

Cytology and Genotyping of High-Risk Human Papilloma Virus from Female Sex Workers in Makassar

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Abstract

Objective: This study aims to determine the results of cytological examination and genotype of high risk HPV in women work as femalesex workers (FSW)at Makassar, South Sulawesi.

Method: This study designed as analytical study with a cross sectional analysis approach and sample came from female sex workers in the Makassar city. Research was conducted in the localization area of sex worker, and conducted from March 2019 - September 2019. The research sample consist of women with reproductive age who sex workers, and meet the inclusion criteria. The cytological examination was performed using the Thin Prep Pap Test and the DNA HPV detection (genotyping) using the reverse dot blot “flow through” hybridization method. Data were analyzed with SPSS.

Results: Our study found that most of the FSWs who were sampled are > 30 years old (61.3%), had <9 years of education (66.3%), were married (77.5%), nulliparous (62.5%), being FSW> 1 year (86.3%), age at FSW ≥ 15 years (73%), age at first coitus ≥15 years (82.3%), number of sex partners within a month ≥5 (61.3%), the last number of sexual partners in the last 1 month<5 (58.8%), did not have an Sexual Transmitted Infection (STI) (96.3%). The number of FSWs who using and not using condoms was comparable (50% each), and all samples had never vaccinated with HPV vaccine. It was found that based on the type of lesion, most FSW samples had non-specific and non-bacterial cervicitis (60%) and the remaining 40% had no lesions. Based on the distribution of HPV genotypes in commercial sex workers, most of the samples had a high risk HPV of 25%. Duration to be FSW connected with high risk HPV (p <0.05).

Conclusion: There is a significant relationship between duration of being FSW and high risk HPV genotype. Our study highlited that FSWs who work for >1 year are prone to having high risk HPV.

Keywords: *Cytology of FSW, HPV genotype of FSW, Makassar.*

Introduction

Cervical cancer is the third most common type of cancer in the world, and is the only cancer that can be

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detected early therefore prevented its development. It is estimated that in 2008 worldwide there were 530,232 women diagnosed with cancer and 275,008 of them died.¹ Most cervical cancers (85%) occur in developing countries such as Indonesia.² The growth rate of cervical cancer has a drastic difference in developed countries compared to developing country. Globally, the burden of cervical cancer in developing countries is around 85% -88% of deaths from cervical cancer. The growth rate of cervical cancer in developing countries is still increasing, by adding the incidence of both of cervical

and breast cancer will be the main cause of death in women of reproductive age by 2025.³

Cervical cancer often found in women aged >50 years, but in developing countries the incidence frequently found in women with reproductive age. In countries that do not have good screening programs, cervical cancer sufferers are mostly diagnosed at an advanced stage with incurable stage.⁴ In addition, the prevalence and genotype of HPV infection differ in each population. There are currently two types of HPV vaccines, namely for types 16 and 18, both of which significantly reduce the prevalence of HPV infection caused by these two types of HPV.⁵ However, the effect of HPV vaccination on the prevention of HPV infection and cervical cancer also differs based on the distribution of HPV genotypes in each region.

Cervical disease is highly contagious and the risk of infection increases with increased sexual activity.^{6,7} Female sex workers are among the high-risk groups who most likely infected and transmit HPV because having more than one sexual partner.⁷ Studies on female sex workers in Cambodia show the highest prevalence of HPV types 51 and 70 dominant compared to types 16, 71 and 81.⁸ However, other research in Turkey shows that female sex workers are mostly infected with HPV types 18, 16 and 50.⁹ A meta-analysis of HPV infections in female sex workers in Asia shows that there are differences in the prevalence of HPV types among southeast and eastern Asia. The predominant types of HPV that infect in eastern Asia consist of types 16, 18, 58, 56 and 52 while HPV infections in southeast Asia are mainly caused by types 52, 16, 58, 18 and 66.¹⁰

Cytological examination has become a standard examination of cervical cancer screening over the last 50 years. Cervical cancer screening examination with cytology has reduced the incidence and mortality rates due to cervical cancer. Screening of cervical cancer is effective step because the nature of this cancer has a long preinvasive phase so that minimal changes can be detected before it turns into a malignancy or metastasis. This examination evaluates the existence of cell abnormalities resulting from cervical epithelial specimens. Conventional cytological examination has a sensitivity level (70% -80%) and specificity (94-97%) to detect high-grade precancerous lesions.¹

The high level sensitivity of HPV DNA testing has the advantage of a very high negative predictive value

even against adenocarcinoma precursors, which are often not detected by cytological examination.¹¹

Research on the cytology and genotype of HPV in commercial sex workers has never been conducted in South Sulawesi, especially Makassar. Therefore, it is necessary to conduct research on the cytology and genotype of HPV in women commercial sex workers in Makassar to stabilized data of this area and for women commercial sex workers regarding the risk of HPV infection and transmission and the importance of educational programs for to prevent the transmission of cervical cancer.

Method and Sample

Our study conducted as an analytical study with cross sectional analysis approach which was carried out in a localization area of Makassar City for 6 months, starting from March 2019 to September 2019. Sampling was done by consecutive sampling i.e subjects in the study sites that meet the requirements inclusions were taken as research samples. The sample of this study consist of women with reproductive age who work as commercial sex workers, who meet the inclusion criteria that is female subjects who work as commercial sex workers, aged 15 - 50 years, actively engaging in sexual relations with more than 1 sexual partner in the past 1 month, the patient is not menstruating, the patient is not using tampons, vaginal medicines, vaginal contraception, vaginal douche for 48 hours before the examination, the patient is not allowed to have sexual course within 24 hours before the examination, and the patient is not pregnant. Our sample including 80 commercial sex workers who were willing to be involved in the study.

Subjects were asked to fill out a questionnaire accompanied by a research assistant. Furthermore, subjects were given an oral explanation of the examination and taking swabs in the endocervix. After the subject understands, it can be asked for willingness to participate in research. Informed consent sheet are provided for patient that willing to participate in this study.

The process of collecting specimens is carried out by the clinician using a broom like brush or endocervical brush and spatula to the ecto-endocervical transformation area. Then the endocervical brush is inserted into a container containing the PreservCyt solution (Cytec, Boxborough, MA) by turning/rinsing about 10 times. By using Thin Prep Processor homogenisation of specimens

with a certain centrifuge speed, and separating materials such as mucus, debris and washing blood from cells without damaging the grouping of cells. After going through the dispersion process, the cells are transferred to specific glass objects in a representative and accurate way through the filtration process and form a thin layer of one layer of cells (thin layer - monolayer cell). The next process of closing and closing the slide is in accordance with the routine process of pap's smear inspection.

Specimens from HPV (cervical swab) examination were put into a sterile 1ml Phosphate Buffered Saline (PBS) solution and then cortized for 3x15 seconds. Furthermore, 0.5 ml of each sample was used for the

extraction of nucleic acids and the remaining preparations were frozen at -20°C . Extraction, detection and determination of HPV type genotypes were performed using the PCR method. Data were analyzed with SPSS.

Result

During the study period, 80 female sex workers were involved in the study. Data distribution were normal by Kolmogorov Smirnov test when the p value is > 0.05 . In this study, the value of $p > 0.05$ was obtained indicating all data were normally distributed therefore chi-square statistical tests would be carried out.

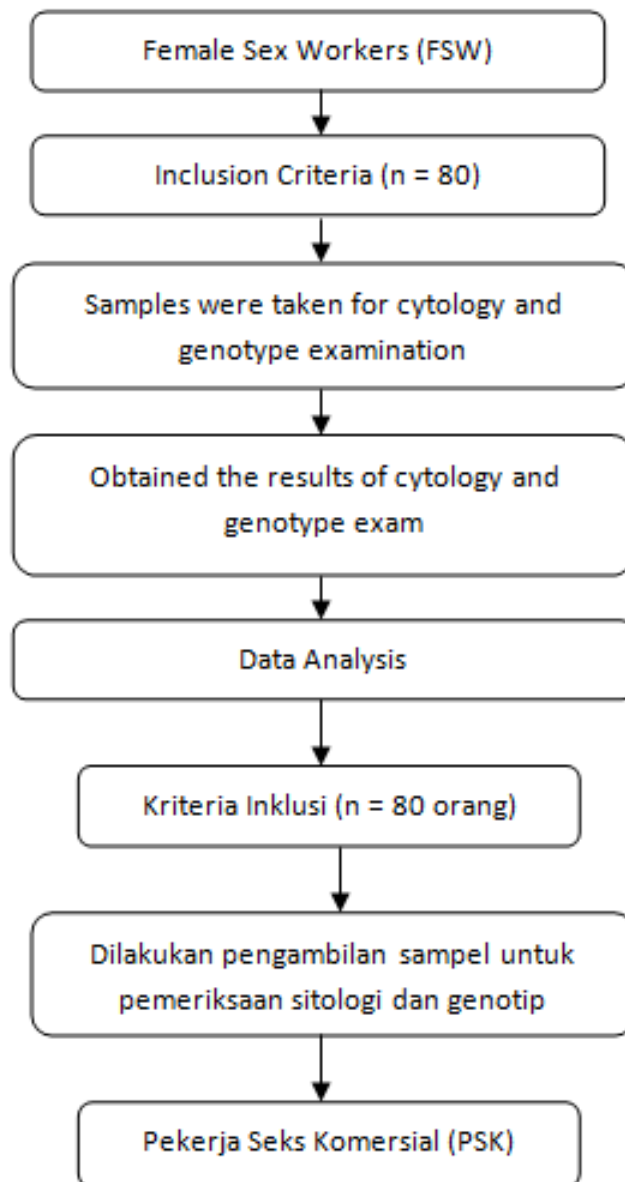


Figure 1 Flow chart

Tabel 1: Frequency Distribution of Variables

Variabel	N	%
Age		
<30 years	31	38,8
>30 years	49	61,3
Education		
<9 years	53	66,3
≥9 years	27	33,8
Marital Status		
Not married	18	22,5
Married	62	77,5
Parity		
Multiparous	16	20,0
Primiparous	14	17,5
Nuliparous	50	62,5
Duration being FSW		
<1 years	11	13,8
≥1 years	69	86,3
Age become FSW		
<15 years	7	8,8
≥15 years	73	91,3
Age ar first coitus		
<15 years	14	17,5
≥15 year	66	82,5
Sex partners in one month		
≥5	49	61,3
<5	31	38,8
Sex partners in last month		
≥5	33	41,3
<5	47	58,8
Condom usage		
Never/occasionally	40	50
Always	40	50
Sexual transmitted disease (STD)		
Yes	3	3,8
No	77	96,3

Table 1 showed that the majority of CSWs who became our sample were > 30 years old (61.3%), had received education in <9 years (66.3%), were married

(77.5%), nulliparous (62, 5%), became CSW >1 year (86.3%), age at CSW ≥15 years (91,3%), age at first coitus ≥15 years (82.5%), number of sex partners in a month ≥ 5 (61.3%), the number of last sexual partners in the last 1 month <5 (58.8%), did not experience sexually transmitted infections (96.3%). The number of CSWs using and not using condoms was comparable (50% each), and all samples has never vaccinated.

Table 2: Pre-Cancer Lesions Categories According to Cervical Cytology Examination Based on Fluid (SSBC) in Female Sex Workers

Tipe Lesi	n	%
Non-specific bacterial cervicitis	48	60,0
Negatif lesion	32	40,0

Our result in table 2 showed that most of samples have Non-specific bacterial cervicitis (60%) and the remaining 40% do not have lesions.

Table 3: Distribution Genotype HPV in Female Sex Workers

Lesion Type	n	%
High-risk HPV	20	25
Low-risk HPV	6	7,5
High-Low risk HPV	24	30
No HPV and other types	30	37,5

Table 3 showed the distribution of HPV genotypes in female sex workers, most of the samples had a 25% high risk HPV, a 7.5% low risk, a 30% high and low risk and 37.5% did not have an HPV.

Based on Figure 2 our study showed that 5 people infected with type 16 HPV, people infected with type 18 HPV were not found, 44 people infected with other types of High Risk HPV, 43 people infected with Low Risk HPV .

In Figure 3 it can be seen that of the 49 HPV types of high risk women of FSWs, HPV type 45 were detected in 6 people, type 51; 6 people, type 16; 5 people, type 33; 5 people, type 35; 5, type 52; 3 people, type 39; 2 people, type 66; 2 people, type 56; 2 people, type 68; 2 persons. And types 5,42,53,58,73 and 82 each in one person, respectively. The other types obtained in 5 people.

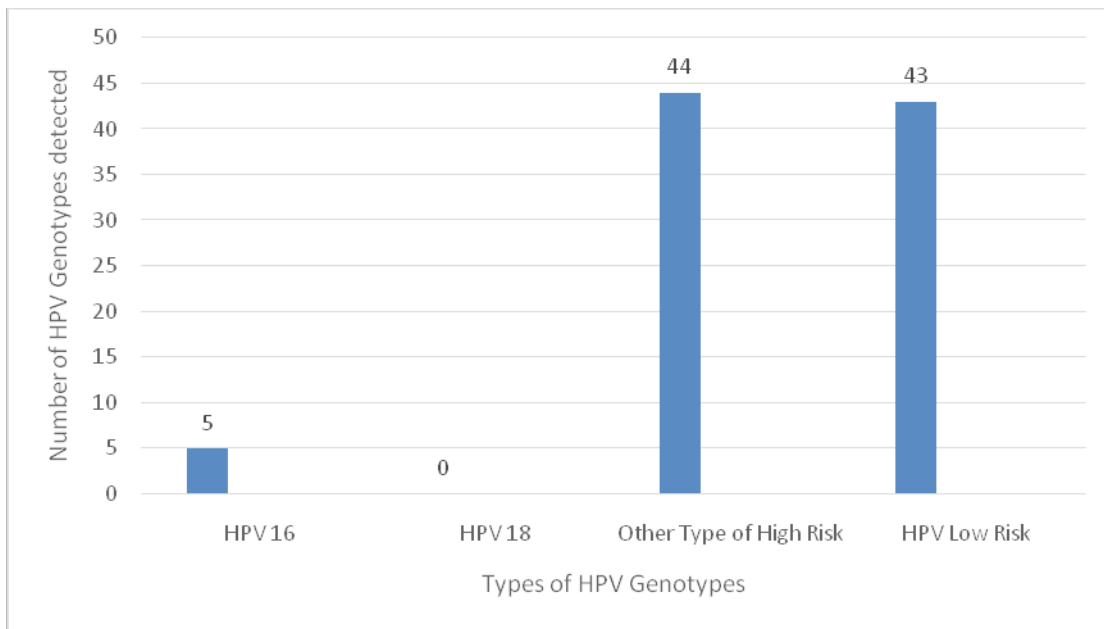


Figure 2 Distribution of HPV type 16, HPV type 18, high risk HPV, low risk HPV in female FSWs in Makassar

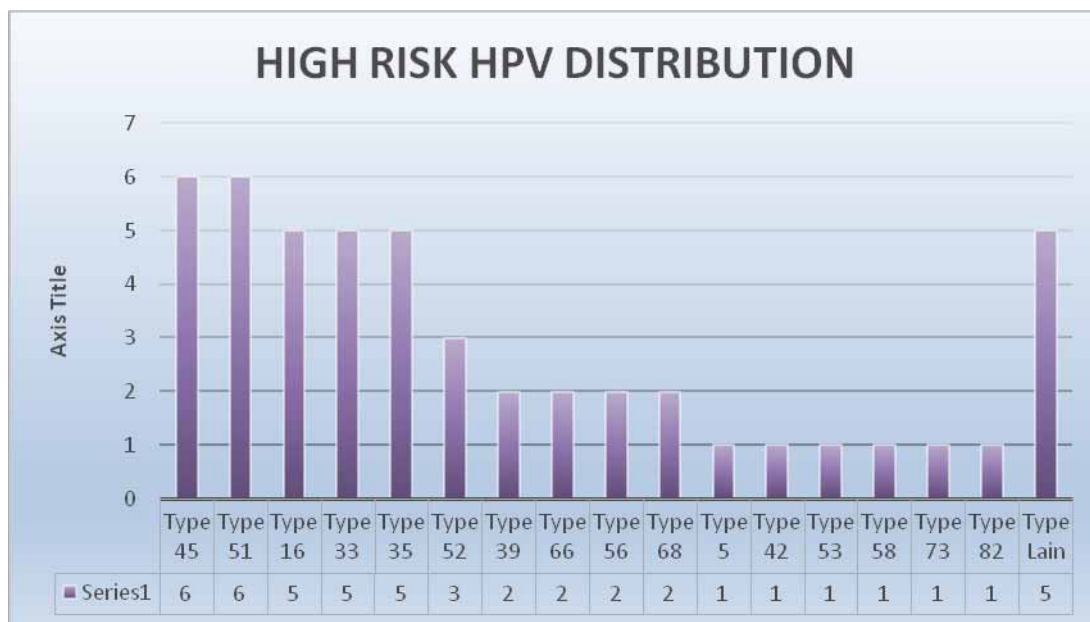


Figure 3 Distribution of High Risk HPV types in women of CSW in Makassar

Table 4: The relationship between the type of cytology of pre-cancerous lesions with the characteristics of female sex workers

Variable	Non-specific cervicitis and bacteria		Negative		p-value	OR (95% CI)
	n	%	n	%		
Age						
<30 years	20	41,7	11	34,4	0,640	1,364 (0,538–3,450)
>30 years	28	58,3	21	65,6		

Variable	Non-specific cervicitis and bacteria		Negative		p-value	OR (95% CI)
	n	%	n	%		
Education						
<9 years	34	70,8	19	59,4	0,412	1,662 (0,649–4,275)
≥9 years	14	29,2	13	40,6		
Marital Status						
Not married	9	18,8	9	28,1	0,477	0,590 (0,205–1,699)
Married	39	81,2	23	71,9		
Parity						
Multiparous	10	20,8	6	18,8	0,232	-
Primiparous	9	18,8	5	15,6		
Nuliparous	29	60,4	21	65,6		
Duration being CSW						
<1 year	9	18,8	2	6,2	0,185	0,289 (0,058–1,437)
≥1 years	39	81,2	30	93,8		
Age became CSW						
<15 years	3	6,2	4	12,5	0,429	0,467 (0,097–2,242)
≥15 years	45	93,8	28	87,5		
First coitus age						
<15 years	9	18,8	5	15,6	0,952	1,246 (0,376–4,130)
≥15 years	39	81,2	27	84,4		
Sexual partner within a month						
≥5	30	62,5	19	59,4	0,963	1,140 (0,456–2,850)
<5	18	37,5	13	40,6		
Sexual partner for last month						
≥5	21	43,8	12	37,5	0,746	1,296 (0,519–3,237)
<5	27	56,2	20	62,5		
Condom usage						
Never/Occasionally	20	41,7	20	62,5	0,110	0,429 (0,171–1,073)
Always	28	58,3	12	37,5		
STD						
Yes	3	6,2	0	0,0	0,271	0,938 (0,871–1,009)
No	45	93,8	32	100		

Uji Chi Square p<0.05

Table 4 illustrates the relationship between the type of cytology of pre-cancerous lesions and the characteristics of commercial sex workers. No association was found between age, education, marital status, parity, length

of time a prostitute, age at prostitution, age of first coitus, number of sex partners in the past 1 month, use of condoms and STIs with the type of cytology of pre-cancerous lesions (p <0.05). While the relationship to vaccine use cannot be analyzed because all samples have

never received an HPV vaccine.

Table 5: Relationship between High Risk HPV genotypes and characteristics of commercial sex workers

Variable	High Risk HPV		Other type-HPV		p-value	OR (95% CI)
	n	%	n	%		
Age						
<30 years	7	8,75	24	30	0,691	0,658 (0,230–1,881)
>30 years	13	16,25	36	45		
Education						
<9 years	15	18,75	38	47,5	0,339	1,737 (0,555–5,432)
≥9 years	5	6,25	22	27,5		
Marital Status						
Not married	5	6,25	13	16,25	0,757	1,205 (0,369–3,937)
Married	15	18,75	47	58,75		
Parity						
Multiparous	3	3,75	13	16,25	0,716	-
Primiparous	3	3,75	11	13,75		
Nuliparous	14	17,5	36	45		
Duration being FSW						
<1 year	6	7,5	5	6,25	0,015	4,714 (1,254–17,717)
≥1 years	14	17,5	55	68,75		
Age became FSW						
<15 years	3	3,75	4	5	0,253	1,941 (0,420–8,976)
≥15 years	17	21,25	56	70		
First coitus age						
<15 years	6	7,5	8	10	0,089	1,408 (0,456–4,351)
≥15 years	14	17,5	52	65		
Sexual partner within a month						
≥5	11	13,75	38	47,5	0,508	1,069 (0,387–2,955)
<5	9	11,25	22	27,5		
Sexual partner for last month						
≥5	8	10	25	31,25	0,895	1,439 (0,505–4,099)
<5	12	15	35	43,75		
Condom usage						
Never/Occasionally	10	12,5	30	37,5	1,000	1,069 (0,388–2,942)
Always	10	12,5	30	37,5		
STD						
Yes	0	0	3	3,75	0,308	1,351 (0,230–7,411)
No	20	25	57	71,25		

Chi Square test $p < 0,05$ Table 5 illustrates the relationship between the High Risk HPV genotypes and characteristics of commercial sex workers. The relationship between the High Risk HPV genotypes and characteristics of commercial sex workers is shown in Table 5. The relationship between the High Risk HPV genotypes and characteristics of commercial sex workers is shown in Table 5. The relationship between the High Risk HPV genotypes and characteristics of commercial sex workers is shown in Table 5.

Risk HPV genotype and the characteristics of female sex workers. No relationship was found between age, education, marital status, parity, age at becoming a sex worker, number of sex partners in the past 1 month, use of condoms and STIs with the HPV genotype ($p < 0.05$).

There was a significant correlation between duration of FSW and High Risk HPV genotype ($p = 0.015$) with an OR value of 4.714. That is, FSWs who have peddled sex for >1 year 4,714 times are more at risk of having high risk HPV.

Discussion

Cervical HPV infection is very contagious and the risk of infection increases with increasing sexual activity. Female sex workers are in a high-risk group infected and transmit HPV because having more than one sexual partner. In this study we tried to analyze the risk factors for pre-cancerous lesions and HPV genotype in female sex workers in Makassar City.

Based on the type of lesion, the majority of samples from FSW had non-specific and non-bacterial cervicitis (60%) and (40%) the rest had no lesions. The results of this study are also not much different from previous studies which showed that female sex workers had more positive cervicitis (80.4%) results compared with negative results (19.6%).¹² Cervicitis is a frequent case of sexually transmitted infections occur in female sex workers where cervical inflammation occurs as a result of infection by sexual organisms, most often caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*.¹³

Based on the distribution of HPV genotypes in female sex workers, most of the samples had high and low risk HPV (62.5%) and 37.5% did not have HPV. A meta-analysis of HPV infection in female sex workers in Asia found that the prevalence of HPV among female sex workers was 12.8 - 84.8%. Female sex workers are 10 times more likely to have HPV infection compared to the general population. The analysis also showed that the prevalence of HPV in the Southeast Asian region was dominated by HPV types 52 (12.9%), 16 (8.5%), 58 (5.2%), 18 (5.0%), and 66 (4.9%).¹⁰

Age is one of the factors causing precancerous lesions and HPV infection. In this study the majority of FSWs with non-specific cervicitis lesions and bacteria aged > 30 years. High-risk HPV infections are also more common in women > 30 years old (16.25%). However, in the bivariate analysis there was no association between

age with lesion type ($p = 0.640$) or HPV genotype ($p = 0.691$). The results of this study are supported by previous studies which showed that women aged 31 - 40 years and 41 - 50 years were most at risk of having pre-cancerous lesions (65% and 63.5%, respectively)¹⁴ However, contrary to the results of studies in women workers sexually reported in the Philippines that age 25 years or younger is more at risk of having an HPV infection.¹⁵

In this study, the majority of samples were low-educated. In samples with non-specific cervicitis and bacterial lesions 70.8% received education for < 9 years, while based on genotype type 18.75% samples with education < 9 years were infected with high-risk HPV. But in the bivariate analysis, education was not significantly related to the type of lesion and HPV genotype ($p = 0.412$ and 0.339 , respectively). The results of this study are supported by research in Nepal which found that HPV infection was lower in educated women compared to uneducated one.¹⁶

Most of the samples in this study were married. The prevalence of nonspecific and bacterial cervicitis lesions is higher in married women (81.2%). High-risk HPV infection is also more common in married women (18.75%). However, in the bivariate analysis no relationship was found between marital status and lesion type ($p = 0.447$) and high-risk HPV genotype (0.757). The results of this study are supported by previous studies which reported that the prevalence of HPV infection was higher in married women, especially married more than once.¹⁶

In this study the majority of samples were nulliparous women. Nonspecific and bacterial cervicitis lesions are higher in nulliparous women (60.4%). Low and high risk HPV infections are also more common in nulliparous women (17.5%). But in the bivariate analysis parity was not related to the type of lesion and HPV genotype ($p = 0.232$ and 0.716 , respectively). Most groups with positive pre-cancerous lesions had children < 4 (34.2%) and they also did not find a significant relationship between parity number and risk of developing cervical pre-cancerous lesions ($p = 0.280$).¹⁷

In this study non-specific and bacterial cervicitis lesion were higher in women who became FSWs for > 1 year (81.2%) but in contrast to high-risk HPV infections were more common in women who had FSWs > 1 year (17.5%). In the old bivariate analysis, FSW was not

related to the type of lesion ($p = 0.185$) but significantly correlated with high-risk HPV genotype ($p = 0.015$) with OR 4,714 (95% CI 1,254-17,717) meaning that being FSW >1 year was at risk. against high-risk HPV infection. This is consistent with the results of previous studies that reported that women who worked as FSWs for 6 months - 2 years were more at risk for HPV infection. But after 2 years, the risk of HPV is proven to decrease. This was hypothesized due to immunity to HPV in women who have worked as sex workers > 2 years.¹⁵

Most of the samples aged ≥ 15 years when they became FSWs, and the age at first coitus was mostly at the age of ≥ 15 years. No significant relationship was found between age at FSW and age at first coitus with nonspecific and bacterial cervicitis lesions as well as with high-risk HPV infection ($p = 0.429$ and $p = 0.952$); ($p = 0.253$ and $p = 0.089$). Women with non-specific bacterial and bacterial lesions and high and low risk HPV genotypes have more sexual partners ≥ 5 . However, no significant relationship was found between the number of sexual partners with the type of lesion and high-risk HPV genotype ($p = 0.963$ and 0.508 , respectively). The benefits of condoms in preventing HPV in women are still a matter of debate. There has been a reported decrease in HPV infection in female sex workers who use condoms, but it is not fully effective in protecting against HPV infection. In this study, no relationship was found. There is an argument that condoms cannot completely cover the infected area

This study has several limitations including the small sample size and cross-sectional design. And in this study the sample collected is still under the institution where the results obtained may be different from the free sample or freelance. Further research is needed with larger samples and better designs to confirm the results of this study.

CONCLUSION

Based on cytological examination of precancerous lesion types in female sex workers in Makassar, most of the FSW samples had non-specific and bacterial cervicitis (60%) and the remaining 40% had no lesions. No relationship was found between age, education, marital status, parity, age at becoming a sex worker, number of sex partners in the past 1 month, use of condoms and STIs with high-risk HPV genotype ($p < 0.05$). There was a significant correlation between duration became FSW and high risk HPV genotype (p

$= 0.015$) with an OR value of 4.714. That is, FSWs who have work as sexual worker for >1 year 4,714 times are more at risk of having high risk HPV.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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