colour (aceto-whiteness), the margins, the surface and the vascular markings.

The degree of whiteness produced by the acetic acid was identified, determined largely by the density of the nuclei of the epithelium. Biopsies were taken from areas that are whitish and denser against the normal background or from any irregular areas and margins, abnormal vascular markings (mosaic or punctation).

For the purpose of this study, we graded the colposcopic findings to:

- **High Grade Lesions (HGL):** CIN 2 and/or CIN 3 or microinvasion.
- **Non-High Grade Lesions (Non HGL):** Cellular changes associated with human papilloma virus (HPV), CIN1 or inflammation.
- **Normal.**

3.4 Punch Biopsy.

A colposcopic impression must always be back up by histological confirmation, thus it became essential to obtain one or more colposcopically directed punch biopsy from the most abnormal looking areas. Most of our colposcopist in this study used an Eppendorfer tip forceps with a simple box joint. Where such forceps is not available the Pistofides' forceps with tooth was used which also has the advantage to prevent slipping of the cervical tissue. All biopsies was done deep enough to contained some stroma and this is later confirmed by the histopathological examination. Patients were not given anaesthesia during this procedure. Bleeding points was