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ORIGINAL ARTICLE

E-cadherin-160 C/A genotypes and cervical intraepithelial neoplasia

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Summary

Purpose: *E-cadherin is a transmembrane glycoprotein with important roles in the maintenance of cervical squamous epithelium integrity. The purpose of this study was to investigate the relationship between E-cadherin-160 C/A polymorphism and cervical intraepithelial neoplasia (CIN).*

Methods: *A case-control study was performed enrolling 70 CIN cases and 107 age-matched healthy controls. Each patient was examined colposcopically, having a cervical specimen. All patients and controls have been genotyped for E-cadherin-160. Data were analysed using odds ratios (OR) and chi-square test at a significance level of p<0.005.*

Results: *The presence of E-cadherin-160 C/A was significantly associated with high-grade squamous intraepithelial lesion (HSIL) (OR=2.7916, 95% CI 1.1495,6.9345, x^2=6.33, p=0.0118) and carcinoma in situ (CIS) (OR=2.5617, 95% CI 1.1676,5.6705, x^2=6.63, p=0.0100). The detection of either CA or AA genotype was also significantly associated with HSIL and CIS.*

Conclusion: *E-cadherin-160 genotype represents a valid risk factor for HSIL and CIS.*

Key words: *E-cadherin, genetic polymorphism, cervical intraepithelial neoplasia, SNP, VEGF*

Introduction

Squamous cervical carcinogenesis has been previously described as a continuous process that takes place above the basal membrane [1]. Briefly, in the presence of persistent high risk human papilloma virus (HR-HPV) in selected women having a poorly defined susceptibility to cervical cancer, the exocervical squamous epithelium is affected starting from the basal layer up to the superficial layer passing through consecutive stages, i.e. CIN I (cervical intraepithelial neoplasia), CIN II or CIN III [1].

Almost 6 decades after Papanicolaou classification [2] for the interpretation of cervical exfoliative cytology, this classification was replaced by the Bethesda classification in order to incorporate the latest data regarding cervical carcinogenesis into everyday practice [3]. Nowadays from the clinical point of view it is very important to discriminate between low grade squamous intraepithelial lesions (LSIL) and HSIL, the two above mentioned categories having a completely different therapeutic approach. LSIL includes koilocytosis and CIN I [3]. Most of the LSILs HR-HPV cases can be found in cytoplasmic episomal state in the so-called productive lesions because high amounts of infective viral particles are produced

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using the host synthesis mechanisms [1]. By contrast, in HSIL patients the HR-HPV are integrated in the nucleus of the squamous cells leading to expression of high amounts of E6 and E7 oncoproteins that will determine genomic instability and cervical cancer [1]. Both LSIL and HSIL are evolutive and potentially reversible states, the percentage of progression of LSIL to cervical cancer being significantly lower compared with HSIL [1].

E-cadherin, a 120 kDa transmembrane glycoprotein, is a member of cadherin superfamily that includes important adhesion molecules [4]. E-cadherin plays an essential role in normal morphogenesis and integrity of the epithelia [5,6]. The cells of squamous epithelia are kept together by tight junctions and desmosomes under the action of adhesion molecules [4].

Normally the expression of E-cadherin is higher in the basal layer of squamous epithelium and becomes lower towards the upper layer [7], allowing the physiological shedding, cell exfoliation and the possibility of performing a Papanicolaou smear [8]. Besides its major role in the preservation of the normal morphology of the cervical epithelia, E-cadherin, far for being a passive molecule, also activates signaling pathways in order to sustain cadherin functions [9].

E-cadherin is encoded by CDH1 gene, located on 16q22.1 [10]. Single nucleotide polymorphisms (SNPs) are abundant in human genome, being associated with no impact/ reduction/incrementing of protein produced from the correspondent gene [11]. Many SNPs have been described at the level of CDH1 gene: +54 C/T, -160C/A, -347 G/A, 797 C/T [12-15]. Previous studies have proved the implications of E-cadherin genetic polymorphism in many epithelial cancers, especially in gynecological cancers: (ovarian and endometrial carcinomas) [15,16]. Moreover, previous studies have demonstrated reduction of E-cadherin secretion in patients with CIN [7,17,18].

The aim of the present study was to analyze the potential implication of E-cadherin promoter polymorphism -160 C/A in patients with CIN

Methods

Study population and specimens

One hundred and seventy seven patients have been enrolled, divided into 70 cases and 107 controls. The study was structured as a case-control study and took place in the 1st Clinic of Obstetrics and Gynecology, Cluj-Napoca, Romania between 1st of January 2014 and 30th of June 2014. The study got approval by the Ethics Committee of the above mentioned University.

All study participants, cases and controls, agreed to participate to the study. An appointment was scheduled after the informed consent was signed.

The inclusion criteria for cases was the diagnosis of CINs. This diagnosis was based on a cervical cytology sample. Every woman with an abnormal cytology was subjected to colposcopy, and also a cervical biopsy when necessary, that allowed to better classify the CINs. The colposcopy was performed by two experienced colposcopists using a digital videocolposcope (MIKRO MZ6) with integrated camera Leica IC80HD (Prague, Czech Republic).

The controls were age-matched women, cytological negative for CIN according with the Bethesda terminology and also negative for HPV testing.

For genetic analyses 2 mL of peripheral blood were drawn from study and control cases. Cervical specimens were sent to the laboratory of the University hospital and the results of the cervical cytology were formulated according to the Bethesda nomenclature [3].

The tube containing peripheral blood was sent to the Genetics Department of the University. From 300µl peripheral blood samples of each patient genomic DNA was extracted using Wizard Genomic DNA Purification Kit (Wizard® Genomic DNA Purification Kit, Promega, MA, USA) and stored at -20°C until tested.

E-cadherin genotyping

E-cadherin-160 C/A SNP was performed according to a RFLP (restriction fragment length polymorphism) reported by Shan K et al. [19]. One hundred ng of genomic DNA were amplified using Eppendorf Mastercycler Thermal Cycler (Hamburg, Germany) in a total volume of 25µl reaction mix (Thermo Fischer Scientific Inc., MA, USA) containing reaction buffer of 1.5nM $MgCl_2$, 20 pmol of each primer, 200µm of each dNTPs and 0.5U of Taq polymerase. The following steps were performed according to the protocol: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C of denaturation for 30 sec, annealing 30 sec at 66°C and elongation 60 sec at 72°C, with a final elongation of 10 min at 72°C. The primers used during the PCR reactions are listed below: 5'-CGCCCCGACTTGTCTCTC-TAC-3' (forward) and 5'-GGCCACAGCCAATCAGCA-3' (Eurogentec®, Belgium).

The PCR amplification products were digested overnight with 4U HincII (Thermo Fischer Scientific Inc., MA, USA). Enzymatic digestion was followed by 2% metaphor gel (Lonza, Basel, Switzerland) electrophoresis. In case of A variant allele- two bands of 368 bp and 80 bp were present, while in the presence of C wild allele only one band of 448 bp was detected.

Statistics

Data was analyzed using Stata Intercooled 10 (College Station®, Texas, USA). Descriptive statistics are given as mean±SD and frequencies. The strength of association between E-cadherin and different classes of CINs was assessed using the x^2 test, with a statistically significant value of $p<0.05$. Odds ratio (OR) with 95% confidence interval (CI) were calculated to estimate the risk for cervical dysplasia.

Results

The average age in the study group was 41.84±11.17 years. The cases included ASC-H: 9, ASC-US: 9, LSIL: 9, HSIL:32 and CIS:11. Overall E-cadherin -160 CC genotype was the most frequently encountered (50.28%), followed by CA genotype (42.37%), while AA genotype was the less detected (7.34%).

E-cadherin-160 CA genotype was first analysed as a potential marker of CINs (Table 1). The detection of CA genotype was associated with a significantly higher risk for high grade disease (OR=2.7916, 95% CI 1.1495, 6.9345, x^2=6.33, p=0.0118). The results were not significant for low grade disease (OR=0.8375, 95% CI 0.1286, 4.1910, x^2=0.06, p=0.8091;Table 1).

Then the impact of any E-cadherin-160 variant genotype (CA or AA) was investigated in relation with CINs, and the results are depicted in Table 2. We found similar results in the analysis of heterozygous genotypes: significant association for HSIL (OR=2.8085, 95% CI 1.1350, 7.2737, x^2=6.07, p=0.0137) and nonsignificant for LSIL (OR=1.0212, 95% CI 0.1915, 5.0364, x^2=0.00, p=0.9760; Table 2).

Discussion

The presence of A allele in E-cadherin -160 has been proved to be associated with a 68% posttranscriptional decreased level [12]. In our study the level of expression of E-cadherin was not assessed but the detection of E-cadherin CA genotype was significantly associated to an increased risk of developing high grade intraepithelial lesions (OR=2.79, 95% CI 1.1495,6.9345, p=0.0118). Moreover, the detection of CA or AA variants was a risk factor for CIN (OR=1.87, 95% CI 0.9839, 3.5737, x^2=4.22, p=0.0399). A constitutional lower E-cadherin level associated with CA genotype could virtually increase epithelial cell motility and worsen the prognosis. The presence of an A allele in E-cadherin-160 interfered with E-cadherin level by altering its postransciptional levels [12].

The number of researches focusing on E-cadherin-160 genotypes in relation with CIN is ex-

Table 1. Analysis of E-cadherin-160 CA genotype - cases vs controls

Comparison	*OR*	*95% CI*	*x^2*	*p value*
Cases/controls	1.6750	0.8689,3.2271	2.76	0.0967
LSIL/controls	0.8375	0.1286,4.1910	0.06	0.8091
HSIL/controls	2.7916	1.1495,6.9345	6.33	0.0118
CIS/controls	2.0100	0.4738,8.8492	1.24	0.2664
HSIL+CIS/controls	2.5617	1.1676,5.6705	6.63	0.0100
HSIL/LSIL	3.3333	0.5635,23.7767	2.43	0.1193
HSIL+CIS/LSIL	3.0588	0.5493-21.0195	2.22	0.1361

OR: odds ratio, CI: confidence interval. For other abbreviations see text

Table 2. Analysis of E-cadherin-160 CA+AA genotype - cases vs controls

Comparison	*OR*	*95% CI*	*x^2*	*p value*
Cases/controls	1.8048	0.9387,3.4803	3.63	0.0567
LSIL/controls	1.0212	0.1915,5.0364	0.00	0.9760
HSIL/controls	2.8085	1.1350,7.2737	6.07	0.0137
CIS/controls	3.4043	0.7569,20.7968	3.33	0.0682
HSIL+CIS/controls	2.9459	1.3094,6.8265	8.20	0.0042
HSIL/LSIL	2.7500	0.4653,16.7199	1.79	0.1811
HSIL+CIS/LSIL	2.8846	0.5149,16.7319	2.11	0.1465

OR: odds ratio, CI: confidence interval. For other abbreviations see text

tremely limited. To our knowledge only one previous study has analyzed the relationship between CIN and cervical cancer [20]. A Korean study published by Kang et al. in 2008 that had enrolled both CIN patients (N=119), cervical cancer patients (N=107) and 112 controls, found no statistically significant differences regarding E-cadherin-160 genotypes between CIN and controls and cervical cancer patients and controls [20].

It is well known that persistent high-risk HPV infection downregulates E-cadherin expression, leading to changes in cell morphology and promoting the transition from epithelial cell morphology to a fibroblast-like morphology known as epithelial-mesenchymal transition, characteristic for malignant transformation [21]. Moreover in cervical cancer cell lines and tumors, E-cadherin level is reduced also by epigenetic alterations, mainly by promoter DNA-methylation [5,18]. Therefore, a supplementary downregulation of a basal low E-cadherin expression induced by a CA or AA genotypes could be crucial. Downregulation of E-cadherin is more severe as the CIN lesion progresses [18]. The detection of at least one A allele at E-cadherin-160 was significantly associated with a high grade lesion or *in situ* carcinoma, but no relationship was found in patients with LSIL, indicating a completely different behavior (Table 2). The explanation may be due to the alteration of E-cadherin level in the presence of HPV oncoproteins, known to be synthetized in high amounts in HSIL lesions with a very low level in LSIL. HPV16 E6 oncoproteins expression causes a reduction of E-cadherin levels [21,22], the loss of E-cadherin in cervical carcinoma being consecutively followed by invasion and metastasis [23].

The results of this study suggest the use of E-cadherin-160 genotype together with E-cadherin expression and p16 [24] in the prognostic algorithm of CIN.

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Conflict of interests

The authors declare no confict of interests.

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