PUBLIC HEALTH RESEARCH

HPV Positivity and its' Influencing Factors among Invasive Cervical Cancer Women in Malaysia

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ABSTRACT

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Introduction Cervical cancer (CC) is the second most prevalent female cancer in Malaysia. Almost 70% of its' causal factors are attributable to oncogenic human papillomavirus (HPV) types 16, 18 and other risk factors. HPV genotypes distributions are also noted to differ by geographical area.

Methods This was cross sectional study conducted in 2007, to determine the influencing factors of HPV positivity and prevalence of HPV infections among patients with cervical cancer in Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Patients' paraffin-embedded cervical tissues kept in the Pathology Department from 1999 to 2007 were randomly selected. A total of 81 medical records with complete information were chosen as samples and patients were contacted for consent. Tissue samples were further derived for PCR DNA for HPV genotyping. Analyses included descriptive statistics; bivariate χ^2 test and correlation were used to determine relationship between factors and HPV positivity. Significance level of less than 0.05 was taken as statistically significant.

Results Mean age of cancer diagnosis was at 52 ± 12.2 years. Women of Chinese ethnicity was the highest ethnicity to be HPV positive at 65.4% and squamous cell carcinoma was more commonly found (59.3%) compared with other types of cancers. The prevalence of HPV positivity was 92.6% with type 16 being the most common (74.1%), followed by type 33 (30.9%) and 18 (22.2%). Multiple HPV infections were a common finding at 54.3%. Factors thought to influence positivity i.e. age of intercourse, number of sexual partners, number of parity, smoking status of patients and their partners, oral contraceptive usage, presence of chronic illnesses and cancer stage were not significantly associated with HPV positivity. Increased CC severity level was not associated with increased number of HPV infections (Pearson correlation 0.58; p =0.607).

Conclusions High HPV positivity at 92.6% was found among ICC patients. Factors thought to influence HPV positivity were not significant. The top three HPV genotypes were type 16 followed by type 33 and 18. However, local women

	HPV serotypes findings need to be replicated in a larger population sample.
Keywords	HPV - invasive cervical cancer – genotyping - HPV distribution - HPV vaccines

INTRODUCTION

World Health Organisation (WHO) reported that in 2002 over 471,000 new cases were diagnosed and 288,000 women died from cervical cancer worldwide. The incidence and mortality rate of cervical cancer were higher in developing countries because of ineffective screenings, lack of knowledge of its benefits among women and unsustainable screening advocates[1]. This can be very damaging especially in under developed and developing countries where the age standardised incidence rate can be higher than the world's incidence rate of cervical cancer [2, 3]. In Malaysia, although various health promotion methods have been utilised to promote female cervical screening through Pap smear; it remained a daunting task where by female population coverage remained low at or less than 40%[4]. Multiple social and cultural barriers hamper this progress and the cervical cancer incidence and mortality remains high causing substantial loss of lives, quality of lives and management costs to the country that provide health services to these cancer inflicted survivors[5].

In Malaysia, the Ministry of Health (MOH) reported cervical cancer as the second leading cause of death after breast cancer among women in year 2003[6] (Malaysia National Cancer Registry 2004). The age standardised incidence rate in 2003 was 19.7/100,000 population and had reduced to 12.2/100,000 in the year 2006[7]. Even with the presence of a nationwide health mitigation effort through opportunistic cervical cancer screening, the screening rate nationwide remained low at 43.7% in a 2006 survey[4]. From the Malaysian National Cancer Registry year 2003 and 2006, cervical cancer incidence rate was highest among the Chinese ethnicity, intermediate among the Malays and lowest among Indians [6,7].

Various risk factors [8,9,10] contributed to cervical cancer development from general cell dysplasia that takes a long time to develop, estimation range from few years to a decade. These risk factors included 'adverse' sexual habits (multiple partners, early intercourse), genital infection (human papillomavirus or HPV, Chlamydia, Herpes), chemical carcinogen (oral contraceptive, cigarette smoking) and women social status (low socioeconomic status). HPV is considered a public health problem, as HPV virus is sexually transmitted and infects men and women alike[11, 12]. HPV acts as a major risk factor for cervical cancer development worldwide [13] but they vary by geographical regions and areas some commented even by ethnicity[14-18]. The International Agency for Research in Cancer reported that HPV types 16 and 18 are carcinogenic and human specific [3]. However more than 100 types of HPV have been discovered; of which approximately 15 are oncogenic while newer types are currently being identified[12, 13]. Approximately 30 types of HPV are associated with anogenital tract infections[13].

The presence of both bivalent and quadrivalent human papillomavirus (HPV) vaccines in Malaysia's private health market have raised concerns on vaccines safety, applicability and suitability of mass vaccinations, the development of various cross protections by different vaccines efficacy by different vaccine types[19]. There are also issues on whether there will be a possibility of other genotypes of HPVs' taking over from types 16 and 18 since vaccine will eradicate these genotypes from Malaysia's women population[20]. Since both novel vaccines have only been tested since 7 years ago, the possibility of a booster is present even though it will be unlikely.

A study in Thailand demonstrated the HPV type 16 was most commonly found in squamous cell carcinoma (SCC), followed by HPV types 18, 58, 52, 31, 33 and 39[17]. In another study, about 80% of HPV types 16, 18, 45, 31 and 33 were distributed in SCC [21]. Among the non-SCC (this include cervical intraepithelial neoplasia (CIN) 3, adenosquamous or adenocarcinoma cell), HPV types 16 was the most common type followed by types 18 and 33. In other studies, it had been showed that HPV types 18 were found in adenosquamous cell at a prevalence rate of ranging between 42.9% [10] to 54.3% [17]. The distribution of HPV types in other geographical area showed the same result that demonstrated that HPV types 16 (44%) and 18 (39%) were the most dominant in Jakarta Indonesia[22, 23]. In Washington, HPV type 16 dominated followed by type 18 and this followed the general world trend [14, 16, 22]. This study was carried out to determine the factors associated with invasive cervical cancer based on cervical tissues. Funding was provided by Merck Sharp and Dohme Malaysia through the National Public Health Physicians Association.

METHODS

This was a cross sectional study in 2006, taking retrospective data of women from the year 1999 to 2007. Eligible women were women whose ICC paraffin-embedded cervical tissues kept in Universiti Kebangsaan Malaysia Medical Centre (UKMMC) Pathology Department. Invasive CC was defined as high-grade squamous intraepithelial lesion **(**HSIL) or CIN3 and above (Stage 1A-4B). Patients excluded were women below the age of 30 or above 80 years of age, who have expired from whatever cause of mortality, patients who were not contactable or women with other concurrent types of cancer.

Two methods of data gatherings were performed. The first was data gathered using data extraction forms that collected patients' sexual behaviour, reproductive history (number of children), past usage of oral contraceptives, occupations and Pap smear screening history. The second method, subjects were contacted through telephone and direct interviews were carried out to gather more information especially when there was insufficient information from the responders' medical records. Patients or caretakers consents to participate in the research were obtained prior the telephone interviews as well as the ethical approval from UKMMC research ethical committee. Cervical cancer tissue samples were analysed using real time Polymerase Chain Reaction (PCR) to determine HPV positivity (defined as presence of at least one type of HPV genotype).

RESULTS

Socio demographic Profiles

Out of 125 patients, 81 patients (64.8% response rate) have complete information required for the study. The block specimens of those 81 patients were subjected to PCR study to identify the HPV types. Table 1 described the socio-demographic characteristic of the ICC patients. From reviews of patients' history from their health record notes, majority of our respondents developed cervical cancer at the age of between 30 to 39 years. However, the mean age of respondents during admission in this study was carried out was at 52.0 ± 12.2 years. About 65.4% of the subjects in this study were of Chinese ethnicity, followed by Malays at 25.9%, Indians at 4.9% and most of them were currently married women at 65.4%.

Risk Factors for HPV Related CC

Table 2 elucidated that 95.5% of patients with early sexual intercourse (defined as sexual intercourse below the age of 18 years) had HPV related cervical cancer compared to 93.0% among those with late sexual intercourse debut. This relationship was not significant.

Of the 66 patients with one sexual partner, 93.9% was infected with HPV and the result was also higher for the patients with more the one sexual partner (85.7%). In term of parity, 90.0% of patients with three children and below developed HPV related cervical cancer. This percentage increased at 95.1% in patients with more than three children. However, this relationship was not significant (p=0.65).

Most of the responders (n=73; 90.1%) stated that they never smoked and all the eight smokers in the study were HPV positive compared to 91.8% among non-smokers. All patients who ever used contraceptive method (n=21; 100%) was HPV positive compared to 90.0% of those not on contraceptive methods. Spouse smoking status was associated with higher HPV positive status at 92.9% compared with 91.4% of spouse who are non smokers. The presences of chronic illnesses (DM, Hypertension, Hepatitis B, Anaemia, Stroke, and Ischemic Heart Disease) were not associated with HPV positivity. There was no significant relationship between HPV positivity with cancer stage ($\chi 2$=0.886; p=0.927). Increased stage of ICC was not significantly associated with increased number of HPV infections (Pearson correlation of 0.058; p=0.607).

Table 1 Socio-Demographic Characteristics of Patients with ICC.

Characteristics	N	%
Age (years) (n=81)	Mean 52.0 ± 12.2	
30 – 39	13	16.0
40 – 49	19	23.5
50 – 59	26	32.1
≥ 60	23	28.4
Income (RM)	Median RM 725 (IQR 1000-1575)	
Ethnic (n=81)		
Malay	21	25.9
Chinese	53	65.4
Indian	4	4.9
Others	3	3.7
Marital Status (n=81)		
Single	2	2.5
Married	53	65.4
Divorcee	11	13.6
Widowed	15	18.5
Education level (n=78)		
Never school	25	32.1
Primary school	29	37.2
Secondary school	20	25.6
Tertiary/University	4	5.1
Patients' occupation (n=81)		
Professional	4	4.8
Business	2	2.5
Agriculture	2	2.5
Factory	2	2.5
Housewife	51	62.9
Others	20	24.9
Smoker (current) (n=81)		
Yes	8	9.9
No	73	90.1
No of sexual partners (n=80)		
Single	66	82.5
Multiple	14	17.5
Usage of oral contraceptives(n=81)		
Yes	21	25.9
No	60	74.1
Pap smear screening done (n=80)		
Yes	44	55
No	36	45

Table 2 Factors associated with HPV Positivity Status.

Factor	HPV Positivity Status n, (%)		$\chi 2$	p
	Positive	Negative		
Early sexual intercourse (n=79)				
Yes	21 (95.5)	1 (4.5)	0.00	1.00
No	53 (93.0)	4 (7.0)		
No. of sexual partner (n=80)				
1	62 (93.9)	4 (6.1)	0.25	0.62
≥ 2	12 (85.7)	2 (14.3)		
Parity (number of children) (n=81)				
0-3	36 (90.0)	4 (10.0)	0.21	0.65
≥4	39 (95.1)	2 (4.9)		
Ever use contraceptive method (n=81)				
Yes	21 (100.0)	0 (0.0)	1.04	0.37
No	54 (90.0)	6 (10.0)		
Smoking (n=81)				
Yes	8 (100.0)	0 (0.0)	0.17	0.9
No	67 (91.8)	6 (8.2)		
*Spouse's smoking (n=77)				
Yes	39 (92.9)	3 (7.1)	0.01	1.00
No	32 (91.4)	3 (8.6)		
Chronic illness (n=81)				
Yes	37 (92.5)	3 (7.5)	0.01	1.00
No	38 (92.7)	3 (7.3)		
Cancer Stage (n=81)				
CIN3/HSIL	18 (90.0)	2 (10.0)	0.886	0.927
Stage 1	22 (91.7)	2 (8.3)		
Stage 2	20 (95.2)	1 (4.8)		
Stage 3	10 (90.9)	1 (9.1)		
Stage 4	5 (100.0)	0 (0.0)		

* Yates correction

Tissue Sample Histology and HPV Type

The most common histology types found in this study was squamous cell carcinomas (SCC) at 73.8%, followed by CIN3 at 23.5%, adenocarcinoma at 18.5% and adenosquamous carcinoma at 1.2%. However, co-infections with types 16/33 were present in SCC at 73.3% and 20% in adenocarcinoma. HPV coinfections with types 16/18 was found to be dominant in SCC at 58.3%, followed by 41.7% in adenocarcinoma. Co-infection with types 18/33 was dominant again in SCC at 66.7%, followed by in adenocarcinoma at 33.3%. The full result of this study was published elsewhere[24].

Table 3 Distribution (%) of HPV Positivity and Genotypes.

HPV Positivity and Types	Percentage (%)							
	n=75*			n=81**				
	SCC (73.8%)	Adeno-squamous (1.2%)	Adeno-carcinoma (18.5%)	CIN3/ HSIL (24.0%)	Stage 1 (28.0%)	Stage 2 (28.0%)	Stage 3 (13.3%)	Stage 4 (6.7%)
Positivity	75.4	1.8	22.8	24.0	29.3	26.7	13.3	6.7
16 (n=60)	75.0	2.1	22.9	20.0	31.7	30.0	11.7	6.7
18 (n=18)	53.3	0	46.7	16.7	33.3	22.2	22.2	5.6
33 (n=25)	77.8	5.6	16.7	28.0	20.0	44.0	4.0	4.0
16/33 (n=20)	73.3	6.7	20.0	25.0	15.0	50.0	5.0	5.0
16/18 (n=15)	58.3	0	41.7	20.0	33.3	20.0	20.0	6.7
18/33 (n=6)	66.7	0	33.3	50.0	0	33.3	0	16.7

**n=75 are among the ICC only*

***n=81 are among pre invasive and ICC*

DISCUSSION

The higher prevalence of Chinese ethnicity among patients diagnosed to have cervical cancer in this study was consistent with the Malaysian Cancer Registry [6,7] (year 2003-2006) that reported that the incidence was highest among the Chinese at age standardised rate of 33.6 per 100 000 and Malays are the lowest ethnic to developed cancer. However this study showed that the Malays were the second group while Indians had the lowest prevalence of cancer. It might be due to sampling bias because UKMMC being a teaching hospital and of public entity was being visited by more Malays than Indian patients.

Women as young as 30 years developed ICC in this study. The development of cervical cancer was common in women older than 35 years age [6,7]. In the Africa region, the mean age of the patients with cervical cancer was 33.9±11.4 years and in southern European region was at 56.5 ±14.3 year[16]. Younger age of sexual debut triggered higher risk of acquiring HPV infection through immaturity of cervical cells and the inability to counter persistent infection[3, 11, 24]. In the Durex sexual survey done in Malaysia, the age of sexual debut for women in this country was as young as 17 years old[25]. This is the results of globalisation and religious /cultural disintegration occurs, where by the age of sexual debut will reduce although the age of actual marriage and commitment may increase[11, 15, and 18]. This sexual novelty of young woman and their male partner/partners puts her at risk of acquiring HPV infection through sexual intercourses. In subsequent sexual activities, these HPV infected women may transmit the infection to other partners later on and possibility to their newborns; presenting with recurrent laryngeal respiratory papillomatosis that needs repeated and intensive procedures to remove the tumour[26]. The unknown local HPV prevalence among women and among men remain as risk factors, unfortunately that information are not readily available as the importance and cost effectiveness are still debated [14,16,18,26]. The sexual activity of men having sex with men in Malaysia may not be as rampant as in other countries but this adds fuel to the fire where the quadrivalent vaccine is highly sought after by these 'high risk' groups as it covers against genital warts [15,20,26]. From previous studies, it has been shown that total HPV positivity among cervical cancer and pre invasive diseases were at 76% [27, 28] .The most dominant types from these studies were types 18 at 68% and type 16 at 58%.

Consistent with other studies [2,9,10], (if HSIL had been omitted) then SCC (73.8%) was most commonly found among our respondents, compared to adenocarcinoma (18.5%) and adenosquamous carcinoma (1.2%). Independent single infection from our result, demonstrated that HPV type 16 (75.0%) and type 33 (77.8%) dominated in SCC. Both HPV 16 and 33 were from A9 species, rather than type 18 which is from the A7 species. HPV type 18 was found to be more dominant in adenocarcinoma at 46.7%. UKMMC being a teaching hospital and as a tertiary referral centre, would reflect the geno-positivity of Malaysian women inflicted with cervical cancers. However this study was a single centre study, thus a wider multi centre study among general women population would be helpful albeit costly as HPV positivity yield might not be very high among 'low risk' women. These current findings stipulated that

genotypes 16 and 18 played a significant role in local cervical cancer development as well. The high prevalence of HPV type 33 remained a deviation against the normal HPV trend except in certain studies from Hong Kong [29], Japan[14], Uganda[15] and Iran[30]. These papers suggested that following cervical infection with HPV type 16, other types may predominate rather than the usual HPV type 18.

However from this study, the results indicated no significant relationships between HPV positivity status and factors (age of first intercourse, number of sexual partners and number of children, contraceptive usage, patients' and spouses' smoking status, presence of chronic illness and CC stage). Other factors could have played significant factors in this study. This might be incongruent with other literatures that suggested the mentioned factors to predict or associated with the development of HPV related disease and positive status. This could be due to the limited sample size among ICC patients taken as responders in this study. Next, enter the question of the effects of mass vaccination. If mass vaccination program are to be implemented, will its properties of cross protection causes serotypes replacement of the HPV epidemiology of the population and change the immunity of local women population? These answers are a long way to be discovered and till then, these possibilities have to further explored and documented.

CONCLUSIONS

HPV infection and positivity was high among women inflicted with ICC in Malaysia. The epidemiology in local setting is extremely important as it may change local HPV epidemiology once mass vaccination is initiated on a mass population scale. There raise another question of will there be a need for second line of HPV vaccines or will the cross protections presumably in HPV vaccines against HPVs in their phylogenetic tree be sufficient to render protection against cancer development.

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