Original article:

Absence of Endocervical / Transformation Zone Component in Cervical Papanicolaou Smears in Northeastern Region of Peninsular Malaysia: Prevalence and Risk Factors

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Abstract:

Background: Negative result from a satisfactory Papanicolaou (Pap) smear without endocervical cells and transformation zone (EC/TZ) components does not increase the chances of cervical cancer. However, a preparation of without EC/TZ components cannot rule out cervical cancer. Therefore, we aimed to study the factor associated with absence of EC/TZ components in Pap smear in Pasir Puteh District. Materials and Methods: A comparative cross-sectional study between groups of presence and absence of EC/TZ components in Pap smear test results was conducted among 114 samples of Pap smear screening who fulfilled study criteria in Pasir Puteh district, Kelantan state of Malaysia. Data were collected from Pap smear registry between 15th December 2019 and 15th January 2020. Descriptive statistics, simple and multiple logistic regressions were used for data analysis. Results and Discussion: The prevalence of Pap smear samples with absence of EC/TZ components was 14.2% (95%CI: 0.11, 0.16). Multiple variable analysis using multiple logistic regression revealed BMI and nurses' working duration as the significant factors associated with absence of EC/TZ components with an adjusted odds ratio (AOR) of 1.08 (95%CI:1.01, 1.16); p<0.05) and 0.85 (95%CI:0.76, 0.96; p<0.05) respectively. Conclusion: Majority of cervical cancer lesion happened in EC/TZ zone. Therefore, it is important to make sure staffs who conduct Pap smear test have received adequate training and used good sampling equipment in difficult patient to ensure the yield of the smear is satisfactory with presence of EC/TZ zone.

Keywords: pap smear, endocervical, transformation zone, cervical cancer, Malaysia.

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Introduction

Cervical cancer is the fourth most common cancer among women after breast cancer, colorectal cancer and lung cancer. According to American Institute for Cancer Research, cervical cancer contributes 6.9% of total number of newly diagnosed cancer in 2018. The main risk factor that contributes to the development of cervical cancer is human papillomavirus (HPV)¹. It can be transmitted through sexual contact either through vaginal, anal or oral sex with carrier of the virus. However, it also has other risk factors such as smoking, overweight, weak immunity and

effect of hormonal treatments². Therefore, cervical cancer is preventable by early screening.

Cervix has two different parts namely endocervix and ectocervix. Ectocervix is an outer part of the cervix. It is covered with non-keratinizing, stratified squamous epithelium. Endocervix is the inner part of the cervix which has an opening into the uterus. It is covered with simple columnar epithelium³. Both cells meet in the middle at transformation zone. This zone consists of mixed type of epithelium. Lesions arising from squamous epithelium constitute 85% of cervical cancer while lesions from columnar epithelium constitute 10%

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of cervical cancer. There is other type of cancer that makes 5% of cases⁴.

Most cervical cancer begins with changes of cells in the transformation zone⁵. Location of this zone varies from person to person. It depends on a few factors such as age, pregnancy, childbirth, hormonal treatment as well as menopause^{3,4}. An American study reported that cells in this zone were more vulnerable to dysplastic differentiation in which this is a precursor to cervical cancer⁵.

Cervical cancer can be screened by using Papanicolaou test or also known as Pap smear test. It is an effective method to detect changes in cervical cells that may indicate potentially precancerous and cancerous cells. Early detection may reduce morbidity and mortality rate among patients since early treatment may be initiated. In Malaysia, women aged 20 to 65 years old who are sexually active are recommended for Pap smear test. If the result is negative, women need to repeat smear the next year. However, after two consecutive negative results, women are recommended to repeat Pap smear every three years⁶.

Nevertheless, the effectiveness of Pap smear screening relies on adequacy of sample taken form the cervix⁷. In grading Pap smear result, Bethesda system is used. In 2001 Bethesda adequacy criteria, specimens deemed as having 'satisfactory for evaluations' must have these criteria; appropriate labeling and identifying information, relevant clinical information, adequate numbers of well-preserved and well-visualized squamous epithelial cells, and an adequate endothelial/transformation zone (EC/TZ) component⁸.

To date, there is still inconclusive association between absence or lack of EC/TZ components with incidence of cervical cancer. However, concern arises when cervical cancer can develop from columnar abnormalities in which, these lesions usually found at endocervical area of cervical epithelium. Therefore, a satisfactory sample without EC/TZ components might lead to overlooking cases with dysplasia or adenocarcinoma⁹.

Currently, there is limited study on factors associated with Pap smear sample technique. To the best of our knowledge, there is no well-published study looking on factors associated with poor outcome of Pap smear samples (in this case, absence of EC/TZ components) in Malaysian setting. However, there is one study conducted in Taiwan that found pregnancy (among other clinical

factors) is associated with absence of endocervical cells or transformation zone⁹. Therefore, we aimed to study other determinants that are associated with this issue including patient and operator factors besides estimating the prevalence of absence of EC/TZ components in cervical Papanicolaou smears in Malaysian setting.

Materials and Methods

From 15th December until 15th January 2020, we conducted a comparative cross-sectional study in eight primary healthcare facilities in the district of Pasir Puteh, Kelantan state of Malaysia. Kelantan is located in northeastern region of Peninsular Malaysia predominated with Malay ethnic population^{10,11}. The clinics involved were Pasir Puteh Health Clinic, Selising Health Clinic, Cherang Ruku Health Clinic, Jeram Health Clinic, Gaal Health Clinic, Banggol Pak Esah Health Clinic, Gong Kulim Health Clinic, and Sungai Petai Health Clinic.

The reference populations were adult (>19 years old) women in Pasir Puteh district, and the study samples were all female patients who fulfilled study inclusion and exclusion criteria in the eight selected health clinics. The inclusion criteria were patients who underwent Pap smear screening at any of the eight recruited health clinics, and the Pap smear procedures were done by healthcare providers. Samples with incomplete variables of 20% were excluded from the study.

The sample size was calculated for each variable of associated factors for poor quality of Pap smear among patients using power and sample size calculation software¹², as well to compare two independent proportions. The largest estimated sample for each group was 57 using the proportion of good quality of Pap smear by the factor of hormonal type of family planning (0.26)¹³, an estimated proportion of 0.06, 5% type 1 error, 80% power and additional of 10% drop-out rate. Therefore, the total sample size required is 114 samples for Pap smear screening. We employed simple random sampling for subject's recruitment from the total patients underwent Pap smear screening in Pasir Puteh district which fulfilled the study criteria.

Data were collected from Pap smear registry (PKW 202C returns) and recorded in patient's proforma. The retrieved information for independent variables included socio-demographic characteristics (age of patient, working experience of operator in year) and clinical characteristics (parity, BMI, type of family planning methods). The dependent variable

will be the outcomes of Pap smear slide either presence or absence of EC/TZ components in cervical Papanicolaou smears.

Statistical Analysis

Data were analyzed using inferential statistics using SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Descriptive statistics with mean and standard deviation (SD), frequency and percentages were calculated. Simple and multiple logistic regression analysis will be used to determine factors associated with absence of EC/TZ components in cervical Papanicolaou smears. All significant variables with a p-value <0.25 from univariable analysis and clinically important variables were chosen for multiple logistic regression analysis. A p-value<0.05 was considered statistically significant.

Results

From 1st January 2019 until 31st December 2019, a total of 1244 samples of Papanicolaou smear were done in the district of Pasir Puteh. Out of these samples, 14.2% (95%CI: 0.11,0.16) or 177 Pap smear samples had no EC/TZ components. Out of 1244 smears, 60 samples were randomly selected for each of the comparison group based on sample size calculation.

Among patients with absence of EC/TZ components on their smears, the mean (±SD) for age, body mass index and parity were 35.88 (±9.07), 26.61 (±6.74) and 3.68 (±1.84), respectively. Moreover, majority of patients with absence of EC/TZ components on their smears used hormonal type of family planning methods. The mean (±SD) duration of service for nurses responsible for smears with no EC/TZ components was 14.92 (±3.06). Details of socio-demographic and clinical characteristics are summarized in Table 1.

In the univariable analysis, body mass index, family planning method and nurses' working duration were the statistically significant and clinically important factors selected for multivariable analysis. Details are summarized in Table 2.

Multivariable analysis using multiple logistic regression revealed body mass index and nurses' working duration were the significant factors associated with absence of EC/TZ components on Papanicolaou smears among patients in Pasir Puteh district with an adjusted odds ratio (AOR) of 1.08 (95%CI:1.01, 1.16); p=0.017) and 0.86 (95%CI:0.76, 0.96; p=0.01) respectively. Details are shown in Table 3.

Discussion and Conclusion

The prevalence of samples with absence of EC/TZ components in Pasir Puteh district was 14.2% (95%CI: 0.11,0.16). It is higher than the prevalence reported by Elias and Linthorst (1983) which was only 6.9% in their study¹⁴. Similarly, a Taiwanese study reported lower prevalence of EC/ TZ components absent among patients who did Pap smear test in one of the Taiwanese hospitals which was 8.7%9. Persistent human papilloma virus (HPV) infection could lead to cervical cancer development especially HPV16², and more than 90% of cervical cancer happened in transformation zone⁵. Deng and Hillpot (2018) found that cells in transformation zones and endocervix have higher susceptibility to dysplastic changes by HPV16 infection and therefore increase risk of developing cervical cancer⁵. Hence, due to the high prevalence of EC/TZ components absent in Pap smear test in Pasir Puteh district, there is a need to study the factors associated with absence of EC/TZ components.

There are many studies reported on factors related to abnormal or even unsatisfactory Pap smear result. However, not many studies reported on factors associated with absence of EC/TZ components. In our study, we found that longer working experience of a nurse (operator) was associated with lower chances of absence of EC/ TZ components in Pap smear preparation (AOR: 0.85; 95%CI: 0.76, 0.96; p<0.05). On the contrary, a Thai study reported that experienced staffs at OB-GYN department in one of Thai hospitals were more likely to get absence of transformation zone cells in Pap smear due to complexity of cases that they received¹⁵. Although staffs' experience in the Thai study might not correlate with duration of working but being experienced means that the staff have received multiple training in conducting Pap smear with correct technique to get satisfactory preparation. A few factors that may increase collection of EC/TZ components are competent technique, good sampling equipment as well as staff's experience¹⁶.

Another factor that is associated with the absence of EC/TZ components in Pap smear preparation is body mass index (BMI) of patients. Findings from our study showed that an increment of 1 kg/m² of BMI is associated with 8% increase in the likelihood of having absence of EC/TZ components of Pap smear preparation (AOR: 1.08; 95%CI:1.01, 1.16; p<0.05). Currently, there is no study found an association between

BMI with absence of EC/TZ components and hence, our findings can be regarded as novel. A study in California, USA reportedthat obesity and overweight increased the risk of cervical cancer¹⁷. However, these overweight and obese women usually had lower rate of Pap smear screening¹⁸. Despite presence of technical difficulty to perform Pap smear in obese women in view of anatomy and equipment adequacy¹⁹, Pap smear test must also be done to this group of women to screen for cancerous and precancerous lesions.

Conclusion and Recommendations

Prevalence of Pap smear result with absence of EC/TZ components in Pasir Puteh district is relatively high and its associated factors were operator's working duration and BMI of the patient. Even though a smear with absence of EC/TZ component is still satisfactory and can be processed, probability to exclude pre-cancerous or cancerous lesion that arise from endocervical and transformation zone area is low. Moreover, majority of cervical cancer lesion happened in this zone.

Therefore, it is important to make sure staffs who will conduct Pap smear test have received adequate training and patient were told to abide to certain routine to make sure the yield of the smear is satisfactory with presence of EC/TZ zone. The need to repeat Pap smear test in negative findings with absence of EC/TZ components depends on one's risk. Generally, it is recommended to repeat smear in 12 months after the last Pap test¹⁶.

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Disclosure statement: The authors declare no conflicts of interest.

Ethical approval issue: This study was approved by the Medical Review and Ethical Committee from National Institute of Health, Ministry of Health Malaysia NMRR-19-3531-52375.

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Table 1: Socio-demographic and clinical characteristics of patients in accordance to Papanicolaou smear outcomes in Pasir Puteh district (n=120)

	Frequency (%)			
Characteristics	Absence of cell (n=60)	Presence of cell (n=60)		
Age (years)*	35.88 (±9.07)	37.23 (±8.40)		
BMI (kg/m²)*	26.61 (±6.74)	24.12 (±5.40)		
Methods of family planning Non-hormonal Hormonal	25 (41.7) 35 (58.3)	14 (23.3) 46 (76.7)		
Parity*	3.68 (±1.84)	3.88 (±2.10)		
Nurses' working duration (year)*	14.92 (±3.06)	16.78 (±3.65)		

^{*}Mean (±SD)

Table 2: Factors associated with the absence of endocervical/transformation zone component in conventional cervical Papanicolaou smears among patients in Pasir Puteh district by simple logistic regression (n=120).

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Factors	β	S.E.	Wald statistics (df)	Crude OR (95% CI)	p-value
Age (years)	-0.02	0.02	0.72 (1)	0.98 (0.94, 1.02)	0.396
BMI (kg/m2)	0.07	0.03	4.66 (1)	1.07 (1.006, 1.14)	0.031
Methods of family planning Non-hormonal Hormonal	-0.85	0.40	4.50 (1)	1.00 0.43 (0.19, 0.94)	0.034
Parity	-0.05	0.09	0.31 (1)	0.95 (0.79, 1.14)	0.577
Nurses' working duration (year)	-0.17	0.06	7.96 (1)	0.84 (0.75, 0.95)	0.005

Table 3: Factors associated with the absence of endocervical/transformation zone component in conventional cervical Papanicolaou smears among patients in Pasir Puteh district by multiple logistic regression (n=120).

Factors	β	S.E.	Wald statistics (df)	Adjusted OR (95% CI)	p-value
BMI (kg/m2)	0.08	0.03	5.66 (1)	1.08 (1.01, 1.16)	0.017*
Methods of family planning Non-hormonal Hormonal	-0.81	0.43	3.52 (1)	1.00 0.45 (0.19, 1.04)	0.061
Nurses' working duration (year)	-0.16	0.06	6.55 (1)	0.85 (0.76, 0.96)	0.010*

^{*}p-value < 0.05.

No multicollinearity and no interaction found.

Hosmer Lemeshow test, p-value=0.071. Classification table 71% correctly classified.

Area under Receiver Operating Characteristics (ROC) curve was 72.5%.

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