Abstract. The aim of this study was to determine whether modulation of expression of cell adhesion molecules may occur in neoplastic transformation of endometrial epithelium. Materials and Methods: E-Cadherin and α-catenin protein expression were evaluated by immunohistochemistry in 124 biopsies representative of normal, hyperplastic and neoplastic endometrium. Results: In normal endometrium (proliferative, secretive and atrophic endometrium) strong homogeneous, E-cadherin and α-catenin reactivity was found; 58.3% and 66.6% of biopsies representative of simple hyperplastic endometrium were homogeneously positive for E-cadherin and α-catenin, respectively, whereas no samples representative of atypical hyperplasia showed evidence of homogeneous E-cadherin or α-catenin expression. No expression of homogeneous E-cadherin was seen in endometrial adenocarcinomas; α-catenin homogeneous immunostaining was observed in 2 G1 and 2 G2 out of 22 adenocarcinoma samples (18.2%). A homogeneous co-expression of both molecules was seen only in normal (70%) and simple hyperplastic (46%) endometrium. Conclusion: These results suggest that E-cadherin and α-catenin down-regulation might be associated with neoplastic transformation of endometrial tissues.

Cell adhesion molecules such as cadherins have been recognized to be important biomarkers of tumor differentiation (1, 2). They regulate epithelial, endothelial and neural cell adhesion with different cadherins expressed on different cell types (3). E-Cadherin, which is an epithelial cell adhesion molecule, is a transmembrane protein with a cytoplasmic domain that connects the actin cytoskeleton through a complex with its associated cytoplasmic proteins, α-, β- and γ-catenins (4, 5). Cell adhesive properties mediated by E-cadherin are regulated by the association with catenins: adhesiveness at cell adhesion sites is enhanced when E-cadherin links to α-catenin (6, 7). Protein p120cas has also been defined as a member of the cadherin–cell–cell adhesion complex and binds directly to the same region of E-cadherin as the catenins (8, 9); p120cas can be phosphorylated after activation by several growth factors and oncogenes, suggesting a possible role for this protein in the regulation of E-cadherin function by these factors (10).

Loss of E-cadherin expression has been correlated with the in vitro invasive phenotype of cancer cell lines (11, 12). Furthermore, previous in vivo studies reported the reduced or aberrant expression of E-cadherin and/or catenins in different human cancers (13); in neoplastic thyroid tissue (14), in esophageal cancer (15), breast cancer (16, 17), gastric (18) and pancreatic carcinoma (19) bladder (20) and prostatic cancer (21), melanoma (22) and meningioma (23). Defects in the E-cadherin/catenin adhesion complex have been described in several gynecologic carcinomas, including cervical (24-26), endometrial (27-30) and ovarian carcinomas (31, 32).

This study evaluated the tissue distribution of E-cadherin and α-catenin in normal, hyperplastic and neoplastic endometrium using immunohistochemical analysis, with the aim to investigate whether differences of expression of these molecules exist. The possibility that modulation of expression of E-cadherin and α-catenin may have a prognostic role is discussed.
Materials and Methods

**Immunohistochemical analysis.** A total of 124 endometrial tissue specimens (biopsy or surgical samples) were fixed in 10% buffered formalin. Hematoxylin end eosin-stained sections from paraffin-embedded blocks were classified as: normal proliferative endometrium (23 cases), normal secreting endometrium (23 cases), atrophic endometrium in postmenopausal women (21 cases), simple endometrial hyperplasia (24 cases), atypical endometrial hyperplasia (11 cases) and 22 endometrial adenocarcinomas. The hormonal phases of all normal endometrium specimens were established according to the criteria of Noyes et al. (33). The endometrial adenocarcinomas were graded according the World Health Organization grading system: seven G1 cases (well-differentiated adenocarcinomas), nine G2 cases (moderately differentiated adenocarcinomas) and six G3 cases (poorly differentiated adenocarcinomas). The patients had not received any preoperative therapy.

Immunohistochemistry was performed according to a standard streptavidin-biotin peroxidase complex method (Zymed, S. Francisco, CA, USA). E-Cadherin and α-catenin expression were assessed using commercially available specific mouse monoclonal antibodies (Zymed, San Francisco, CA, USA): HECD-1, specific for human E-cadherin and α-CAT-7A4 raised against a synthetic peptide corresponding to the C-terminus of mouse α-catenin (dilution 1:50). Sections (4 μm) were deparaffinized in xylene, rehydrated in graded ethanol and washed with phosphate-buffered saline (PBS), then treated in a microwave oven for 15 min in 0.01 M citrate buffer (pH 6.0) and allowed to cool for 20 min to room temperature. Endogenous peroxidases were quenched by incubation in 0.3% hydrogen peroxide and sections were then incubated in serum blocking solution to reduce non-specific labeling. Anti E-cadherin primary antibody and anti α-catenin primary antisera were added and sections were then incubated for 1 h at room temperature. Positive controls were known E-cadherin- and α-catenin-expressing epithelial tissues; negative controls were carried out by using unrelated isotype matching antibody. The secondary biotinylated antibody was incubated as previously described (26). The reaction was revealed by adding diaminobenzidine-tetrahydrochloride (DAB) chromogen mixture (Zymed). After hematoxylin counterstaining, slides were permanently mounted and analyzed for the presence and distribution of the immunostaining. Staining was scored based on semiquantitative assessment of the distribution pattern of staining (plasma membrane and cytoplasm) and number of immunoreactive epithelial cells. Following a previous study (17), homogeneous expression was present when immunostaining of E-cadherin and α-catenin was observed more than 70% of epithelial cells for each tissue section in (with strong membrane immunostaining) as a normal control; heterogeneous expression was presentation immunostaining of less than 70% of epithelial cells was observed. Samples were considered negative when they showed no immunoreactivity.

**Statistical analysis.** Pearson χ2 test was used to assess the statistical difference of E-cadherin and α-catenin expression in relation to the histological classification (p<0.05 was accepted as statistically significant).

**Results**

The results of the investigations on E-cadherin and α-catenin expression and their different patterns of distribution in normal, hyperplastic and neoplastic endometrium are summarized in Table I.

**Table I. E-Cadherin and α-catenin immunohistochemical expression.**

<table>
<thead>
<tr>
<th>Histology</th>
<th>E-Cadherin</th>
<th>α-Catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Heterogeneous</td>
</tr>
<tr>
<td></td>
<td>(17.4%)</td>
<td>(17.4%)</td>
</tr>
<tr>
<td></td>
<td>(17.4%)</td>
<td>(13%)</td>
</tr>
<tr>
<td>Atrophic endometrium</td>
<td>3/21</td>
<td>0/21</td>
</tr>
<tr>
<td></td>
<td>(14.3%)</td>
<td>(0)</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>6/24</td>
<td>4/24</td>
</tr>
<tr>
<td></td>
<td>(25%)</td>
<td>(16.7%)</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>4/11</td>
<td>7/11</td>
</tr>
<tr>
<td></td>
<td>(36.4%)</td>
<td>(63.6%)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>7/22</td>
<td>15/22</td>
</tr>
<tr>
<td></td>
<td>(31.8%)</td>
<td>(68.2%)</td>
</tr>
</tbody>
</table>
cases in postmenopausal women interestingly showed the same pattern for E-cadherin and α-catenin expression. A homogeneous distribution was seen in 18 out of 21 specimens (85.7%), whereas only 14.3% cases were negative for both molecules.

A total of 24 cases of simple hyperplasia and 11 cases of atypical hyperplasia were also analyzed. Homogeneous E-cadherin and α-catenin expression was seen in 58.3% cases and 66.6% cases respectively (Figure 1B). Homogeneous expression of E-cadherin and its associated cytoplasmatic molecule was not seen in atypical hyperplasia. E-Cadherin heterogeneous expression was seen in 63.6% cases of atypical hyperplasia; moreover 45.5% of cases of atypical hyperplasia showed heterogeneous α-catenin expression (Figure 1C) whereas 54.5% of cases were unreactive for α-catenin.

Twenty-two cases of endometrial adenocarcinoma were analyzed: seven cases were classified as G1, 9 cases were classified as G2 and six cases were classified as G3. E-Cadherin homogeneous expression was never seen (68.2% of adenocarcinoma cases showed an heterogeneous expression and 31.8% were unreactive); α-catenin homogeneous expression was seen in four cases (2 G1 and 2 G2 tumor). Immunostaining was restricted to focal areas of well-differentiated carcinoma (Figure 1D).
A homogeneous co-expression of both adhesion molecules was seen in 70% and in 46.0% of cases of normal and simple hyperplastic endometrium, respectively; no atypical hyperplasia or adenocarcinoma samples showed such a co-expression.

The histological diagnoses were correlated with the presence of immunostaining and the pattern of distribution (normal endometrium, simple hyperplasia, vs. atypical hyperplasia and adenocarcinoma). The correlation between E-cadherin expression \( (p<0.0001) \), \( \alpha \)-catenin expression \( (p<0.0001) \) and the histological diagnosis was statistically significant; furthermore, no co-expression of both adhesion molecules was observed in atypical hyperplasia and adenocarcinoma samples \( (p<0.01) \) (Table II).

### Discussion

Alterations in cell adhesion are among the hallmark characteristics of a malignant tumor, including irregularities in expression and distribution of adhesion molecules (1, 2). Decreased expression of E-cadherin, a protein essential for the establishment of cell–cell contacts, has been detected in a significant number of tumors of different epithelial origin (12). Since the association of E-cadherin with catenins is essential for a proper anchorage to the cytoskeleton and is necessary for E-cadherin binding function (13), this study investigated the co-expression of E-cadherin and \( \alpha \)-catenin in normal, hyperplastic and neoplastic endometrium.

According to previous studies (31, 32), E-cadherin immunostaining showed features similar to that of \( \alpha \)-catenin in endometrial samples. In normal endometrium, a strong and homogeneous expression was seen in contrast to that observed in atypical hyperplastic or neoplastic samples, where the intensity of staining was clearly decreased. The expression of E-cadherin and \( \alpha \)-catenin did not change during the menstrual cycle, which is consistent with the results of a previous study (32). Morphologically normal endometrial cells showed a polarized E-cadherin and \( \alpha \)-catenin expression which was uniformly localized at cell-to-cell borders. It is worth emphasizing that such a localized expression was never seen here in atypical or neoplastic endometrial cells, where the immunostaining was weak and found mainly in the cytoplasm. This may be linked to the fact that such an altered localization can be due to abnormal accumulation of E-cadherin and \( \alpha \)-catenin in the cytoplasm so that no adhesion function can be expressed. Moreover different areas of adenocarcinoma samples showed different pattern of E-cadherin and \( \alpha \)-catenin expression thus reflecting the intratumoral heterogeneity of neoplastic epithelium.

In simple hyperplastic endometrium, homogeneous E-cadherin (58.3%) and \( \alpha \)-catenin (66.6%) expression was decreased; although no direct evidence for a correlation between the E-cadherin/\( \alpha \)-catenin status has been reported, this evidence suggests that the lost of E-cadherin interactions with the cadherin–catenin complex might be impaired early in the hyperplastic process.

No atypical hyperplasia or adenocarcinoma samples showed a homogeneous co-expression for both adhesion molecules, suggesting that their expression might provide an additional criterion to define endometrial malignancies.

These findings indicate that alterations of these adhesion proteins are involved in endometrial cancers. Further studies are necessary in order to evaluate the biologic significance of adhesive functions, during endometrial carcinoma progression.

### Acknowledgements

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### References


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