UNIVERSA MEDICINA

*September-December, 2014* *Vol.33 - No.3*

# Epidermal growth factor polymorphism most prevalent in stage II cervical carcinoma

**Kevin Dominique Tjandraprawira*, Ramdan Panigoro**, Yudi Mulyana Hidayat***, Herman Susanto***, and Edhyana Sahiratmadja****

*Faculty of Medicine, Padjadjaran University
**Department of Biochemistry, Padjadjaran University
***Department of Obstetrics and Gynecology, Dr. Hasan Sadikin General Hospital

**Correspondence**
Kevin Dominique Tjandraprawira
Faculty of Medicine, Padjadjaran University
Jalan Raya Jatinangor KM 21
Jatinangor
West Java, Indonesia
Email: kevintjandraprawira@gmail.com
Phone: +62813-20221221

*Univ Med 2014;33:192-8*

## ABSTRACT

**BACKGROUND**
Cervical cancer ranks second among female cancers worldwide and is widely associated with human papilloma virus (HPV) infection. However, HPV infection progression is influenced by various host factors. Epidermal growth factor (EGF) is a host factor important for proper epithelial proliferation and development, and may play a role in cervical cancer progression. A functional A61G polymorphism in the EGF gene has been hypothesized to alter EGF concentration in vivo with increasing guanine content associated with greater EGF level. However, a map of A61G polymorphism distribution is not available for any population, including Indonesia. This study aims to determine the distribution of EGF A61G polymorphism among cervical cancer patients at Dr. Hasan Sadikin General Hospital.

**METHODS**
A retrospective cross-sectional study was conducted between July-November 2010. Included were 61 cervical cancer patients of various stages at Dr. Hasan Sadikin hospital, who had previously undergone blood sample collection, DNA isolation and finally genotyping for EGF gene using Illumina BeadXpress®. Chi-square test was used to analyse the data.

**RESULTS**
The EGF A61G polymorphism was exhibited by 88.5% of patients (as genotypes A/G and G/G). The majority of patients with this polymorphism were of moderate severity (FIGO stage II and III, 42.6% and 38.1% respectively). Patients with the polymorphism but with the lightest severity (FIGO stage I) accounted for 22.2% of the population.

**CONCLUSION**
EGF A61G polymorphism affected the majority of cervical cancer patients and that once stratified, the patients showed intermediate severity in terms of their cancerous growth.

**Keywords:** Cervical cancer, EGF, A61G polymorphism

# *Polimorfisme A61G pada gen EGF paling banyak pada karsinoma serviks stadium II*


***ABSTRAK***

***LATAR BELAKANG***

*Kanker serviks merupakan kanker kedua terbanyak pada perempuan di seluruh kanker serviks hampir selalu diasosiasikan dengan infeksi* human papilloma virus *(HPV). Akan tetapi, berbagai faktor penjamu (*host*) telah dibuktikan dibutuhkan untuk memungkinkan progresi infeksi ini kedalam suatu massa neoplastic.* Epidermal growth factor *(EGF) merupakan sebuah faktor pertumbuhan dari penjamu yang diperlukan untuk proliferasi dan diferensiasi dari jaringan epitel. Sebuah polimorfisme A61G fungsional pada gen EGF diduga dapat mengubah konsentrasi EGF* in vivo *di mana konsentrasi guanin yang lebih tinggi pada genotip seseorang dikaitkan dengan konsentrasi EGF yang lebih besar pula. Akan tetapi, hingga sekarang belum ada peta distribusi polimorfisme A61G untuk populasi Indonesia. Penelitian ini bertujuan untuk menentukan distribusi polimorfisme A61G pada pasien kanker serviks stadium I-IV di Rumah Sakit Dr. Hasan Sadikin. Uji* chi-square *digunakan untuk analisis data.*

***METODE***

*Sebuah rancangan potong silang dilakukan antara bulan Juli-November 2010. Pengambilan darah serta isolasi DNA dilakukan pada 61 pasien kanker serviks dengan berbagai stadium di RS Dr. Hasan Sadikin dan kemudian, uji genotyping untuk gen EGF menggunakan BeadXpress® Illumina.*

***HASIL***

*Hasil analisis menunjukkan bahwa polimorfisme A61G dimiliki oleh 88,5% pasien (dengan genotip A/G dan G/G). Di antara pasien-pasien yang memiliki polimorfisme ini, mayoritas memiliki tingkat keparahan sedang (stadium II dan III FIGO, 42,6% dan 31,8%). Kemudian, hanya 22,2% pasien dengan polimorfisme ini yang memiliki tingkat keparahan ringan.*

***KESIMPULAN***

*Penelitian ini menunjukkan bahwa polimorfisme A61G pada gen EGF meliputi mayoritas pasien kanker serviks dan setelah distratifikasi, mayoritas pasien menunjukkan tingkat keparahan sedang.*


***Kata kunci:*** *Kanker serviks, EGF, polimorfisme A61G*

## INTRODUCTION

Cervical cancer forms a huge burden in the epidemiology of female cancer, as it currently ranks second worldwide. Its prevalence is estimated at 500,000 cases, with 270,000 deaths annually.[1] Southeast Asia, Sub-Saharan Africa and South America today form the hot zones for high HPV prevalence. In Indonesia, cervical cancer reached a crude incidence rate of 12.1 per 100,000 women back in 2008 and this corresponds to more than 13,000 cases of new cervical cancer cases every year in Indonesia alone.[2] Cervical cancer, as any other type of cancer, requires both host and environmental factors. The human papilloma virus is the prerequisite environmental factor and both single and multiple HPV infections in a single patient is no longer an oddity.[3] In fact, from a local study done at Dr. Hasan Sadikin General Hospital, HPV 16 is the most prevalent virus among patients, followed by HPV 18, 45 and 52.[3] Once the virus infects the cervical epithelial cells, it then has its genes expressed and mature viral particles synthesized via the expression of various early proteins, namely E5, E6 and E7

proteins.[4-6] The early proteins allow the inactivation of the pro-apoptotic genes inherent to the host, which then allows an uninhibited mitotic proliferation of cervical epithelium.[7]

HPV is a necessary yet insufficient factor,[8] as only interaction with host factors will allow such infection to proceed to a neoplastic growth. One of the proposed factors is epidermal growth factor (EGF), a protein encoded by a gene on chromosome 4q25-2. Once it binds to its receptor, epidermal growth factor receptor (EGFR), it activates several signaling pathways, namely ras/raf/ mitogen-activated protein kinase (MAPK) or phospho-inositide 3-kinase (PI3K), which under normal conditions are required to ensure proper proliferation and differentiation of epithelial tissues.[8-10] However, excessive activation of EGFR or heightened production of EGF may also incite tumorigenesis in cells of epithelial origin.[8]

As EGF is a protein, the promoter region of the EGF gene often has the final say in determining the circulating levels of EGF. There are variations, known as single nucleotide polymorphisms (SNPs) [11] in the promoter region that may be responsible for the increased levels of EGF and up to this day, only one functional SNP has been described. Shahbazi et al.[12] were the first to describe the rs4444903 or A61G polymorphism in the 5'-untranslated region of the gene's promoter. Their results revealed that this particular SNP, associated with prompting increased guanine content in one's genotype, had a high correlation with malignant melanoma development. Since then, various authors have also accredited this SNP as being involved in the development of esophageal adenocarcinoma, glioma, colorectal cancer and hepatocellular carcinoma.[13] However, protective effects of this particular SNP have also been noted in ovarian cancer and gastric cancer.[13, 14]

Cervical cancer is derived from epithelial cells and therefore its development has also been suggested to be influenced by this SNP. Until recently, however, results have been conflicting. A study in Korea performed by Kang et al.[15] did not find any correlation between this SNP and greater cervical cancer development while a study in the USA performed by Araujo et al. [16] revealed the opposite results. Furthermore, it has also been revealed that the prevalence of this polymorphism is somewhat governed by racial origins, with Asian populations possessing a greater prevalence of the G allele in their genotypes.[17] However, until recently there has been no studies to confirm this finding on Indonesian populations. Thus, this study seeks to discover the distribution of the A61G polymorphism in cervical cancer patients presenting to Dr. Hasan Sadikin General Hospital, Bandung.

## METHODS

### Research design

This study employs a retrospective observational study design and was conducted between July – November 2010.

### Research subjects

Included were patients who presented to Dr. Hasan Sadikin General Hospital with cervical carcinoma of various severity and had undergone histologic examinations at the Department of Anatomic Pathology resulting in an established diagnosis of cervical cancer. Subtypes of cervical cancer, namely keratinizing squamous cell carcinoma, nonkeratinizing squamous cell carcinoma, small cell squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma, were all included in the diagnosis of cervical cancer. Patient details regarding age, age at first sexual intercourse, parity and abortion were collected. Of the initial study population, 135 patients had undergone genotyping and were initially included in the study. However, lack of data on patients' age, age at first sexual intercourse, parity and abortion, led to the inclusion of only 61 patients in the end. Total inclusive sampling was then employed on the final study population.

Since this study used secondary data obtained from an earlier study done under the supervision of the Oncology Working Group of Dr. Hasan Sadikin General Hospital, the following steps were not carried out in the present study and are here included for completeness only.

**DNA isolation**

DNA is extracted from collected blood samples by means of salting out methodology. The extracted DNA, adjusted to 50 ng/ìL and transferred onto 96-well plates, is then been analyzed with the Illumina BeadXpress system.

**SNP array analysis**

Three specific oligonucleotide primers (OPA) are prepared for each SNP, comprising two ASPs (allele specific primers) and one LSP (locus specific primer). These primers are then allowed to hybridize with the genomic DNA and PCR is then carried out, generating multiple amplicons with labels attached for all the different SNPs. The PCR products are then combined with the VeraCode bar-coded beads. These beads contain sequences corresponding to the LSPs and they emit fluorescence. The fluorescence on each bead is then quantified and the signal emitted by the fluorescence indicates a particular address sequence. Each address is an indicator toward a specific locus and the signals emitted by the beads are then translated to AA, BB or AB genotypes. The wild-type genotype for the EGF gene is A/A while the non-wild type genotypes are A/G and G/G respectively.

**Statistical analysis**

For statistical analyses the SPSS 17.0. software package (SPSS Inc., Chicago, IL) was used. Patients were stratified according to their genotypic profile as well as their diagnostic staging as determined according to the classification laid out by FIGO in 2008.[18] The influence of the EGF genotype on the main outcome variable, severity of cervical carcinoma, was assessed with Chi-square test.

Table 1 Ditribution of the demographic and clinical characteristics of the patients (n=61)

| Characteristic | n (%) |
|---|---|
| Patient's age, mean (SD) | 48.43 (8.74) |
| Age at first intercourse, mean (SD) | 16.56 (2.22) |
| Parity, mean (SD) | 3.62 (1.72) |
| Abortions, mean (SD) | 0.56 (0.79) |
| Stage at diagnosis (FIGO) | |
| I | 13 (21.31) |
| II | 24 (39.34) |
| III | 22 (36.07) |
| IV | 2 (0.03) |
| Genotype | |
| AA | 7 (11.5) |
| AG | 29 (47.5) |
| GG | 25 (41.0) |

FIGO: International Federation of Gynecology and Obstetrics
SD: standard deviation

**Ethical clearance**

The ethical clearance required for this study was granted in July 2011 by the Health Research Ethics Committee, Medical Faculty, Padjadjaran University – dr. Hasan Sadikin General Hospital Bandung.

**RESULTS**

This study involved 61 cervical carcinoma patients of varying severity (stages I-IV) with diverse sociodemographic backgrounds, concerning aspects of age, age at first sexual intercourse, parity and abortion. In this study, the majority of patients (88.5%) had the A61G polymorphism on the EGF gene, resulting in a skewness toward the alleles with guanine nucleotides. Table 1 presents a summary of the demographic and clinical features of the study population along with the population's genotypic distribution.

Furthermore, among those patients who possessed A61G polymorphism in their genotype, most were accounted as either stage II or stage III in their carcinomic severity, as there were 12 and 9 patients for A/G genotype, and 11 and 8

Table 2 Distribution of the EGF genotypes (wild-type vs. guanine-containing genotypes), stratified according to the diagnostic staging by FIGO

| Genotype | Stage I FIGO (%) | Stage II FIGO (%) | Stage III FIGO (%) | Stage IV FIGO (%) | p |
|---|---|---|---|---|---|
| A/A | 1 (14.3) | 1 (14.3) | 5 (71.4) | 0 (0) | 0.390 |
| A/G & G/G | 12 (22.2) | 23 (42.6) | 17 (31.8) | 2 (3.6) | |

FIGO: International Federation of Gynecology and Obstetrics

patients for G/G genotype, classified under stage II and III respectively. The severest of all, stage IV, contributed the smallest number of patients, as out of 61 patients only 2 were reported to fall in this category. Moreover, once the patients under A/G and G/G genotypes were merged together, it is evident that those diagnosed under stage II and III were still highest in number (Table 2).

## DISCUSSION

Overall, the results of this study managed to demonstrate that there was no uniform distribution of EGF gene polymorphisms among patients with cervical carcinoma at Dr. Hasan Sadikin General Hospital. The results were toward a majority of patients possessing A61G polymorphism, which seemed to confirm the notion that there is a greater proportion of this polymorphism in Asian populations.[17]

To date, there have only been two other studies that investigated the relationship between the A61G polymorphism of the EGF gene with regard to cervical cancer. Both of these studies differed much in their subjects as one was done in Korea and the other one was done in the United States. Studies investigating the role of EGF gene polymorphism on cervical cancer development have been very few in number and efforts to search for greater support regarding EGF gene polymorphism distribution in cervical cancer patients reaped few results.

The first of the two studies was done back in 2007and this was the first ever study to investigate the possible role of A61G polymorphism in affecting the development of cervical cancer.[15] This study involved 337 patients, with 168 patients acting as cases and 169 as controls matched for age. This study discovered that there was no significant difference in terms of the polymorphism distribution between the two groups and eventually the study concluded that the A61G polymorphism exerted protective effects in reducing individual susceptibility toward cervical carcinoma. Nevertheless, the study did point out that the presence of A61G polymorphism was associated with an increased risk toward metastasis in those patients with established carcinoma, albeit without statistical significance.

Another contribution made by the study performed by Kang et al.[15] is that they managed to analyze the distribution of EGF genotypes between Caucasian and East Asian populations from previous reports. Based on their analysis, they confirmed that there was a significant difference between these two populations in terms of EGF genotype distribution, with results in tune with our study, as in their study, Kang et al.[15] also observed that the majority of their patients had A61G polymorphism.

The second study was performed in 2012 in the United States of America.[16] Similar to the first study, this study also involved cancer vs. noncancer patients. The study was replete with the indication that the A61G polymorphism had a role to play in increasing the likelihood of developing more advanced cervical carcinoma and that this likelihood was more evident in G carriers.

Our current study is the first of its kind to be done on Indonesian population and it is also an addition to an already limited population of studies conducted on A61G polymorphism and its role in cervical cancer. Thus, there is the

urgency to conduct more studies on this particular issue as there has not been an unequivocal conclusion on the overall effect of having more guanine on one's genotype toward one's risk of developing a more severe neoplastic growth.

However, our study is not without limitations. The first limitation is the number of the samples, which was limited to 61 individuals. This number is significantly smaller than the calculated minimum requirement of 97 samples and this number is also considerably minute compared to the studies done by previous researchers.[15,16,19] Furthermore, lack of similar publications concerning EGF gene and cervical cancer development proved to be a hindrance in terms of coming up with greater support for the study. Finally, the status of this study, despite being the first of its kind in Indonesia, is a problem in itself, as the results of this study cannot be compared with, nor confirmed by, other similar studies.

## CONCLUSION

The A61G polymorphism is evident in the majority of cervical carcinoma patients and most of these patients exhibited moderate severity in their carcinoma staging.

## ACKNOWLEDGEMENT

The authors wish to thank Dr. Ani Melani Maskoen, drg.,M.Kes and Ms. Nurul Melani Rahayu of Molecular Genetics Laboratory at Dr. Hasan Sadikin General Hospital for having performed the initial sequencing of genetic material using the Illumina BeadXpress®.

## REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55:74-108.
2. WHO/ICO Information Center on HPV and Cervical Cancer (HPV Information Centre). Human papillomavirus and related cancers in Indonesia. Barcelona;2010.
3. Sahiratmadja E, Tobing MDL, Dewayani BM, Hernowo BS, Susanto H. Multiple human papillomavirus infections predominant in squamous cell cervical carcinoma in Bandung. Univ Med 2014;33:58-64.
4. Chauhan S, Jaggi M, Bell M, Verma M, Kumar D. Epidemiology of human papilloma virus (HPV) in cervical mucosa. Methods Mol Biol 2009;471:439-56. doi: 10.1007/978-1-59745-416-2_22.
5. zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Nat Cancer Inst 2000;92:690-8.
6. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342-50.
7. Ibeanu OA. Molecular pathogenesis of cervical cancer. Cancer Biol Ther 2011;11:295-306.
8. Narayanan R, Kim HN, Narayanan NK, Nargi D, Narayanan B. Epidermal growth factor-stimulated human cervical cancer cell growth is associated with EGFR and cyclin D1 activation, independent of COX-2 expression levels. Int J Oncol 2012;40:13-20.
9. Wells A. EGF receptor. Int J Biochem Cell Biol 1999;31:637-43.
10. Singh AB, Harris RC. Autocrine, paracrine and juxtacrine signaling by EGFR ligands. Cell Signal 2005;17:1183-93.
11. Bag A, Jyala NS, Bag N. Indian studies on genetic polymorphisms and cancer risk. Ind J Cancer 2012;49:144-62.
12. Shahbazi M, Pravica V, Nasreen N, Fakhoury H, Fryer AA, Strange RC, et al. Association between functional polymorphism in EGF gene and malignant melanoma. Lancet 2002;359:397-401.
13. Li TF, Ren KW, Liu PF. Meta-analysis of epidermal growth factor polymorphisms and cancer risk: involving 9,779 cases and 15,932 controls. DNA Cell Biol 2012;31:568-74.
14. Araujo AP, Ribeiro R, Pereira D, Pinto D, Sousa B, Catarino R, et al. Ovarian cancer and genetic susceptibility: association of A61G polymorphism in the EGF gene. Exp Biol Med 2009;234:241-5.
15. Kang S, Kim JW, Park NH, Song YS, Park SY, Kang SB, et al. Epidermal growth factor 61 A/G polymorphism and uterine cervical cancer. Int J Gynecol Cancer 2007;17:492-6.
16. Araujo AP, Catarino R, Ribeiro R, Pereira D, Pinto D, Medeiros R. Epidermal growth factor genetic variation associated with advanced cervical cancer in younger women. Am J Clin Oncol 2012;35:247-50.

17. Lanuti M, Liu G, Goodwin JM, Zhai R, Fuchs BC, Asomaning K, et al. A functional epidermal growth factor (EGF) polymorphism, EGF serum levels, and esophageal adenocarcinoma risk and outcome. Clin Cancer Res 2008;14:3216-22.
18. Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. Int J Gynecol Obstet 2009;105:107-8.
19. Dahlan MS. Besar sampel dan cara pengambilan sampel. Jakarta: Salemba Medika;2013.