Research Article

The Risk of Infection Human Papilloma Virus Infection in Acceptors of Depot Medroxyprogesterone Acetate Contraceptions

Risiko Infeksi Human Papilloma Virus pada Akseptor Kontrasepsi Depot Medroxyprogesterone Acetat (DMPA)

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Abstract

Objective: Cervical cancer is the second most prevalent cancer in women around the world and the most common cancer in women causing death. This study aims to analyze the connection between infection of human papilloma virus (HPV) 16/18 and cervical changes in the acceptors of Depot Medroxyprogesterone Acetate (DMPA) Contraceptions and nonacceptors of Depot Medroxyprogesterone Acetate (DMPA) Contraceptions.

Methods: The research was conducted at the Public Service Institution of Dr. Wahidin Sudirohusodo hospital, and private midwife clinics for seven months from December 2015 to June 2016. The research design is cross-sectional with. The samples were forty acceptors of Depot Medroxyprogesterone Acetate (DMPA) and forty non-acceptors of Depot Medroxyprogesterone Acetate (DMPA) contraception. Prevalence of HPV 16/18 and cervical cytology changes were examine using the polymerase chain reaction and liquid base cervical cytology.

Results: The results showed there was no significant relationship between long-term use of DMPA contraceptives with HPV 16 and 18. There was no significant relationship between long-term use of DMPA contraceptives with cervical cytology changes. There was no significant relationship between HPV 16 and 18 infections with the occurrence of cervical cytology changes in long-term use of DMPA contraceptives.

Conclusion: The long-term use of DMPA contraceptive does not increase the risk of HPV 16 and 18 infections. Also does not cause cervical cytology changes that lead to cervical malignancy.

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Keywords: cervical cytology changes, Depot Medroxyprogesterone Acetate (DMPA) contraception, HPV 16/18 infection

Abstrak

***Tujuan**: Kanker serviks merupakan kanker kedua terbanyak pada wanita di seluruh dunia saat ini dan merupakan kanker terbanyak pada wanita yang menyebabkan kematian. Penelitian ini bertujuan mengetahui hubungan antara infeksi human papilloma virus 16/18 dan perubahan sitologi serviks pada akseptor kontrasepsi DMPA dibandingkan dengan non akseptor kontrasepsi DMPA.*

***Metode**: Penelitian ini menggunakan rancangan potong lintang. Penelitian ini dilakukan di BLU RS Dr. Wahidin Sudirohusodo, rumah sakit jejaring serta Bidan Praktek Swasta. Penelitian ini dilakukan selama 7 bulan, yaitu mulai bulan Desember 2015- Juni 2016. Sampel sebanyak 40 akseptor kontrasepsi DMPA dan 40 non akseptor kontrasepsi DMPA. Infeksi HPV 16/18 dan perubahan sitologi serviks diperiksa melalui polymerase chain reaction dan Sitologi Serviks Berbasis Cairan.*

***Hasil**: Hasil penelitian menunjukkan tidak terdapat hubungan bermakna antara penggunaan kontrasepsi DMPA jangka panjang dengan infeksi HPV 16 dan 18. Tidak terdapat hubungan bermakna antara penggunaan kontrasepsi DMPA jangka panjang dengan terjadinya perubahan sitologi serviks. Tidak terdapat hubungan bermakna antara infeksi HPV 16 dan 18 dengan terjadinya perubahan sitologi serviks pada penggunaan kontrasepsi DMPA jangka panjang.*

***Kesimpulan**: Penggunaan kontrasepsi DMPA jangka panjang tidak meningkatkan risiko terjadinya infeksi HPV 16 dan 18. Juga tidak menyebabkan terjadinnya perubahan sitologi serviks yang mengarah kepada keganasan serviks.*

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***Kata kunci**: infeksi HPV 16/18, kontrasepsi Depot Medroxyprogesterone Acetate (DMPA) Contraceptions, perubahan sitologi serviks*

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INTRODUCTION

Cervical cancer is the second most prevalent cancer in women around the world today and the most common cancer in women that causes the death of primarily young women. Based on the World Health Organization (WHO) statistical data, there are about 500,000 new cases and 250,000 deaths each year.[1] In general, higher incidences are found in developing countries, and these countries contribute 83% of reported cases annually. Economically advantaged countries have significantly lower cervical cancer rates and add only 3.6% of new cancers. This incidence disparity highlights successes achieved by cervical

cancer screening programs in which Papanicolaou (Pap) smears are regularly obtained. In 2006, the American Cancer Society estimated 9,710 new cases and 3,700 deaths from this malignancy.[2] Data from the Ministry of Health in Indonesia, cervical cancer and breast cancer still the highest prevalence among malignant gynecologic tumors. In 2013, the prevalence of cervical cancer was 0.8%, and breast cancer is 0.5%.

HPV 16 and HPV 18 because this is a high-risk HPV found in almost all cervical carcinoma. In the United States and Europe, HPV 16 is the most prevalent type found in approximately 50% of cases, while types 18, 31 and 45 found in approximately 25 - 30% of cases.[3]

Progesterone contained in the Combined Oral Contraceptives (COCs) causes depletion of squamous epithelium thus it is more susceptible to HPV infection.[4] The relationship between the use of DMPA contraceptive with HPV infection is debatable. Research says that epithelial atrophy becomes more susceptible to damage, so it becomes more susceptible to HPV infection. This may explain that DMPA is positively associated with oncogenic HPV infection. We found that the use of DMPA results in depletion of the epithelium.[5]

Progesterone is previously considered as a major candidate hormone in the cervical neoplastic caused by the immunosuppressive effects and the likely associated with HPV infection. Human papilloma virus tends to infect the cells with progesterone receptors. Both HPV 16 and 18 contain progesterone and glucocorticoid response elements that increase the expression of oncogenic HPV E6 and E7, which are considered crucial in the transformation of cells with the gestagenic stimuli.[6]

By knowing HPV as the primary cause of cervical cancer, there is new development for the detection of HPV as screening for uterine cervical carcinoma. Therefore, HPV cannot be grown in culture, the HPV DNA testing with the methods of molecular biology is an accurate way to detect HPV infection and HPV typing by PCR (Polymerase Chain Reaction). In addition, early detection of cervical cytological changes in contraceptive DMPA acceptors is also important for the change in cervical cytology is a risk factor for HPV infection. For a screening of cervical cytological changes, the most commonly used today is conventional Pap smear. However, the conventional Pap smear has the limitations, i.e., the false negative rate of 14 - 33% and two-thirds are caused by the process of sampling or sample preparation. This leads to inaccurate and equivocal diagnosis. Currently, the major advanced screening technique is liquid-based cytology. The transition from conventional cytology to liquid-based cytology techniques is due to the increased sample quality, reproducibility, sensitivity, and specificity similar to molecular testing.[7]

METHOD

This research was conducted at several hospitals in Makassar, health centers and in Private Practice Midwife (BPS) Hj. Markarmah. Preparations that have been taken from the research subjects were sent to the Laboratory of Anatomical Pathology for liquid-based cervical cytology and the Laboratory of Microbiology for PCR. The research began in December 2015 - June 2016.

This was a cross-sectional study. The population in this research was contraceptive DMPA acceptors, and controls were those who did not receive DMPA acceptors. The sample was injected contraceptive DMPA acceptors, and the control group were women who did not use progestin injection hormonal contraceptive and met the inclusion criteria and have signed the informed consent to participate in the study.

All subjects who met the inclusion criteria were taken in accordance with the estimated sample size. Two examinations, i.e., PCR and liquid-based cytology with LC-Prep, were done on the subjects. Test results were recorded and then analyzed.

The data that have been collected and analyzed were then processed by computer using SPSS for windows. The statistical method used was Bivariate Analysis Chi-square test and Fisher's exact.

RESULTS

The basic characteristics of the samples showed that of the 80 research subjects involved, the largest age group was 31 - 45 years old (57.5%) with education level of more than nine years (71.25%). Employment status of the research subject was generally work (56.25%). The age at the first coitus was generally 20 - 25 years

(73.75%) with multiparity (63.75%). The length of contraceptive use was over three years (52.5%) with complaints of menstrual disorders (47.5%) and a complaint of menstrual disorders with vaginal discharge (50%). The research subjects using contraceptive DMPA were mostly aged 31 - 45 years (60%), with education level of more than nine years (55%). Employment status was not working (55%). All contraceptive DMPA acceptors were already married and the age at the first coitus was 20 - 25 years (77.5%) with multiparity (80%). The length of contraceptive use was mostly more than three years (52.5%) with the majority of complaints of menstrual disorders (97.5%). Based on the results of Chi-square test and Fisher's exact, the homogeneous characteristics of the research subjects obtained were age group, education, occupation, marital status, age at the first coitus, duration of contraceptive use and the type of complaint (p> 0.05). (Appendix, Table 1).

HPV 16 infection based on PCR showed that the research subjects who used contraceptive DMPA had positive for HPV 16 of 1 people (2.5%) and negative for HPV 16 of 39 people (97.5%). The research subjects who did not use contraceptive DMPA all had negative for HPV 16 of 40 people (100%) (Appendix, Table 2).

Table 1. Characteristics of the Subjects

Characteristics	DMPA Contraceptive					
	Yes		No		Total	
	n	%	n	%	n	%
Age (year)						
20 - 30	16	40	18	45	34	42.5
31 - 45	24	60	22	55	46	57.7
Education Level						
≤ 9 year	22	55	1	2.5	23	28.75
> 9 year	18	45	39	97.5	57	71.25
Employment						
Unemployment	18	45	27	67.5	45	56.25
Employment	22	55	13	32.5	35	43.75
Age first time coitus (year)						
20 - 25	31	77.5	28	70	59	73.75
> 25	9	22.5	12	30	21	26.25
Parity						
Nulli/Primipara	8	20	21	52.5	29	36.25
Multipara	32	80	19	47.5	51	63.75
Duration contraceptive use (year)						
≤ 3	19	47.5				
> 3	21	52.5				
Complain						
No complain	1	2.5				
Menstrual disorder	19	47.5				
Menstrual disorder and flor albus	20	50				

Table 2. HPV 16 Infections

Subject	HPV 16						p
	Positive		Negative		Total		
	n	%	n	%	n	%	
Contraceptive DMPA acceptors	1	2.5	39	97.5	40	100	
Non Contraceptive DMPA acceptors	0	0	40	100	40	100	1.000
Total	1	1.2	79	98.8	80	100	

HPV 18 infection based on PCR showed that the research subjects who used contraceptive DMPA had positive for HPV 18 of 1 people (2.5%) and negative for HPV 18 of 39 people (97.5%). The research subjects who did not use contraceptive DMPA all had negative for PCR 18 of 40 people (100%). Fisher's exact test results obtained by value p = 1.000 (p> 0.05). It means there is no relationship between hormonal contraceptive use in combination with the incidence of HPV 18 infection (Appendix, Table 3).

Cervical cytology based on liquid-based cervical cytology examination showed that the research subjects who used contraceptive DMPA found eight people who experienced cervical cytological changes (20%). While for non-contraceptive DMPA acceptors, there were six people who experienced cervical cytological changes. Most of the samples from both groups that did not experience changes in cervical cytology were 66 people (82.5%) (Appendix, Table 4).

DISCUSSION

This study shows that the use of contraceptive DMPA has not been associated with HPV 16/18 infection and cervical cytological changes. This study is conducted on contraceptive DMPA acceptors for this contraceptive type is one risk factor for cervical carcinoma with HPV infection.

In this study, we examined HPV 16 and HPV 18 because this is a high-risk HPV found in almost all cervical carcinoma. In the United States and Europe, HPV 16 is the most prevalent type found in approximately 50% of cases, while types 18, 31 and 45 found in approximately 25 - 30% of cases.[3]

Based on the results of Chi-square test and Fisher's exact, the homogeneous characteristics of the research subjects obtained were age group, education, occupation, marital status, age at the first coitus, duration of contraceptive use and the type of complaint (p> 0.05). Most of the research subjects had multiparity and used contraceptive DMPA.

To determine the effect of contraceptive DMPA to the HPV infection clearly, the risk factors that may increase HPV infections such as first sexual intercourse before age 20 years, parity more than four, having multiple sexual partners and smoking habits are excluded.

In this study, 80 subjects of the research found one case of HPV 16 infection based on PCR on contraceptive DMPA acceptor group with incidence rates of 1.2% and one case of HPV 18 infection with the incidence rate of 1.2%. There was no HPV infection in the control group. After Fisher's exact test to determine the relationship between contraceptive DMPA with HPV 16 and HPV 18 obtained results that were not statistically significant. This is consistent with research conducted by Morgan *et al*., which conducted follow up to 1,135 women (376 COC acceptors, 331 DMPA acceptors, and 428 non- contraceptive acceptors) for 18 months. They found new HPV infection in 269

Table 3. HPV 18 Infections

Subject	HPV 18						p
	Positive		Negative		Total		
	n	%	n	%	n	%	
Contraceptive DMPA acceptors	1	2.5	39	97.5	40	100	
Non Contraceptive DMPA acceptors	0	0	40	100	40	100	1.000
Total	1	1.2	79	100	80	100	

Table 4. Cervical Cytological Changes

Subject	cervical cytological changes						p
	Positive		Negative		Total		
	n	%	n	%	n	%	
Contraceptive DMPA acceptors	8	20	32	80	40	100	
Non Contraceptive DMPA acceptors	6	155	34	85	40	100	0.77
Total	14	17.5	66	82.5	80	100	

women and high-risk HPV infection in 157 women. However, after adjusting for age, number of sexual partners, new sexual partner, bacterial vaginosis infection and duration of the use of COCs and DMPA, the relation between the detection of new HPV infection with the use of COCs reduced even not statistically significant.[4]

The use of hormonal contraceptives such as depomedroxyprogesterone acetate (DMPA) has been associated with an increased risk of cervical cancer and is considered as a co-factor in cervical carcinogenesis. The increased risk of disease is observed in women who use hormonal contraceptives of long-term progesterone injection such as DMPA. Progesterone contained in the Combined Oral Contraceptives (COCs) causes depletion of squamous epithelium thus it is more susceptible to HPV infection.[4] Based on the research results, Tiffany G *et al.*, stated that use of DMPA within one year or more is related significantly to the detection of oncogenic HPV.[5]

Progestin contained in contraceptive DMPA can affect cervical cytology. The relationship between the use of DMPA contraceptive with HPV infection is debatable. Research says that epithelial atrophy becomes more susceptible to damage, so it becomes more susceptible to HPV infection. This may explain that DMPA is positively associated with oncogenic HPV infection. We found that the use of DMPA results in depletion of the epithelium.[5]

Progesterone is previously considered as a major candidate hormone in the cervical neoplastic caused by the immunosuppressive effects and the likely associated with HPV infection. Human papilloma virus tends to infect the cells with progesterone receptors. Both HPV 16 and 18 contain progesterone and glucocorticoid response elements that increase the expression of oncogenic HPV E6 and E7, which are considered crucial in the transformation of cells with the gestagenic stimuli.[6]

In this study, of the 80 research subjects found two cases of HPV infection, each infection with HPV 16 and HPV 18 in contraceptive DMPA acceptors. On the samples with positive HPV 16 infection found cervical cytological changes and on the samples with positive HPV 18 infection also found cervical cytological changes in the form of endocervical cells undergoing squamous metaplasia and found halo-perinuclear representation. Based on Fisher's exact test results, there was no association between HPV 16/18 infection with cervical cytological changes in the contraceptive DMPA acceptors.

Normal cervical cytology can be found in the latent phase of virus infection. In these circumstances, there are no lesions, but there is exposure to the virus without causing infection. In this phase, the virus cannot attach to the surface of cells or penetrate cells since there are no or less cell surface receptors specific for HPV. It can also occur in cases where the virus has entered the cell but has failed to do multiplication or no maturation of viral particles. In this phase, the HPV virus can only be detected with bio-molecular methods.

Genital HPV infection is very common, mostly asymptomatic, does not cause a change in the tissue and therefore is not detected on Pap smear. The prevalence of HPV in cervical cytology in women with normal Pap smears peaked at the age of 20 - 24 years.

Not all women infected with human papillomavirus (HPV) 16/18 have detectable levels of anti HPV-16/18 antibodies. Women who seroconvert develop low antibody levels and seroconversion occurs within months and varies among women. The slow and weak antibody response generated by HPV infections could be explained by its life-cycle in the host. HPV is shed within intact cells lining mucosal surfaces, which limits exposure of the host immune system to the virus. HPV infected cells that undergo lysis (i.e., koilocytes) are shed to the environment and infections do not produce viremia. Finally, infections produce a limited load of HPV antigenic proteins.[8]

Cervical cytological changes by HPV infection are also associated with higher levels of viral load in the host and the type of HPV. Previous studies conducted on cervical cell of women with cervical lesions (cancerous or pre-cancerous lesions) found that the average level of viral load is higher in women who are positive HPV.

This study has advantage and disadvantage. The advantage of this study is that confounding factors which can lead to a high incidence of HPV infection, primarily high-risk HPV such as age at the first coitus, parity, and number of sexual partners, are removed. This is done so that the effect of contraceptive DMPA against HPV infection,

primarily high-risk HPV, can be seen clearly. However, HPV DNA bio-molecular examination in this study is devoted to the high-risk HPV types 16/18 so that other high-risk HPV types cannot be detected.

CONCLUSION

The researcher concludes that out of 80 samples found HPV 16 infection of 1.2% and HPV 18 infection of 1.2%. The role of contraceptive DMPA against HPV 16/18 infection and cervical cytological changes cannot be proven through PCR and cervical cytology examination. In addition, the relationship between HPV 16/18 infection with cervical cytology changes in hormonal contraceptive DMPA acceptors has also not been proven. Despite of this study found no relationship between duration of use of contraceptive DMPA with HPV infection and cervical cytological changes, the two samples infected with HPV 16/18 are found in contraceptive DMPA acceptor groups that are also experiencing cervical cytological changes. The researcher suggests that further research on HPV types 16/18 and other HPV types of hormonal contraceptive DMPA acceptors is required. Further research with greater samples to determine the prevalence of HPV infection and cervical cytology changes in contraceptive DMPA users is also required.

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