A NEW APPROACH IN THE SCREENING FOR CERVICAL CANCER USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

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by

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ

(النور: 35)

Light upon light! God doth guide whom He will to His light: God doth set forth parables for men: and God doth know all things [Al Nur, 35]

DEDICATION

To my beloved parents, may ALLAH Al-mighty save them

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Absorbance
ANNs	Artificial neural networks
ANOVA	Analysis of Variance
ASCUS	Atypical squamous cell of undetermined significance
BaF$_2$	Barium fluoride
CI	Confidence Interval
DNA	Deoxyribonucleic acid
DTGS	Deuterated Triglycine Sulphate
FTIR	Fourier Transform Infrared
HeNe	Helium Neon laser
HPV	Human Papilloma Virus
HSIL	High grade squamous intra-epithelial lesion
IR	Infrared
IUD	Intrauterine contraceptive device
KBr	Potassium bromide
LBC	Liquid based cytology
LSIL	Low grade squamous intra-epithelial lesion
MCT	Mercury Cadmium Telluride (HgCdTe)
PLS	Partial least squares
SCC	Squamous cell carcinoma

SD	Standard Deviation
SPSS	Statistical Package for social sciences
STD	Sexually transmitted diseases
T	Transmittance
WHO	World Health Organization
ZPD	Zero Path Difference

LIST OF APPENDICES

Page

PENDEKATAN BARU DALAM PENYARINGAN KANSER SERVIK MENGGUNAKAN SPEKTROSKOPI FOURIER TRANSFORM INFRAMERAH

ABSTRAK

PENGENALAN:

Pap smear telah digunakan untuk mengesan petanda awal dan kanser serviks sejak 1940-an dan terdapat bukti yang jelas bahawa ujian Pap telah menurunkan kadar mortaliti/kematian disebabkan kanser serviks. Malangnya, terdapat keputusan negatif palsu yang mampu mencecah sehingga 69%. Spektroskopi FTIR digunakan dengan meluas dalam pelbagai bidang industri untuk menyukat dan mengesan kompaun kimia. Terbaru, ia digunakan untuk mengkaji perubahan struktur pelbagai sel kanser manusia pada peringkat molekular.

OBJEKTIF:

Kajian ini dijalankan untuk menilai spektroskopi FTIR sebagai kaedah baru untuk penyaringan kanser serviks sebagai perbandingan dengan ujian sitologi.

METODOLOGI:

Serpihan serviks diambil dan ditempatkan di dalam medium ThinPrep^{TM}. Untuk analisis spektroskopi FTIR, sampel dikeringkan dan dipancarkan dengan sinaran infra merah pada frekuensi dari 4000 sehingga 400 cm^{-1}. Data serapan dicerap menggunakan spektrometer Spektrum BX II FTIR dan dibandingkan dengan bacaan serapan rujukan sampel yang diketahui menggunakan perisian spektroskopi FTIR. Bacaan spektroskopi FTIR dibandingkan dengan keputusan ujian sitologi.

KEPUTUSAN:

Spektroskopi FTIR dapat membezakan sel normal daripada sel abnormal pada 800 sampel yang dikaji. Spektrum normal dicirikan dengan jalur glikogen yang jelas/kuat pada 1,029 (±4.5) cm^{-1}, jalur meregang C-O karbohidrat yang agak jelas/kuat pada 1,156.4 (±4.7) cm^{-1} dan jalur asimetrik fosfodiester asid nukleik pada 1,234 (±4.60) cm^{-1}.

Spektra abnormal dalam kecerunan berbeza menunjukkan penurunan pada intensiti jalur glikogen pada 1,029 (±4.5) cm^{-1} dan intensiti jalur fosfodiester yang lebih jelas/kuat pada 1,234 (±4.6) cm^{-1}. Spektra abnormal juga dilihat apabila terdapat anjakan jalur simetri fosfodiester pada 1,078 (±1.6) cm^{-1} kepada frekuensi yang lebih tinggi dan penurunan intensiti jalur C-OH pada 1,156 (±4.7) cm^{-1}. Sensitiviti untuk mengesan sel serviks abnormal adalah 85%, spesifisiti 91%, nilai jangkaan positif 19.5% dan nilai jangkaan negatif 99.5%.

KESIMPULAN:

Melalui kajian ini, kami mendapati bahawa FTIR mungkin berguna untuk penyaringan kanser serviks. Teknik ini mudah, pantas dan murah serta tidak memerlukan pengawetan dan pewarnaan. Kaedah ini dapat diaplikasi di negara yang tidak mempunyai sitopatologis yang mencukupi. Sepanjang pengetahuan kami, ini merupakan percubaan pertama menggunakan spektroskopi FTIR sebagai kaedah alternatif dalam penyaringan kanser serviks di Malaysia.

A NEW APPROACH IN THE SCREENING FOR CERVICAL CANCER USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

ABSTRACT

INTRODUCTION:

Pap smear has been used successfully to detect precursor and cancer lesions of the uterine cervix since 1940s. There is clear evidence that Pap test has reduced mortality from cervical cancer. Unfortunately false negative smears occur and may reach up to 69%. Fourier transform infrared (FTIR) spectroscopy has been utilized to measure and detect chemical compounds in many industrial fields. Recently it has been used to study the structural changes of cells at the molecular level on various human cancers.

OBJECTIVES:

This study is to evaluate FTIR spectroscopy as new tool for screening of cervical cancer in comparison with cervical cytology.

METHODOLOGY:

Cervical scrapings were taken by cytobrush and placed in ThinPrep^{TM} medium. The samples were dried over infrared transparent matrix. Beam of infrared light were directed at the dried samples at frequency from 4000 to 400 cm^{-1}**.** The absorption data were produced using Spectrum BX II FTIR spectrometer. Data were compared with the reference absorption data of known samples using FTIR spectroscopy software. FTIR spectroscopy was compared with cytology (gold standard).

RESULTS:

FTIR spectroscopy could differentiate normal from abnormal cervical cells in the 800 samples examined. The normal spectrum was characterized by strong glycogen band at 1,029 (±4.5) cm^{-1}, relatively strong C-O stretching band of carbohydrates at 1,156.4 (±4.7) cm^{-1} and relatively weak band of the asymmetric phosphodiester stretching modes of nucleic acids at 1,234 (±4.6) cm^{-1}.

The abnormal spectra in different gradients showed a decrease in glycogen band intensity at 1,029 (±4.5) cm^{-1}, a stronger and broader intensity of phosphodiester stretching band at 1,234 (±4.6) cm^{-1}. Abnormal spectra were also seen when there was a shifting of symmetric phosphodiester band at 1,078 (±1.6) cm^{-1} to a higher frequency and a decrease in the intensity of C-OH stretching band at 1,156 (±4.7) cm^{-1}. The sensitivity to detect abnormal cervical cells was 85%, specificity 91%, positive predictive value 19.5% and negative predictive value of 99.5%.

CONCLUSION:

With this preliminary study we conclude that FTIR may be a useful tool for screening of cervical cancer. The technique is simple, rapid and cheap. The cervical scrapings require no fixation or staining. This method could be adopted in countries where sufficient number of cytopatholgists is not available. To the best of our knowledge this is the first attempt at using an alternative method for screening of cervical cancer using FTIR spectroscopy in Malaysia.

CHAPTER ONE

INTRODUCTION

INTRODUCTION

This work deals with a new screening test, Fourier Transform Infrared Spectroscopy (FTIR), an important and new technique for detection of cervical cancer at its early stages. Screening for cervical cancer is currently carried out by the Papanicolau (PAP) smear test. Squamous and glandular epithelial cells of transformation zone are exfoliated using Cytobrush or spatula, fixed in ethanol and stained with the Papanicolau stain (Patten, 1978).

Until the early 1990s, cervical cancer was the most frequent neoplastic disease among women in developing countries, before breast cancer became the predominant cancer. Each year, over 400,000 new cases of invasive cervical cancer are diagnosed world wide, representing nearly 10% of all cancers in women and was responsible for over 250,000 deaths in 2005 (WHO, 2006).

A definitive diagnosis is obtained by cervical biopsy and examination of the stained tissue. The predictive value of a biopsy is higher than that of the Pap test because the anatomical arrangement is preserved allowing evaluation of pathological features in relation to histological architecture (Yeoh and Chan, 1997).

Cervical disease is classified cytologically using the two-tier Bethesda system for Pap smears low- (LSIL) and high-grade squamous intraepithelial lesions (HSIL) and the three-tier cervical intraepithelial neoplasia (CIN I,II and III) system for surgical samples (LSIL=CIN I, HSIL= CIN II and CIN III) (Cestero, 2006).

Samples diagnosed as CIN II and CIN III, have a higher risk of proceeding to carcinoma in situ (CIS). For the past 20 years the presence of high-risk Human Papilloma Virus (HPV) genotypes has been associated with cervical dysplasia and its progression to cancer. This is now being used as an adjunct to detect cervical lesions in conjunction with Pap smear (Solomon *et al*, 2002).

The previous decade has witnessed the evolution of Fourier transform infrared (FTIR) spectroscopy as an independent modality to discriminate between diseased and normal tissue. Several comprehensive books and articles outlined the field (Pavia *et al*, 2001).

Early studies reported spectral differences between normal and malignant tissues by cytological and histological methods. The essential differences were related to changes in glycogen, nucleic acid, and protein content (Wood *et al*, 2004).

FTIR spectroscopy is used primarily for qualitative and quantitative analysis in chemistry and environmental sciences especially for determining the chemical

structure of many inorganic and organic compounds, drugs, pollutants, materials evaluation and identification. It is also used in deformulations, forensics purposes and measuring the level of purity of a compound (Wood *et al*, 2004).

This study presents preliminary results obtained from the analysis of 800 FTIR spectra obtained for cervical smears. The aim of this study is to demonstrate that this new technology can differentiate between different cytological results in cervical smears, and distinguish normal from dysplastic and neoplastic samples.

FTIR spectroscopy could be used an alternative to Pap smear, which is known to have low sensitivity. The clusters of spectra produced have the potential to be used as input data for the development of a diagnostic algorithm based on an Artificial Neural Network in future.

This study demonstrates that subtle differences between normal and cancer samples can be distinguished spectroscopically. The importance of this study is based on the fact that the spectroscopic changes precede the morphological changes of carcinogenesis which can be seen under the microscope.

1.1 Statement of the current problem

The shortage of trained cytopathologists, cytotechnologists and cytopathology laboratory facilities in developing countries, the long period (1-3 months) between the Pap screenings and the declaration of the test results (Jeronimo *et al*, 2005).

Pap test involves examination of the specimens under light microscope which is tedious, laborious and time-consuming. Highly skilled personnel are required as the reliability of the test depends upon human judgment.

The large variation in sensitivity between 30-80%, due to subjective observation was another problem limiting the success of screening program.

With these problems in mind, we embarked on an alternative method of screening using FTIR spectroscopy.

CHAPTER TWO

OBJECTIVES

OBJECTIVES

2.1 General objectives

The aim of this study was to evaluate the utility of a new, rapid, more cost-effective procedure for cervical cancer screening.

2.2 Specific objectives

1   To identify and characterize the infrared spectra of normal, pre-malignant (LSIL and HSIL) and malignant cervical scrapings.

2   To study the level of agreement between the results of FTIR spectroscopy with liquid based cervical smear cytology (gold standard).

3   To evaluate effectiveness of Fourier transform infrared FTIR spectroscopy in screening for pre-neoplastic lesions of cervical smears.

2.3 Hypothesis

FTIR spectroscopy is as effective as cervical cytology in detecting precursor lesions of cervical cancer.

CHAPTER THREE

LITERATURE REVIEW

Literature Review

3.1 Cervical Cancer

3.1.1 Background

In 2005, according to WHO projections, over 500,000 new cases of cervical cancer were diagnosed, of which over 90% were in developing countries. It is estimated that over 1 million women worldwide currently have cervical cancer; most of whom have not been diagnosed, or have no access to treatment that could cure them or prolong their life.

In 2005, almost 260,000 women died of the disease, nearly 95% of them in developing countries (WHO, 2006), making cervical cancer one of the gravest threats to women's lives.

In many developing countries, access to health services is limited and screening for cervical cancer either is non-existent or reaches few of the women who need it. In these areas, cervical cancer is the most common cancer in women and the leading cause of cancer death among women (WHO, 2006).

3.1.2 Burden of suffering

Cervical cancer has a major impact on women's lives worldwide, particularly in developing countries where it is the leading cause of cancer deaths among

women. Four out of five new cases, and a similar proportion of deaths, occur in developing countries where screening programs are not well established or effective.

Experience in developed countries has shown that well planned, organized screening programs with high coverage can significantly reduce the number of new cases of cervical cancer and the mortality rate associated with it.

There is also evidence that general awareness about cervical cancer, effective screening programs, and the improvement of existing health care services can reduce the burden of cervical cancer for women and for the health care system. There is a huge difference in the incidence of and mortality from cervical cancer between developed and developing countries, as shown in figures 3.1 and 3.2 (WHO, 2006).
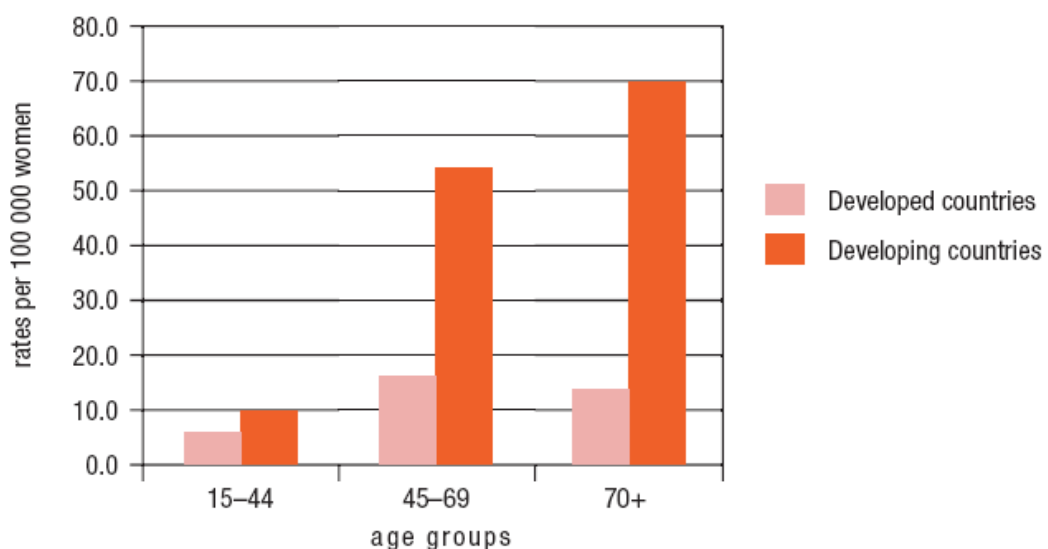
Fig. 3.1 Age-standardized incidence rates of cervical cancer in developed and developing countries for the year 2005 (WHO, 2006)
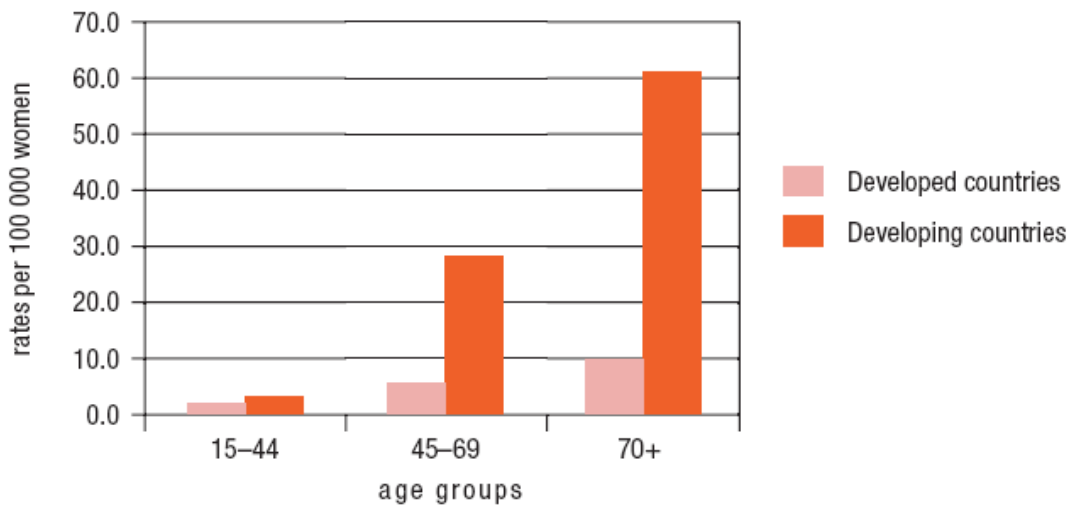
Fig. 3.2 Age-standardized mortality rates of cervical cancer in developed and developing countries for the year 2005 (WHO, 2006)

The hardest-hit regions are among the world's poorest. Central and South America, the Caribbean, sub-Saharan Africa, and parts of Oceania and Asia have the highest incidence rates over 30 per 100,000 women. These rates compare with no more than 10 per 100,000 women in North America and Europe.

Because the disease progress over many years, an estimated 1.4 million women worldwide are living with cervical cancer, and two to five times more –up to 7 million worldwide –may have precancerous conditions that need to be identified and treated.

If it is not detected and treated early, cervical cancer is nearly always fatal. The disease, which affects the poorest and most vulnerable women, sends a ripple effect through families and communities that rely heavily on women's roles as providers and caregivers.

3.1.3 Etiology

3.1.3 (a) Human Papillomavirus (HPV)

Epidemiologic and clinical data indicate that HPV, especially HPV-16 and HPV-18, play a major role in the etiology of cervical cancer (De Marco *et al,* 2006). Expression of HPV-specific oncoproteins, E6 and E7, are considered essential in maintaining malignant growth of cervical cancer cells (Nubia *et al*, 2006).

However, HPV infections are widespread in the general population yet cannot be found in every patient with cervical cancer, nor do all infections with HPV result in cervical cancer. There are many investigators who argue that HPV is necessary etiologic factor, but few who maintain that it alone is sufficient to cause cervical cancer (Zur Hausen, 2002).

In the 1970s, Harald zur Hausen postulated a role for, and then found HPV-DNA in cervical cancers. In the 1980s, his group was the first to isolate HPV-16 and HPV-18 from cervical cancer tissues. Several epidemiologists have subsequently shown highly statistically significant associations between HPV and development of cervical intraepithelial neoplasia (CIN) grade 2 or 3, and with development of cervical cancer (Zur Hausen, 2002).

In 1995, a World Health Organization consensus panel collected biologic and epidemiologic data and concluded that at least HPV-16 and HPV-18 infection caused cervical cancer. HPV can be found in more than 90% of patients with cervical cancer worldwide, most frequently HPV-16 (50%), HPV-18 (12%), HPV-45 (8%), and HPV-31 (5%) (Schiffman and Brinton, 1995).

HPV is DNA tumor virus whose genome is organized in three regions: the early gene (E1 to E7), the late gene (L1 and L2) regions and the upper regulatory region (URR). The early and late gene regions are both protein-encoding, but the URR is non-encoding (Aggarwal *et al,* 2006).

The URR has numerous binding sites for many repressors and activators of transcription, and it may play a part in determining the range of hosts for specific HPV types.

In the protein-encoding regions, the E6 and E7 are considered to play the most major roles. These two units encode for oncoproteins that allow replication of the virus, increasing genomic instability, accumulation of oncogene mutations, further loss of cell-growth control, and ultimately cancer.

Both the E6 and E7 proteins alter the pathways that regulate tissue growth, by interfering with growth receptors or growth factors. Production of cytokines has been shown to be altered in cells infected with HPV-16. The E6 protein increases degradation of the p53 tumor suppressor protein, thereby interfering with apoptosis (programmed cell death).

The E7 protein disrupts complexes of the transcription factor E2F with the tumor-suppressor protein pRb and related proteins which are involved in control of the cell cycle, thus causing their degradation, altering control of transcription and progression of the cell cycle. The E7 protein has been shown to cause abnormal synthesis and duplication of centrosomes, resulting in abnormal mitotic division (Peter *et al*, 2006).

The late region units, L1 and L2 encode for viral capsid proteins during the late stages of virion assembly. The protein encoded by L1 is highly conserved among different papilloma virus species; therefore, antibodies against the bovine papilloma virus have been used to identify HPV capsid proteins in human tissues.

The minor capsid protein encoded by L2 has more sequence variations than that of the L1 protein; hence, antibodies against the L2 protein had been a source of antigen for specific types of HPV antibodies (Ho *et al,* 2006).

HPVs vary genetically not only between but also within types. Intra-type variants are defined as HPVs that vary by 2% or less in specified regions of the genome (Cheah, 1994).

Previous studies have reported intra-type sequence variations of the most common type, HPV-16, and have included specimens from different geographic areas. The major groupings of HPV-16 variants are designated European (E), Asian (As), Asian American (AA), African 1 (Af1), African 2 (Af2), and North

American 1 (NA1). Variants of these groups are denoted by HPV-16 E6 nucleotide position and substituted nucleotide (Wheeler *et al*, 1997).

3.1.3 (b) Sexually transmitted disease (STD)

The specific role of other infectious agents in the pathogenesis of cervical cancer has been investigated in many epidemiological surveys. The most studied sexually transmitted infectious agents in relation to cervical cancer are Herpes Simplex Virus (HSV-2), Chlamydia Trachomatis (CT) and Human Immunodeficiency Virus (HIV). Repeated exposure of the uterine cervix to the male partner's asymptomatic urogenital infection can lead to precancerous or cancerous lesions.

Zur Hausen was also the first to recognize that HPV was not sufficient for cancer induction, and proposed that HSV-2 and HPV act synergistically to induce cervical cancer (Zur Hausen, 2002).

Others have postulated a role for HSV-2 infection in the etiology of cervical cancer, while acknowledging the primary role for HPV, Hildesheim and colleagues studied women with invasive cervical cancer in Latin America and compared viral and behavioral characteristics with controls.

Compared with women negative for both HPV 16/18 and HSV-2, those positive for HSV-2 alone had a relative risk of 1.2. Those positive for HPV 16/18 DNA alone had a relative risk of 4.3 (95% CI=3.0, 6.0), and those positive for both

HSV-2 and HPV 16/18 had a relative risk of 8.8 (95% CI= 5.9, 13.0), suggesting a possible biological interaction. Furthermore, HSV-2 was persistent in a few selected cervical cancer tumors (Hildesheim *et al*, 1991).

Chlamydia trachomatis was associated with a two-fold increased risk for cervical cancer (OR=1.8; 95% CI, 1.2-2.7) in the large IARC multi- center case-control study. It was likely due to the inflammatory response with generation of free radicals and development of genetic instability (Smith *et al*, 2004).

Immunocompromised patients with HIV infection or organ transplantation are at high risk of HPV-associated anogenital cancers compared with age matched healthy individuals. HIV-positive women are at an increased risk of cervical cancer when compared with their HIV-negative counterparts.

This association appears to be stronger for women with a low CD4 T-lymphocyte count. As HIV infection is related to an immunocompromised state, it shows the importance of the host's immunological cofactors in HPV carcinogenesis (Alcina *et al*, 2005).

3.1.3 (c) Nutritional factors

The few published studies assessing the role of diet on HPV persistence have shown a possible protective effect of diets rich in fruits, vegetables, Vitamins C and E, beta- and alpha-carotene. Evidence for a protective effect against cervical neoplasia was probable for folate, retinol, and Vitamin E, and possible

for vegetables, Vitamins C and B12, alpha-carotene, beta-carotene. Evidence for an increased risk of cervical neoplasia associated with high blood homocysteine was considered probable (Anna, 2000).

The current available evidence for an association between diet, nutritional status and cervical HPV carcinogenesis is not yet convincing, even though there is some support for the hypothesis that antioxidant nutrients may play a protective role in cervical carcinogenesis.

3.1.3 (d) Oral contraceptives and IUDs

The effects of oral contraceptives on the occurrence of cervical neoplasia are controversial (Smith *et al*, 2003). Cervical carcinogenesis may be hormone-dependent as judged by:

(a) the histological sensitivity of the cervix to hormonal influences

(b) the decreasing incidence of cervical cancer after menopause; and

(c) the results of animal experiments with estrogens and progesterones

Oral contraceptives cause atypical squamous epithelium, increase squamous metaplasia, basal cell and glandular hyperplasia of the cervix that could be misdiagnosed as malignant, but proved to be benign by using light and electron microscopy. Prolonged usage of an intrauterine device (IUD) is often associated with exfoliation of atypical cells that might be mistaken to represent carcinoma (Lech and Ostrowska, 2006).

3.1.3 (e) Genetic aspects

Several structural aberrations were observed in the chromosomes of patients, e.g. deletions and marked variability in size and shape of homologous chromosomes (Kate *et al*, 2004). The consistently observed chromosomal abnormalities in different stages of carcinoma of the cervix confirm the diagnostic value of the presence of such aberrations in cases of equivocal diagnosis. Thus, the presence of an abnormal clone of cells with chromosomal aberrations may indicate the presence of malignancy (Harry, 2005).

3.1.3 (f) Other risk factors

Tar-based vaginal douching may have a role with the development of cervical cancer. In 1931, Smith reported use of Lysol douches was significantly more common among cases than among controls. In 1950, Lombard and Potter discovered that douching with coal-tar derivatives was reported by more cervical cancers cases than controls (Lombard and Potter, 1950).

In 1967, Rotkin found a significant association between Lysol vaginal douches and cervical cancer compared with hospital-based controls matched for age, race, religion, and hospital (Rotkin, 1973).

Cigarette smoking also plays a role with cervical carcinogenesis. In 1977, Winkelstein reached a conclusion that cigarette smoking was a causative factor for cervical cancer (Harry, 2004). Other groups of investigators at John Hopkins

University have published data supporting passive cigarette smoking as a risk factor for cervical cancer (Winkelstein, 1977).

Another carcinogens generated by burning wood in kitchen stoves or ovens are tar-based compound and correlated with cervical cancer. In a follow-up study by a group of investigators, 125 women with CIN (CIN I, II, III) in Honduras were compared with 241 controls.

They concluded that chronic exposure to wood smoke significantly increased the risk of CIN III, and that chronic inhalation of carcinogens derived from wood smoke could have an effect on the progression to cervical cancer, similar to that observed from cigarette smoking (Winkelstein, 1977).

The natural history and risk factors that influence acquisition of persistent HPV infection that mediate progression in the continuum of cervical lesion grades is shown in the figure 3.3 (Bosch *et al*, 1997).

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                    ┌─────────────────────┐
                    │    Normal Cervix     │
                    └─────────────────────┘
                          │      ▲              • HPV infection
                          ▼      │
                    ┌─────────────────────┐
                    │        LSIL          │
                    └─────────────────────┘

about 60% regress; 15%                      Coexisting factors:
progress within 2-4 years
                                            • Smoking
                                            • Oral contraceptive use
                                            • Parity
                                            • Other STDs
                          │                 • Nutrition
                          ▼
                    ┌─────────────────────┐
                    │        HSIL          │
                    └─────────────────────┘

30% to 70% progress
within 10 years
                          │
                          ▼
                    ┌─────────────────────┐
                    │  Invasive cervical   │
                    │       cancer         │
                    └─────────────────────┘
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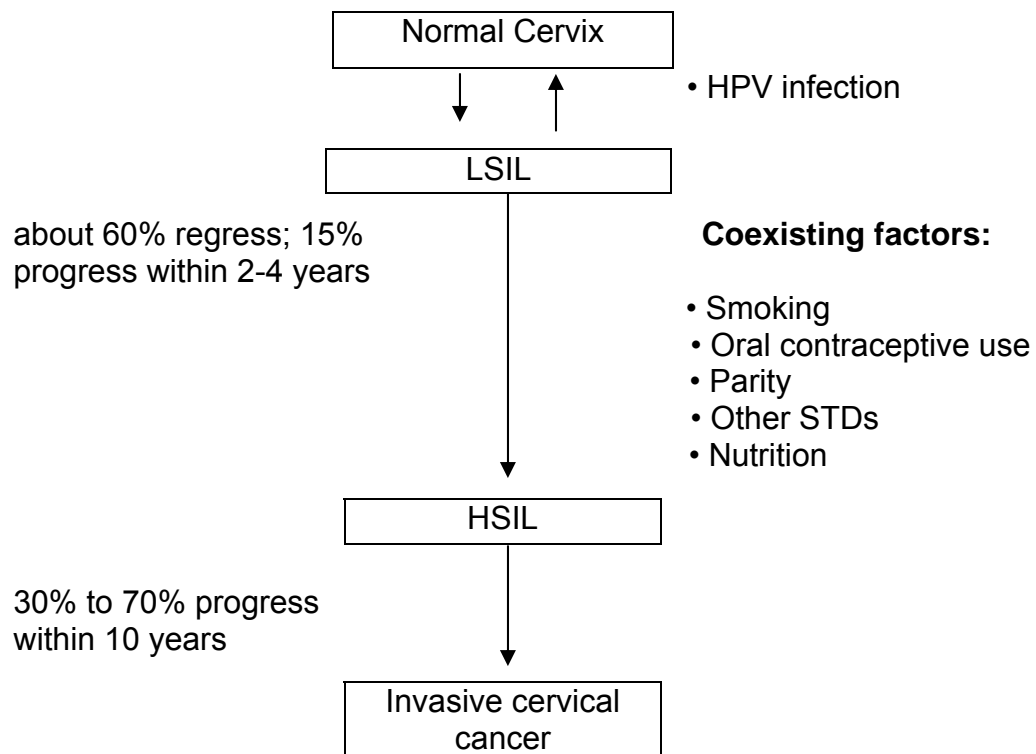
Fig. 3.3 Diagram represents a current understanding of cervical cancer natural history (Bosch *et al*, 1997)

3.1.4 Epidemiologic Studies

The first observations relating to the incidence, distribution and possible causative factors of carcinoma of the uterine cervix were made in 1842 by Rigoni-Stern, an Italian physician who examined the records of deaths in Verona from 1760 to 1839. He noted that uterine cancer was less common in unmarried women and extremely rare in nuns (Hafez, 1982).

Uterine cancer was one of the most prevalent neoplasms reported in Western Europe during the mid 19th century. It is thought that the cervix was the primary site in most of these cases. An increased risk was noted in the lower socioeconomic classes, among multiparous women and in non-white women (WHO, 2002).

Previous studies on Jewish and non-Jewish populations conducted in USA, Europe indicated that Jewish women had a decreased incidence of cervical cancer despite being a part of the lower socioeconomic classes residing in New York City (Ezra *et al*, 2000).

The inference that circumcision of the Jewish males maybe a contributing factor prompted the examination of other ethnic groups such as Muslims, who also practice circumcision. The results of these investigations suggest that the variation in incidence between the compared ethic groups cannot be attributed solely to circumcision but maybe due to associated cultural differences or other factors (Das *et al*, 2000).

The incidence of carcinoma of the cervix seems to be associated with low income and limited education, meanwhile in most countries of the Middle East conservative Muslim mode of life, male circumcision, sexual conservatism, and ritual hygiene probably operate in reducing incidence of carcinoma whereas aesthetic factors and complacency in seeking medical advice operate in delaying diagnosis (Fadua, 2001; Wang, 2004).

3.1.5 Cancer of the cervix in Malaysia

In 2004, the national Cancer Registry reported that cancer of the cervix was the second most common cancer among women in Malaysia for the year 2003 (Figure 3.4). It constituted 12.9% of total female cancers (NCR website, accessed on January 2006).

Cancer cervix was the eighth leading cause of death among medically certified deaths in 1998. Nearly 80% of patients with cervical cancer seen with advanced stages of IIB - IV A3. Despite the availability of screening program, 10.5% of female cancer deaths at government hospitals are due to cervical cancer, meanwhile 7.9% of all cancer admissions in government hospitals are diagnosed as cervical cancer (Othman, 2003).
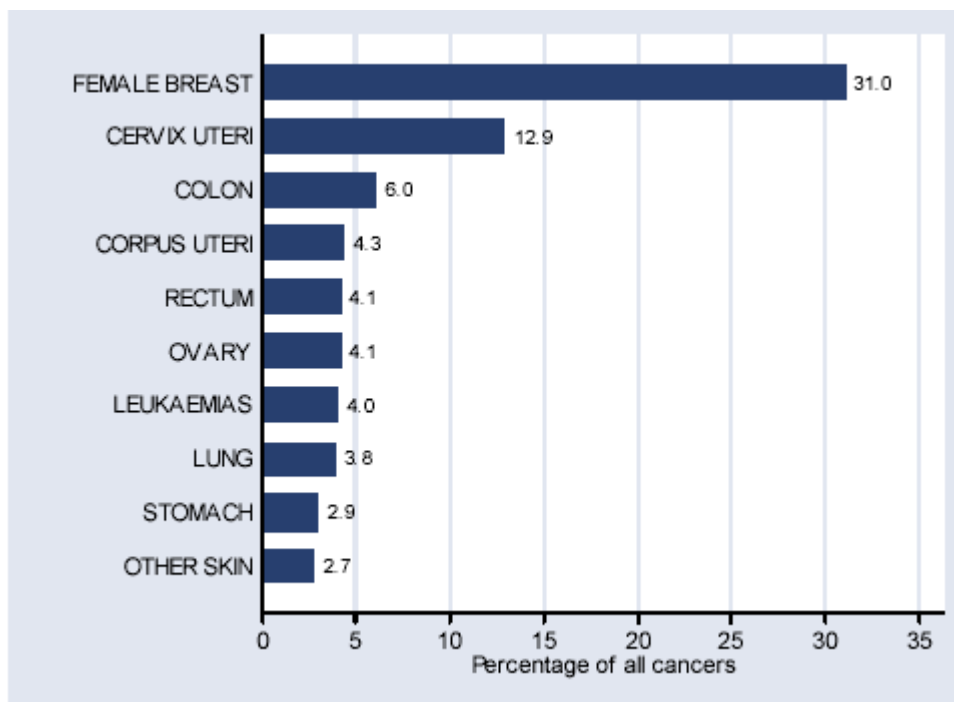
Fig. 3.4 Ten most frequent cancers in females, Peninsular Malaysia 2003 (Othman, 2003)

There were a total of 1,557 cases of cancer cervix, with an ASR of 19.7 per 100,000 population (Table 3.1).

Table 3.1 Cervix uteri cancer incidence per 100,000 population (CR) and Age- standardized incidence (ASR), Peninsular Malaysia 2003 (Othman, 2003)

Sex	No.	%	CR	ASR
Female	1557	100	16.5	19.7

Cervical cancer incidence rate increased with age after 30 years. It has a peak incidence rate at ages 60-69 years, and declined thereafter (Figure 3.5).
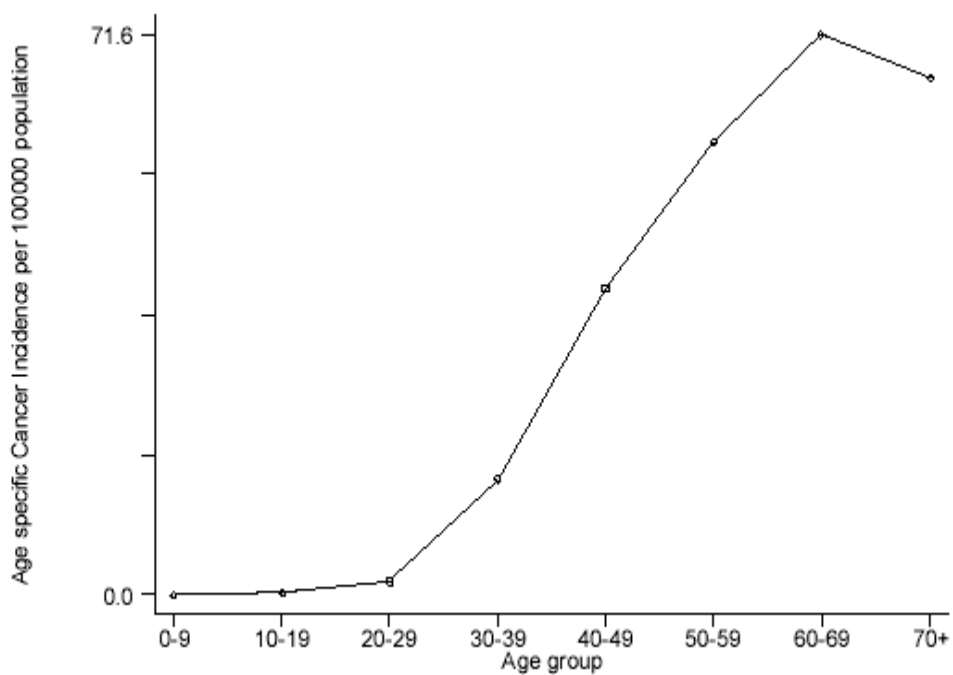

Fig. 3.5 Cervix uteri Age- specific cancer incidence per 100,000 population, Peninsular Malaysia 2003 (Othman, 2003)

Chinese women had the highest ASR of 28.8 per 100,000 population, followed by Indians with ASR of 22.4 per 100,000 population and Malays with ASR of 10.5 per 100,000 population (Table 3.2).