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Association between +874 T>A polymorphism IFNG gene and cervical cancer patients in Bandung.

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ABSTRACT

Introduction : Many women are infected with human papillomavirus (HPV), however, only a subset of women infected with persistent high-risk types of HPV will ever develop cervical cancer. Interferon gamma (IFN-γ) is one of the key regulatory cytokines that influence the HPV clearance. The production and the function of IFN-γ may impaired by the defect of the *IFNG* gene, leading to the cervical malignant progression.This study aimed to examine the association between *IFNG* +874 T>A polymorphism and cervical cancer.
Methods: In a case-control study design, consecutive untreated women with cervical cancer who showed for the first time in HasanSadikin Hospital Bandung were enrolled (n =98). Their controls were women who came for PAP smear (n = 81), and were not matched in ages and ethnicities. DNA extracted from blood was amplified by amplification refractory mutation system - polymerase chain reaction (ARMS – PCR) to detect *IFNG* +874 T>A polymorphism.
Results: The distribution of *IFNG* genotypes TT, TA and AA for women with cervical cancer who met the inclusion criteria (n = 64) and with negative intraepithelial lesion or malignancy (n = 42) were 14.1%, 50.0%, 35.9% and 7.1%, 52.4%, 40.5%, respectively. No significant differences could be observed between both groups (p= 0.64). Stratifying the cervical cancer women into a group of squamous cell carcinoma (n = 54) revealed no statistical different.
Conclusions: In this study, *IFNG* +874 T>A polymorphismseems not to contribute in susceptibility to cervical cancer. Larger participants for genetic study are required to detect true association for this polymorphism. Identification of other variants in *IFNG* gene signaling and its role in the development of cervical cancer diseases need to be further examined to provide information for the biological marker, useful for the development of diagnostic and therapeutic strategies.

Introduction

Cervical cancer is the second highest incidence rate in the world after breast, and this cancer continues to be main health issue among women,[1,2] In Indonesia, cervical cancer is the most common gynecologic cancer.[3]Chronic infection of human papillomavirus (HPV) is associated with cervical cancer.[4]Recent studies show that there are more than 100 types of HPV have been identified. More than 30 types infect the human genital tract, classified in high and low risk HPV according to the lesion they cause, i.e. high risk HPV genotype (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82, 26, and 53) and low risk HPV genotype(6, 11, 40, 42, 43, 44, 54, 61, 72, and 81).[5]In cervical cancer tissue, single infection of high risk HPV, or multiple infection high risk HPV with low risk HPV genotypes can occur.[6,7]

A meta analysis has shown that HPV 18 was the type most commonly found in adenocarcinoma of cervix, while HPV 16 was more commonly found in squamous cell carcinoma cervix.[5,8]HPV infection was found to be associated with some risk factors such as having multipartner sexual and early age of first marriage which younger than 16 years old..[12,13]Study in India exploring the risk factors for having cervical cancer such as married at a very young age i.e. less than 16 years old, multipartner sexual or have been married more than once were found to have no significant relationship in determining which type of infection the patients.[9]In contrary, study in Costa Rica showed that the number of women with multiple HPV infection was significantly higher in women with multipertner sexual.[10]

Interferon gamma (IFN-γ) is a pro-inflammatory cytokineplays a role in antiproliferative, antitumor and antiviral activities, and both innate and adaptive immune responses that may influence the HPV clearance, suggesting that IFN-γ play a key role in the development of cancers.[13]The susceptibility to HPV infection that leads to cervical cancer may influenced by the *IFNG*gene encoding IFN-γ. Study in HPV-infected patients point out the role of the IFN-γ in the infection control, for example low IFN-γ production or IFN-γ function impair may lead to the cervical malignant progression.[14]Our study aimed to investigate the possible association between of *IFNG*in particular +874T>A polymorphism and cervical cancer in Bandung, Indonesia.

Method

In a cross sectional study with retrospective approach, DNA was isolated from venous blood collected by EDTA tubes according to the manufacture's protocol (Qiagen Blood Mini Kit, Germantown, MD). Clinical data were obtained from medical records and histopatologically confirmed cervical cancer data was collected. In brief, bi-allelic *IFNG*+874 T>A polymorphism was conducted by amplification refractory mutation system method, modification of polymerase chain reaction technique (ARMS – PCR).This technique was then compared with other machine, BeadXpress Reader (Illumina)®. For ARMS-PCR, condition was 95ºC for 1min, 10 cycles of denaturation 95ºC for 15s, annealing 62ºC for 50s, extension 72ºC for 40s, followed by 20 cycles of denaturation 95ºC for 20s, annealing 56ºC for 50s, and extension 72ºC for 50s, and final extension 72ºC for 10 min then 4ºC for eternity, then analysed by electrophoresis (1.5%), showed 261 bp band. For BeadXpress (Illumina)®, was performed according to manufacture's protocol. Written consent was obtained from all subjects, and the study protocol was approved by the Institutional Review Board from University Padjadjaran, Bandung.

Statistical Analyses

The Hardy-Weinberg equilibrium (HWE) and program CONTING was used to calculate χ^2.SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was analysed for data questionnaires and genotypings. OR is determined to calculate the possible significant differences in genotypes. All statistical analyses were two-sided and *P* values <0.05 were considered as statistically significant.

Results

In total, 98 subjects with cervical cancer were recruited of whom 29 had missing histological data, 5 had no malignant cells found in the samples thus, only 64 participants were furtheranalysed. Squamous cell carcinoma (SCC) non-keratinizing were more prevalent in the study population (*n* 53 of 64; 82.8%). The analysis was only based on the histopathological data because the clinical data were not written in some of the medical record, such as age, marriage status, number of children, smoking status and other high risk sexual behavior making the data analyses difficult. In the control group, 18 had missing histological data. 21 participants showed abnormal findings in the cytology examination such as low-grade squamous intraepithelial lesion (n=16) and high-grade squamous intraepithelial lesion (n= 5) therefore those were excluded.

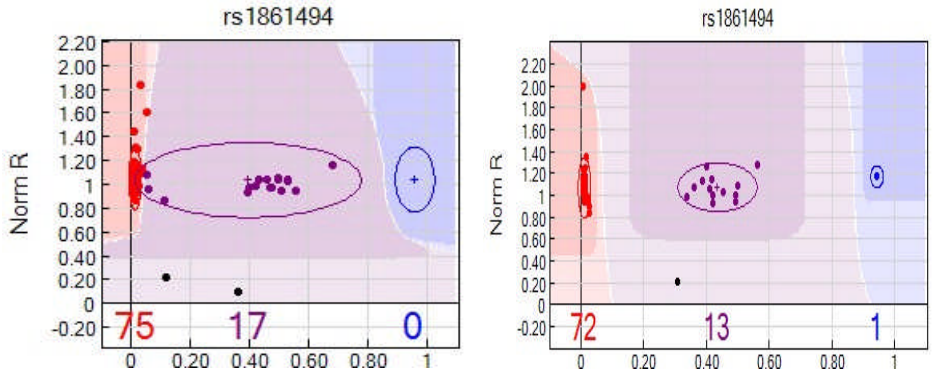
IFNG +874 T>A polymorphism

The genotype of the *IFN* +874 T>A polymorphism was in Hardy – Weinberg equilibrium in the total group of individuals as well as in the patients and controls group. The distribution of *IFNG* genotypes TT, TA and AA for women with cervical cancer who met the inclusion criteria (*n* 64) and with negative intraepithelial lesion or malignancy (NILM) (*n* 42) were 14.1%, 50.0%, 35.9% and 7.1%, 52.4%, 40.5%, respectively, and no significant differences could be observed between both groups (*p* 0.64). The result using ARMS-PCR confirmed the result using BeadsXpress (Illumina)®, as shown in table 1 and figure 1

Tabel1.
Distribution *IFNG* T874A in cervical cáncer and control

SNP	alelle / genotype	Cases n (%)	Control n (%)	*P*
IFN	T	50 (39.1)	28 (33.3)	0.39
+874 T/A	A	78 (60.9)	56 (66.7)	
	TT	9 (14.1)	3 (7.1)	0.64
	TA	32 (50.0)	22 (52.4)	
	AA	23 (35.9)	17 (40.5)	

Figure 1.
DistributiononBeadXpress Reader (Illumina)®



Discussion

IFN gene encodes the cytokine IFN-γ, thus, host genetic in immune response to HPV infection may play a role in the susceptibility to disease risk.[16] A single nucleotide polymorphism located in the first intron of the *IFNG* gene can influence the secretion of cytokine.[17] The *IFNG* genotype TT is determined of high production of IFN-γ, and *IFNG* genotype TA and AA are for intermediate and low production of IFN-γ, respectively. [11] Interestingly, ethnic background may influence the distribution of cytokine gene polymorphism.[18] For example, studies in various populations had showed that there was a clear correlation between ethnicity and distribution of *IFNG* polymorphism across different population groups.[19]*IFNG*+874T>A polymorphism are frequently studied in intracellular infectious disease such as tuberculosis[20] and cancer.[21]

Here we examined the *IFNG*+874T>Ausing PCR-ARMS technique. Next to PCR-ARMS technique,we also examine the same genotype by BeadXpress system. Interestingly, different or discordant result occurs, however, both texhnique resulted in no association between the SNP and the cancer susceptibility. Although meta analyses of *IFNG*+874T>A polymorphism were not associated with cancer in general, this polymorphism showed an association with cervical cancer.[14, 21] In contrary, studies on *IFNG*polymorphism and cervical cancers showed conflicting result, for example study from South Africa revealed no significant different between cervical cancer patients and control.[19]The susceptibility to HPV infection that leads to cervical cancer may influenced by the variations in *IFNG*gene that encodes cytokine IFN-γ. Our study showed

no significant difference between cervical cancer patients and their control with negative intraepithelial lesions (NILM).

Our limitation in this study is the incomplete data on medical records i.e. age and ethnicity. It seems that the ethnicity is associated with the chance of getting cervical cancer. The distribution of the T and A allelic and genotypic frequencies in our study result is in concordance with the study in Brazil,[22] however, in contradictory to this study, after stratifiying in a more homogenous group e.g. only squamous cell carcinoma (SCC) and NILM, *no* significant difference was found. Interestingly, the TT genotype, designated for IFN-γ high levels producer, were less than the AA genotype i.e. the IFN-γ low levels producer in our population similar to the studies in India and in Brazil.[14,122]Different in ethnicity background may play a role in the distribution of *IFNG* +874 genotype.

Because the number of participants recruited is also limited, the distribution of *IFNG* +874 T/A polymorphism in our population may not reflect the cervical cancer in Indonesia. Better management in medical record may be valuable for future studies.

To conclude, *IFNG* polymorphism at +874 T>A seems not to contribute to susceptibility to cervical cancer in population from Bandung. Dissecting the mechanism in immunological pathways against HPV infection may give a clear insight for future immunotherapy.

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