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ORIGINAL ARTICLE

HER-2 expression in uterine cervix carcinogenesis

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Summary

Purpose: *To assess the expression and clinical significance of HER-2 protooncogene in the uterine cervix carcinogenesis.*

Patients and methods: *We examined 69 tissue samples of low grade cervical squamous intraepithelial lesions (SIL) (n=16), high grade SIL (n=11) portio vaginalis uteri (PVU) carcinoma in situ (n=11) and PVU invasive carcinoma, stage IA-IIA (n=13; study group) and 18 samples without SIL or malignancy (control group). The expression of HER-2 was detected immunohistochemically using a monoclonal antibody. Fisher's exact test was used to assess statistical significance. By establishing sensitivity and specificity of the test, the level of reliability of these analyses was determined as a possible screening method for early detection of changes in the uterine cervix.*

Results: *Overexpression of HER-2 was found to increase in direct relation to the grade of the cervical lesions. Statistically significant difference was found in the frequency of overexpression in patients with high grade SIL, PVU carcinoma in situ and PVU invasive carcinoma compared with the control group. High sensitivity was of great diagnostic significance for the detection of these types of changes in the uterine cervix. On the basis of high predictive values it can be concluded that in patients with HER-2 overexpression there is a great possibility that they have premalignant or malignant changes in the uterine cervix.*

Conclusion: *Our results indicate that overexpression of HER-2 oncogene may play an important role in cervical carcinogenesis. However, more extensive series of samples is required to establish the prognostic significance of HER-2 in cervical carcinogenesis.*

Key words: carcinogenesis, cervical neoplasia, HER-2, immunohistochemistry

Introduction

Squamous cell carcinoma of the uterine cervix is currently one of the most common malignancies in women worldwide.

In searching ways of prevention, early diagnosis and effective treatment of premalignant lesions and malignant tumors of the uterine cervix, molecular-genetic researches play a significant role in the last few years.

Analysis of oncogenes' expression in human cancer is increasingly important to gain a better insight in the process of tumorigenesis and to identify new markers for early diagnosis of malignant transformation. Abnormal expression of different cellular oncogenes in various cancers assessed by hybridization and immunological techniques has been previously reported [1]. The results of these analyses do not appear to be of any early diagnostic value since oncogene expression is, in general, only demonstrable in tumors that can already be classified as malignant.

The key difference between normal and malignant cell is in a subtle change of specific genes that control, by their products, the processes of growth,

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division and differentiation of a cell and are called protooncogenes. These genes that are present in the genotype of every normal cell possess oncogenic potential because their disturbed expression may lead to malignant transformation.

The HER-2 oncogene encodes a transmembrane tyrosine kinase receptor ($p185^{HER-2}$) with extensive structural homology to the epidermal growth factor receptor. In response to ligand specific binding, these receptors act by forming hetero- or homodimers and thereby initiate tyrosine kinase activity in the intracellular domain. This amplification of tyrosine kinase activity results in the phosphorylation of several tyrosine residues in the C-terminus. These phosphorylated tyrosines then serve as binding sites for signal transducers and adapter molecules that initiate signaling pathways resulting in cell proliferation, migration, adhesion, protection from apoptosis, and transformation [2]. These receptors activate signaling pathways responsible for initiation and development of some malignant tumors.

This oncogenic pathway of some cells is thought to be initiated as a result of HER-2 mutation, overexpression, structural rearrangements, and/or relief of normal regulatory or inhibitory pathways. The presence of HER-2 receptors has also been associated with accelerated tumor progression and resistance to therapy for multiple types of malignancies [3]. The causal relationship of this receptor network to disease progression and resistance to therapy provides a rationale for targeting this signaling system with tumor-selective strategies.

Amplification of HER-2 oncogene or disturbances of complex mechanisms that control its expression may be the cause of a far larger than usual number of HER-2 molecules on the cellular membrane, which may suggest that such a cell has malignant potential [4].

Many studies have shown that poor prognosis is in positive correlation with the degree of expression of this oncogene (ovary, uterine cervix, lung, prostate, breast, colon) [5-7].

HER-2 oncoprotein is normally present at low levels in all cells of the human body.

The purpose of our study was to assess the expression and clinical significance of HER-2 oncogene in the uterine cervix carcinogenesis.

Patients and methods

This prospective study was carried out during 2004 and 2005 at the Department of Obstetrics and Gynecology, Faculty of Medicine and the experimental part was performed at the Laboratory for Experimental and Clinical Immunology of the Faculty of Medicine and at the Immunological Laboratory of the Public Health Institute, Kragujevac.

In patients operated at the Department of Obstetrics and Gynecology because of premalignant and/or malignant changes of the uterine cervix, some tissue sections were taken from the operative material (hysterectomy, punch biopsy or conization) for pathological verification and used for this research.

The control group consisted of 18 patients in whom ambulatory biopsy of the uterine cervix was performed (Papanicolaou test was indicated) and where malignant changes or SIL were not found by histopathology (cervicitis chronica of mild to moderate degree).

The study group consisted of 16 patients with pathological diagnosis of low grade SIL, 11 with high grade SIL, 11 with PVU carcinoma *in situ* and 13 with PVU invasive carcinoma, stage IA-IIA.

Determination of HER-2 oncogene expression followed in patients in the study and the control group.

Cryostat sections were sent for intraoperative diagnosis. Extra sections of cervical lesions were snap-frozen in liquid nitrogen and stored at –70° C until used for immunohistochemistry.

C-erbB2/HER-2 monoclonal antibody (clone 3B5, Pharmingen, San Diego, USA) was used, which was diluted with phosphate buffered saline (PBS, pH 7.2).

Four-micrometer frozen sections were fixed in 100% acetone for 5 min and the endogenous peroxidase activity was quenched by 10 min incubation in 0.5% hydrogen peroxide. After fixation, the slides were washed with PBS. Nonspecific antibody binding was blocked with PBS containing 10% rabbit serum for 10 min at room temperature in the humidity chamber. The slides were blotted and primary antibodies applied. For monoclonal antibody C-erbB2/HER-2 the incubation was 60 min at room temperature (concentration of 5 μg/ml). The slides were washed 3 times in PBS. Secondary antibody, diluted with PBS, was applied for 60 min in the humidity chamber at room temperature. Streptavidin/horse-radish-peroxidase complex technique was used for visualization with diaminobenzidine as chromogen. Sections were counterstained with hematoxylin for 30 sec and washed in cold tap water. Finally, the slides were dehydrated in alcohol, cleared in xylene and mounted in Canada balsam.

HER-2 positive breast carcinomas were used as positive controls. For negative controls, the samples were taken through the procedure with omission of the primary antibody.

Slides were evaluated by two of the authors, unaware of immunohistochemical or clinical data using a semiquantitative method on a Zeiss AXIOSKOP 2 light microscope. The percentage of immunopositive cells in representative areas of the sections was assessed. The intensity of immunostaining was divided into 4 categories, namely: negative – (0-5%); 1+(5-25%); and positive 2+(25-50%), 3+(50-100%).

Tissue sections from the operative material or material got by biopsy were taken after obtaining informed consent of patients in accordance with the Declaration of Helsinki and recommendations of the World Health Organization (WHO) for experiments on human material and after getting approval of the Ethics Committee.

Statistics

Based on the frequency of the lesions found, patients were split into 4 subgroups and 2×2 contingency tables were formed and specificity and sensitivity were calculated. Also, based on the ROC (receiver operating characteristic) curve which represents the relation between specificity and sensitivity, the discrimination power of the test was determined.

Fisher's exact test was used to assess statistical significance. By establishing sensitivity and specificity of the test, the level of reliability of these analyses was determined as a possible screening method for early detection of changes in the uterine cervix.

Results

Table 1 and Figure 1 show the expression levels of HER-2 oncogene in the study and control group. Overexpression of HER-2 was present in 27.8% of women with normal cervix and in 56.3% of patients with low grade SIL (Fisher's exact test, p=0.163; Photo 1).

On the basis of the frequency of patients with HER-2 overexpression, a 2×2 table of contingency was formed (Table 2), in which sensitivity and specificity were calculated. The sensitivity of determining

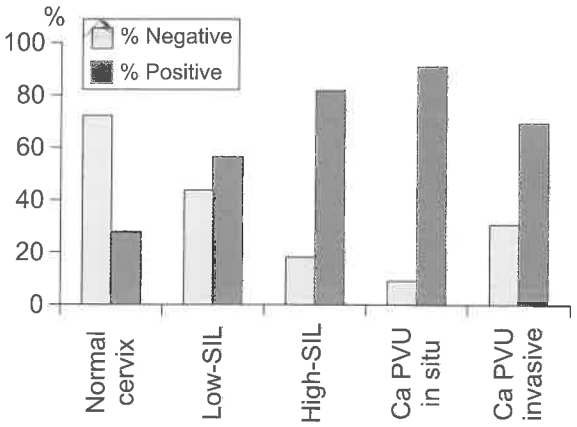


Figure 1. Percentage of positive and negative HER-2 expression. For abbreviations see text.

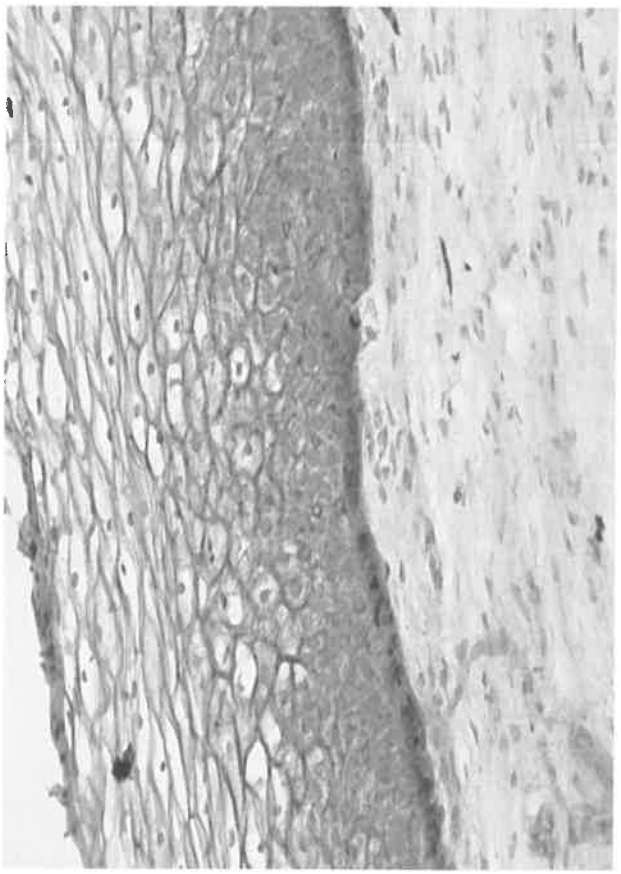


Photo 1. HER-2 overexpression (3+) in low grade SIL (× 40).

Table 1. Expression of HER-2 in the control and study group

HER-2 expression	*Normal cervix n (%)*	*Low grade SIL, n (%)*	*High grade SIL, n (%)*	*PVU Ca in situ, n (%)*	*PVU invasive Ca, n (%)*
Negative (–)	4/18 (22.2)	1/16 (6.2)	0/11 (0)	0/11 (0)	1/13 (7.6)
Negative (1+)	9/18 (50.0)	6/16 (37.5)	2/11 (18.2)	1/11 (9.1)	3/13 (23.1)
Positive (2+)	1/18 (5.6)	4/16 (25.0)	2/11 (18.2)	7/11 (63.6)	3/13 (23.1)
Positive (3+)	4/18 (22.2)	5/16 (31.3)	7/11 (63.6)	3/11 (27.3)	6/13 (46.2)

For abbreviations see text

Table 2. Table of contingency (low grade SIL)

Test	*Disease present (low grade SIL)*	*Disease absent (normal cervix)*	*Total*
HER-2 overexpression	9	5	14
HER-2 negative	7	13	20
Total	16	18	34

HER-2 as a method for detection of low grade SIL was 56.25% and specificity was 72.2% (Figure 2).

The positive predictive value was 64.32%, which means that this percentage of patients with HER-2 overexpression may be expected to harbor low grade SIL in the cervix. The negative predictive value was 65%, meaning that this percentage of patients with HER-2 negative expression are not expected to have this type of change.

Table 1 and Figure 1 show that HER-2 overexpression was present in 27.8% of women with normal cervix and in 81.8% of patients with high grade SIL (Fisher's exact test, $p = 0.008$) (Photo 2).

The sensitivity of determining HER-2 for detecting high grade SIL changes was 81.82% and specificity was 72.2% (Table 3 and Figure 3).

Regarding the obtained values of sensitivity and specificity, the discrimination power of overexpression of this oncogene with the aim of determining the existence of change of this type was greater than the discrimination power of negative expression in patients with normal findings in the cervix.

The positive predictive value in high grade SIL changes was 64.18% and the negative predictive value was 86.65%.

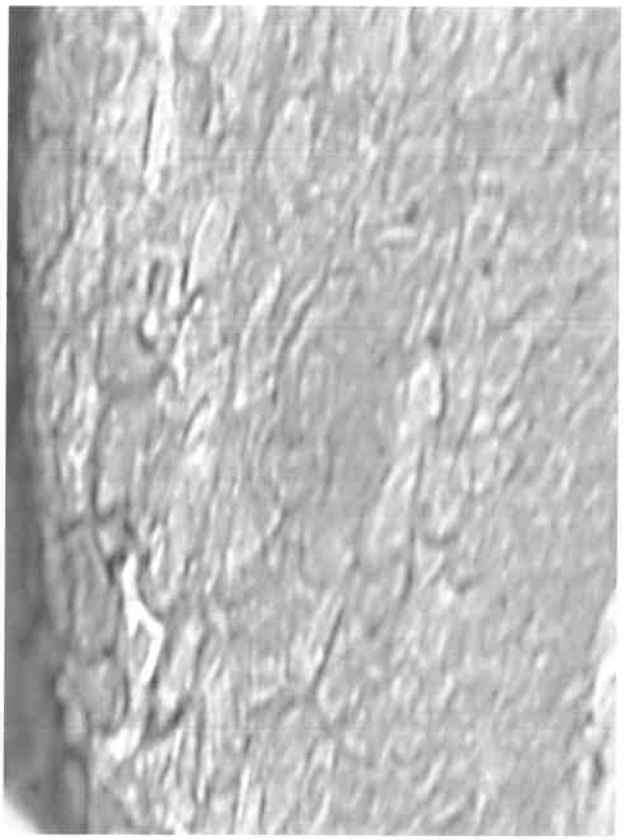


Photo 2. HER-2 overexpression (3+) in high grade SIL (× 40).

Table 3. Table of contingency (high grade SIL)

Test	*Disease present (high grade SIL)*	*Disease absent (normal cervix)*	*Total*
HER-2 overexpression	9	5	14
HER-2 negative	2	13	15
Total	11	18	29

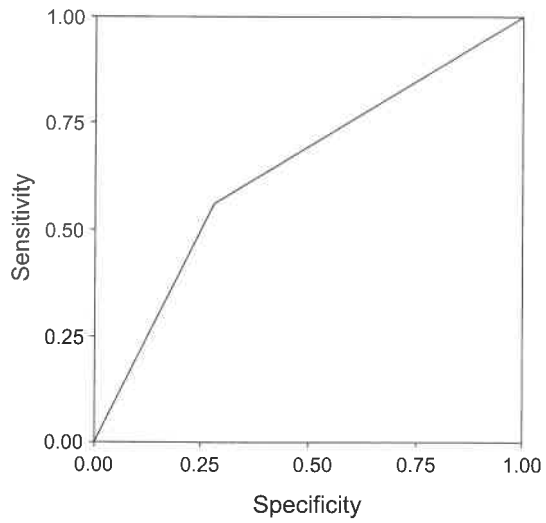


Figure 2. ROC (receiver operating characteristic) curve for HER-2 overexpression in patients with low grade SIL.

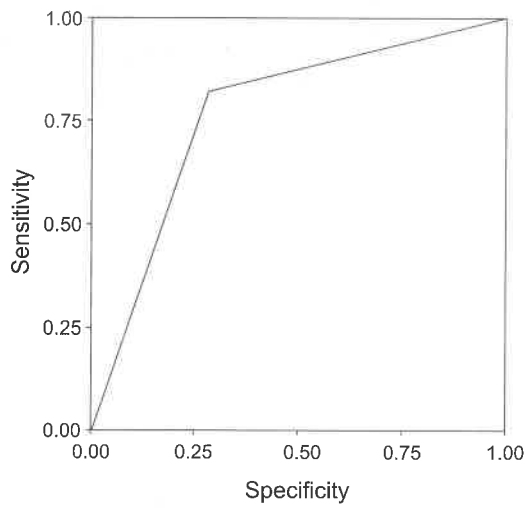


Figure 3. ROC (receiver operating characteristic) curve for HER-2 overexpression in patients with high grade SIL.

Table 1 and Figure 1 show that HER-2 overexpression was present in 27.8% of patients with normal cervix and in 90.9% of patients with PVU carcinoma *in situ* (Photo 3).

Overexpression of HER-2 oncogene was significantly different in patients with normal cervix than in patients with PVU carcinoma *in situ* (Fisher's exact test, p=0.008). The sensitivity of this oncogene for diagnosing this type of lesions was 90.9% and the specificity was 72.2% (Table 4 and Figure 4).

The positive predictive value was 64.18% and the negative predictive value was 86.65%.

Table 1 and Figure 1 show that HER-2 overexpression was present in 27.8% of patients with normal cervix and in 69.3% of patients with PVU invasive carcinoma (Fisher's exact test, p = 0.033) (Photo 4).

The sensitivity and specificity of the test were 69.3% and 72.2%, respectively (Table 5 and Figure 5), and the positive and negative predictive values were 64.13% and 76.46%, respectively.

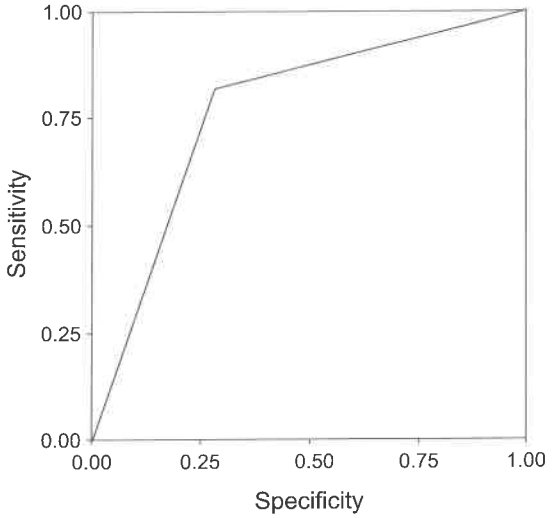


Figure 4. ROC (receiver operating characteristic) curve for HER-2 overexpression in patients with PVU carcinoma *in situ*.

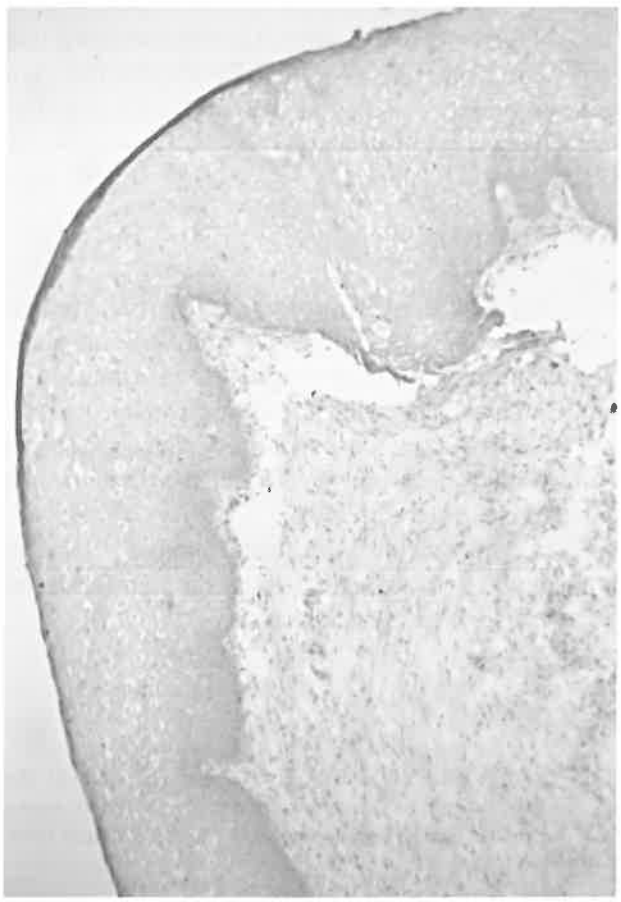


Photo 3. HER-2 overexpression (3+)in PVU cancer *in situ* (×40).

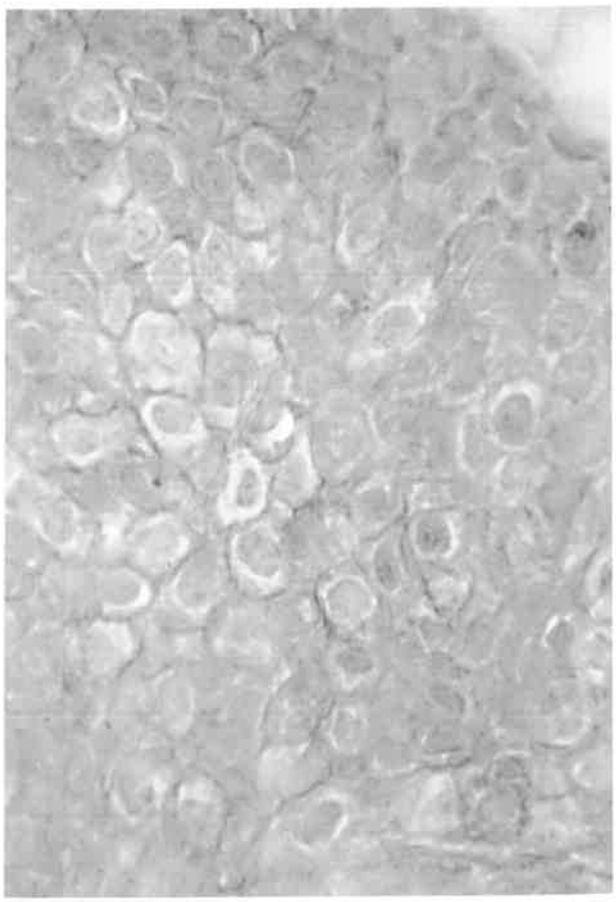


Photo 4. HER-2 overexpression (3+) in PVU invasive cancer (×40).

Table 4. Table of contingency (PVU carcinoma *in situ*)

Test	*Disease present (PVU Ca in situ)*	*Disease absent (normal cervix)*	*Total*
HER-2 overexpression	10	5	15
HER-2 negative	1	13	14
Total	11	18	29

Discussion

HER-2 is included among oncogenes that have been investigated for prognostic value in various malignancies, including carcinoma of the cervix. Controversial results have been published as to the prognostic value of its expression.

In some studies HER-2 has been shown an independent predictor of poor prognosis in carcinoma of the cervix [8-10]. On the other hand, other studies have also revealed no statistically significant correlation with an adverse outcome [11-13].

Contradictory results have also been published on the prognostic significance of this oncogene in other gynecological sites [3,14,15].

The conflicting results may be due to differences in institutional treatment standards and to varied subjective interpretations of staining intensity and distribution between centers. Because staining is judged on a continuum, differences in institutional "cut-off" determinations for positive staining may also affect correlation with clinicopathological results.

Potti et al. [16] found that HER-2 overexpression was a favorable prognostic factor on univariate analysis for both overall and disease-free survival; however, it was not significant on multivariate analysis.

Table 5. Table of contingency (PVU invasive carcinoma)

Test	*Disease present (PVU invasive Ca)*	*Disease absent (normal cervix)*	*Total*
HER-2 overexpression	9	5	14
HER-2 negative	4	13	17
Total	13	18	31

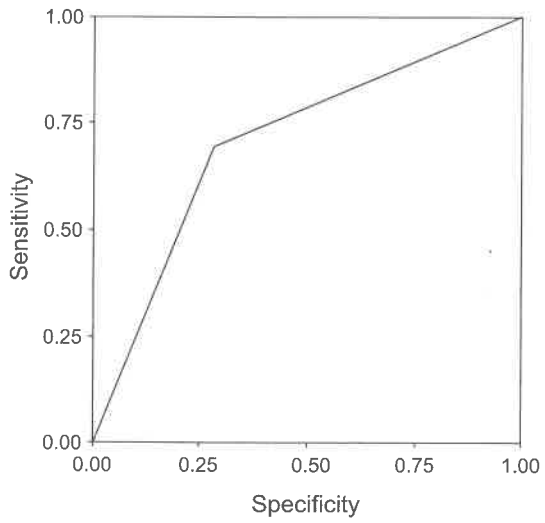


Figure 5. ROC (receiver operating characteristic) curve for HER-2 overexpression in patients with PVU invasive carcinoma.

Kihana et al. [17] in their analysis of 44 patients with cervical adenocarcinoma found that increased expression of HER-2 correlated with a poorer prognosis.

Absence of correlation between clinical prognosis and HER-2 expression was described by Kersemaekers et al. [18].

In our study, statistically significant difference was not found in the frequency of overexpression of HER-2 between the control group and patients with low grade SIL. However, statistically significant difference was found in the frequency of overexpression in patients with high grade SIL, PVU carcinoma *in situ* and PVU invasive carcinoma in relation to the control group. High sensitivity values speak in favor of great diagnostic significance for the detection of these types of changes in the uterine cervix. On the basis of high predictive values it can be concluded that in patients overexpressing HER-2 oncogene there is a great possibility that they have premalignant or malignant changes in the uterine cervix.

Conclusion

The results of the present study indicate that evaluation of HER-2 expression may provide additional and independent prognostic information to predict the clinical course of cervical cancer.

Positive expression of this oncogene suggests with much certainty that there are premalignant or malignant changes in the uterine cervix.

However, studies with greater number of samples are required to establish the prognostic significance of HER-2 in cervical carcinogenesis.

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