Immunohistochemical study of extracellular matrix metalloproteinase inducer expression and nuclear morphometry in endometrial hyperplasia and endometrioid adenocarcinoma
Rasha M. AbdRabh, Nehal S. Zafer, Nashwa M. Emara, Ebtehal M. Abdel Aal

Department of Pathology, Faculty of Medicine, Benha University, Banha, Egypt
Correspondence to Rasha M. AbdRabh, MD, PhD, Pathology Department, Faculty of Medicine, Benha University, Banha, 11311, Egypt. Tel: +20 114 938 7855; e-mail: rashahamza32@yahoo.com
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Background
Endometrial cancer is the commonest gynecological cancer in developed countries and the second most common in developing ones.

Aim
This study aimed to evaluate the immunohistochemical expression of extracellular matrix metalloproteinase inducer (EMMPRIN) in endometrial hyperplasia and endometrioid adenocarcinoma and its relation with various clinicopathological variables. Moreover, we studied nuclear morphometry to assess the potential risk of patients to develop cancer endometrium.

Materials and methods
A total of 50 paraffin-embedded tissue blocks from endometrial hyperplasia (15 cases) and endometrioid adenocarcinoma (35 cases) specimens were immunohistochemically studied for EMMPRIN expression. Nuclear morphometric analysis was carried out by means of Olympus soft imaging system to measure the mean nuclear area (MNA), the mean nuclear minimum diameter (Mmnd) and mean nuclear maximum diameter, and mean nuclear ellipsoidy for all cases.

Results
There was a significantly increased EMMPRIN expression ($P<0.001$) in the endometrioid adenocarcinoma cases compared with the endometrial hyperplasia. Statistically significant relationships were detected between EMMPRIN expression score regarding tumor grade ($P<0.01$), myometrial invasion ($P<0.01$), lymph node metastasis ($P<0.01$), and International Federation of Gynecologists and Obstetricians stage ($P<0.05$). Kaplan–Meier analysis indicated that EMMPRIN overexpression was related to disease-specific survival ($P<0.05$). The nuclear morphometric parameters (MNA, Mmnd, mean nuclear maximum diameter, and mean nuclear ellipsoidy) could significantly differentiate between endometrial hyperplasia without atypia, atypical hyperplasia, and endometrioid carcinoma ($P<0.001$, $<0.01$, $<0.05$, and $<0.01$, respectively). The MNA and Mmnd had a significant positive correlation with tumor grade ($P<0.01$ and $<0.05$, respectively). The MNA was correlated with deep myometrial invasion ($P<0.05$), lymph node metastasis ($P<0.01$), International Federation of Gynecologists and Obstetricians stage ($P<0.01$), and disease-specific survival ($P<0.01$) of endometrioid adenocarcinoma cases.

Conclusion
EMMPRIN expression score and MNA may be used as prognostic markers to predict poor outcome in patients with endometrioid adenocarcinoma.

Keywords:
extracellular matrix metalloproteinase inducer, endometrioid carcinoma, nuclear morphometry

Introduction
Endometrial hyperplasia is a pathological condition characterized by increased glandular/stromal ratio. The recent classification, WHO 2014, classifies it into two groups: benign hyperplasia and atypical hyperplasia/endometrial intraepithelial neoplasia (Radhika, 2016).

Endometrial carcinoma is the commonest gynecological cancer in developed countries and the second most common gynecological one in developing countries (Goel and Kumar, 2012). In Egypt, it comes in the 13th rank among cancers in women, representing 1.6% (El-Bolkainy et al., 2013). The extracellular matrix metalloproteinase inducer (EMMPRIN) is the main
inducer of matrix metalloproteinases (MMPs). It is also known as cluster of differentiation 147 (CD147) or basigin which is encoded in human by the BSG (basigin) gene (Monteiro et al., 2014). It is involved in a range of processes, including angiogenesis, inflammatory diseases, and cancer progression (Xiong et al., 2014).

Image analysis (IA) is a technology that has undergone rapid development since 1960s. IA includes automated or semi-automated computer-based methods in which image information is digitized, captured, stored, and subjected to quantitation of image features. This process might also be referred to as “morphometry” (Wang et al., 2011). Nuclear morphometry may complement cytological diagnosis and give helpful information. The potential benefit from this technique is to discriminate between benign and malignant lesions, for objective grading of invasive neoplasms, prediction of prognosis, and therapeutic response (Priya and Sundaram, 2011).

This study was designed to evaluate the role of EMMPRIN and nuclear morphometry in endometrial hyperplasia and endometrioid endometrial adenocarcinoma cases in predicting survival and the potential risk of tumor progression.

Materials and methods
Study groups
This work included 50 cases of endometrial lesions (endometrial hyperplasia and endometrioid adenocarcinoma) obtained through collection of archived paraffin blocks of endometrial specimens during the period from March 2011 till December 2014 from the Pathology Department, Faculty of Medicine, Benha University.

Histopathological evaluation
Formalin-fixed paraffin-embedded blocks were cut into 5-μm thickness and examined microscopically using H&E stain to assess tumor grade, myometrial invasion, lymph node metastasis, and tumor International Federation of Gynecologists and Obstetricians (FIGO) stage. A total of 15 cases were endometrial hyperplasia and 35 cases were endometrioid adenocarcinomas. The endometrioid adenocarcinoma cases were classified according to FIGO grading and revised FIGO staging systems (Amant, 2010). All of the patients underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy with or without pelvic and/or para-aortic lymphadenectomy, except for four cases of endometrial hyperplasia, which underwent dilatation and curettage. Cases were selected on the basis of availability of demographic and clinicopathological data and approval by the ethical committee.

Immunohistochemical procedure
According to the manufacturer’s instructions, the sections were deparaffinized and then hydrated through a series of descending alcohols. Then, they were put in 10-mmol/l citrate buffer (pH 6) and were twice pretreated in a microwave oven for antigen retrieval. After a 25-min cooling period, the endogenous peroxidase activity was inactivated by incubation in 3% hydrogen peroxide (H2O2) for 5 min. Then the slides were incubated with the primary antibody at room temperature overnight. The primary antibody was mouse monoclonal antibody, anti-EMMPRIN, concentrated antibody diluted to 1 : 100 (Midco Trade Co., USBiological life Sciences, San Diego, CA, USA). The sections were incubated with avidin-biotin-peroxidase system (Dako, Dako, Santa Clara, CA, United States) for 30 min. Two or three drops of streptavidin enzyme label were put on each slide for 30 min at room temperature. Peroxidase reaction was detected by addition of diaminobenzidine tetrahydrochloride. Slides were rinsed well in tap water for 5 min then slightly counterstained with Mayer’s hematoxylin for 1–2 min and dehydrated in ascending alcohol. The slides were cleared in xylene for three changes, and cover slides were applied.

Evaluation of extracellular matrix metalloproteinase inducer expression scoring index
EMMPRIN expression was assessed using a scoring system that includes two variants, the intensity and extent of staining, as described by Zhang et al. (2012) and Yuan et al. (2015). The intensity of staining was scored as 2 (weak intensity), 3 (moderate intensity), and 4 (strong intensity). The extent of staining was based on the percentage of positive tumor cells. Samples were scored from 0 to 3 based on the percentage of cells showing positive membranous staining as follows: 0, 0–5%; 1, 6–50%; 2, 51–75%; and 3, more than 75%. An overall score index (SI) was calculated for each sample by multiplying the intensity score by the percentage score. Tumors were grouped into one of three groups based on their SI: score 1, negative or weak expression (SI, 0–4); score 2, moderate expression (SI, 5–8), and score 3, strong expression (SI, 9–12).

For negative controls, the primary antibodies were omitted. Ovarian carcinoma tissue was used as an external positive control.
Nuclear morphometry
Morphometric analysis was carried out by means of Olympus soft imaging system (Olympus Corporation, Hamburg, Germany). Approximately 30 nuclei were examined in the most representative areas of the slide, and their contours were traced manually using 40× objective to measure the mean nuclear area (MNA), mean minimum nuclear diameter (Mmnd), and mean maximum nuclear diameter (MMND). Mean nuclear ellipsoidy (MNE) was calculated by dividing MMND by Mmnd.

Statistical methods
Statistical analysis was performed using statistical package for the social sciences, version 16 (SPSS Inc., Chicago, Illinois, USA) for windows. Continuous variables were analyzed as mean±SD values or median (range) as appropriate, and percentages were calculated for categorical data. For categorical variables, differences were analyzed with $\chi^2$-tests and Fisher’s exact test. Differences among continuous variables with normal distribution were analyzed by Student t-test. Receiver operator characteristic (ROC) curve was used to determine the optimum sensitivity and specificity in prediction of endometrial lesions. $P$ value of up to 0.05 was considered statistically significant (Table 1).

Results
Immunohistochemical results
There was a highly significant statistical correlation between score of EMMPRIN expression and the histopathological type. Moreover, a significant positive statistical correlation between score of EMMPRIN expression and tumor grade, myometrial invasion, lymph node metastasis, FIGO stage, and 2-year survival was found (Table 2).

Nuclear morphometric results
Mean nuclear area
There was a highly significant statistical correlation between MNA and the histopathological type. MNA increases with the progress from endometrial hyperplasia without atypia (88.4 μm$^2$) to atypical hyperplasia (103.0 μm$^2$) to endometrioid adenocarcinoma (144.7 μm$^2$). There was a significant positive statistical correlation between MNA and tumor grade, myometrial invasion, lymph node metastasis, FIGO stage, and 2-year survival (Table 3).

Short axis of the nucleus (mean minimum nuclear diameter)
A significant statistical correlation was present between Mmnd and the histopathological type ($P<0.01$). Mean short axis of the nucleus increases with the progression from endometrial hyperplasia without atypia (9.58 μm) to atypical hyperplasia (11.72 μm) to endometrioid adenocarcinoma (13.0 μm). There was a significant statistical positive correlation between Mmnd and the tumor grade ($P<0.05$) (Table 4).

Long axis of the nucleus (mean maximum nuclear diameter)
There was a significant statistical correlation between MMND and the histopathological type ($P<0.05$). The mean long nuclear axis decreases with the progress from endometrial hyperplasia without atypia (20.6 μm) to atypical hyperplasia (12.9 μm) to endometrioid adenocarcinoma (12.8 μm) (Table 5).

Mean nuclear ellipsoidy
A significant statistical correlation was found between MNE and the histopathological type ($P<0.01$). It was noticed that the MNE decreases with the progression from endometrial hyperplasia without atypia (2.17 μm) to atypical hyperplasia (1.10 μm) to endometrioid adenocarcinoma (0.99 μm). There was a significant correlation between MNE and age ($P<0.05$), but there was no significant correlation between MNE and each of the following: tumor grade, myometrial invasion, lymph node metastasis, FIGO stage, and the 2-year survival ($P$ value for all >0.05) (Table 6).

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Table 1 Clinicopathological data of the studied cases

<table>
<thead>
<tr>
<th>Variables</th>
<th>N=50 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial hyperplasia (N=15)</td>
<td></td>
</tr>
<tr>
<td>Atypia</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma (N=35)</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7 (20)</td>
</tr>
<tr>
<td>II</td>
<td>23 (65.7)</td>
</tr>
<tr>
<td>III</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td></td>
</tr>
<tr>
<td>&lt;50%</td>
<td>21 (60)</td>
</tr>
<tr>
<td>≥50%</td>
<td>14 (40)</td>
</tr>
<tr>
<td>LNM</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>29 (82.9)</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (17.1)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>22 (62.9)</td>
</tr>
<tr>
<td>II</td>
<td>7 (20)</td>
</tr>
<tr>
<td>III</td>
<td>6 (17.1)</td>
</tr>
<tr>
<td>2-year survival</td>
<td></td>
</tr>
<tr>
<td>Survived</td>
<td>19 (54.3)</td>
</tr>
<tr>
<td>Recurrent</td>
<td>6 (17.1)</td>
</tr>
<tr>
<td>Died</td>
<td>10 (28.6)</td>
</tr>
</tbody>
</table>

FIGO, International Federation of Gynecologists and Obstetricians; LNM, lymph node metastasis.
Diagnostic accuracy

The diagnostic accuracy of EMMPRIN expression score and nuclear morphometry was determined by using ROC plots. These plots show the specificity (true negative fraction) and sensitivity (true positive fraction) of the test for all possible thresholds. The accuracy of the test is given by the area under the curve (Table 7).
Using ROC curve, EMMPRIN expression score has 77.1% sensitivity and 100% specificity, with area under the curve of 0.88, so it was very good for prognosis of endometrial cancer. However, MNA has 100% sensitivity and 100% specificity, with area under the curve of 1.0, denoting that MNA was excellent for endometrial cancer prognosis.

There was a statistically significant correlation between score of EMMPRIN expression and MNA among the endometrioid adenocarcinoma cases \((P<0.001)\) \((\rho=0.531)\), as seen in Graph 1. On the contrary, there was no statistically significant correlation between score of EMMPRIN expression and other nuclear morphometric parameters (Mmnd, MMND, and MNE) \((P=0.29, 0.96, \text{and 0.06, respectively})\)

### Discussion

Endometrial cancer is the most common gynecologic cancer in developed countries, and its prevalence is increasing. The most frequently occurring subtype is endometrioid adenocarcinoma (Morice et al., 2016).

EMMPRIN is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily. It is a MMP inducer that is expressed on the tumor cell surface (Zhang et al., 2012).

In this study, the levels of EMMPRIN expression were significantly increased in the endometrioid...
adenocarcinoma cases compared with the endometrial hyperplasia with and without atypia ($P<0.001$). This is in agreement with the studies by Nakamura et al. (2012) and Yuan et al. (2015). This may be explained by the fact...
that the endometrial hyperplasia is a noninvasive benign lesion with no invasion of either the basement membrane or the stroma (Radhika, 2016).

In this study, there was a significant increase of EMMPRIN expression and grades of endometrioid adenocarcinoma cases ($P<0.01$). These results were in agreement with Riethdorf et al. (2006), Ueda et al. (2007), Stewart and Crook, (2011), and Nakamura et al. (2012), who reported that the intensity with the pattern of EMMPRIN staining was significantly higher in poorly differentiated endometrial carcinoma.

On the contrary, Yuan et al. (2015) showed no correlation between score of EMMPRIN and grade of endometrial carcinoma. This different result may be owing to the study of both GI and GII as one item named low grade, and GIII as high grade and owing to the difference in number of the studied cases.

There was a significant statistical correlation in our study between EMMPRIN expression score and the presence of deep myometrial invasion ($P<0.01$). This result can be explained by that the expression of EMMPRIN on the neoplastic cells stimulates the production of various MMPs in cancer cells and fibroblasts, causing degradation of extracellular matrix, facilitating cancer cell invasion. This was in agreement with Nakamura et al. (2012) who found a positive relation between high

![Fig. 2](image-url)
EMMPRIN expression score and depth of myometrial invasion. However, Ueda et al. (2007) found that the staining scores for EMMPRIN were not associated statistically with depth of myometrial invasion. This disagreement may be explained by presence of different antibodies and different interpretations of EMMPRIN.

High EMMPRIN expression score in our study was statistically related to presence of lymph node metastasis ($P<0.01$), which matched with the work by Yuan et al. (2015) on endometrial cancer.

Our study stated that high EMMPRIN expression was associated with high FIGO stage ($P<0.01$), which is in agreement with the results obtained by Gabison et al. (2005) and Nabeshima et al. (2006) who studied the role of EMMPRIN in cancer progression.

On the contrary, different results were reported by Yuan et al. (2015), who found no significant difference in EMMPRIN expression observed between high and low FIGO stages.

The contrast in findings may be owing to the use of different scoring systems for EMMPRIN expression. Furthermore, there were variations in methodology between these studies, including differences in primary antibodies (monoclonal vs. polyclonal Ab).

On the contrary, in our study, there was no significant statistical correlation between EMMPRIN score and lymphovascular invasion ($P>0.05$). This disagrees with the results reached by Ueda et al. (2007) and Nakamura et al. (2012) who found a significant statistical correlation between them. The contrast in findings may be owing to the smaller sample size and rarity of lymphovascular invasion in our examined cases.

Endometrial biopsy is used frequently to differentiate benign endometrial lesions from malignant ones. Mostly, there is no difficulty in this differentiation, but in some cases, such as differentiation of complex atypical endometrial hyperplasia from low grade endometrial carcinoma, it is still difficult (Cermik et al., 2011).

In our study, computer-assisted IA of endometrial hyperplasia and carcinoma was based on measurement of four nuclear variables: MNA, short nuclear axis/ Mmnd, long nuclear axis/MMND, and MNE.

According to our results, the nuclear morphometric parameters (MNA, Mmnd, MMND, and MNE) could significantly differentiate among endometrial hyperplasia without atypia, atypical hyperplasia, and carcinoma ($P<0.001$, $<0.01$, $<0.05$, and $<0.01$, respectively). So, we can report that nuclear morphometric parameters are potentially useful tools in the diagnosis of endometrioid adenocarcinoma.

This is in agreement with Cermik et al. (2011) who found significant differences between atypical complex-type endometrial hyperplasia and low-grade carcinoma for all nuclear parameters. On the contrary, El-Sharkawy et al. (2015) found that the nuclear area and the nuclear roundness could differentiate significantly among simple hyperplasia, atypical hyperplasia, and carcinoma. However, short and long axes and nuclear ellipsoidy could not differentiate among the three groups.

It may be explained by the fact that a variety of factors exert their influence on nuclear morphology. The main factor is the concentration of the formaldehyde fixative. Overheating on a stretching plate is another factor. So, interlaboratory variations could blur the discriminating power of a morphometric classification or even result in essential differences.

In this study, the MNA and Mmnd were increasing with the progression from endometrial hyperplasia without atypia to atypical hyperplasia to endometrioid carcinoma (88.4, 103, and 144.7 $\mu$m$^2$ for MNA and 9.58, 11.72, and 13 $\mu$m for Mmnd).

However, the MMND and MNE were decreasing (20.6, 12.9, and 12.8 $\mu$m for MMND and 2.17, 1.10, and 0.99 $\mu$m for MNE). This is explained by the fact that the nuclei of the endometrioid adenocarcinoma glands are larger, more rounded, and pleomorphic than those of the hyperplastic glands.

Similarly, Kashyap et al. (2017) found that there was a gradual increase in nuclear size parameters from benign breast lesions to atypical ductal hyperplasia, ductal carcinoma in situ, and further to invasive carcinoma.

We found that both the MNA and Mmnd had a significant positive correlation with tumor grade. However, MMND and MNE were not correlated.

Similarly, Kalhan et al. (2010) and Gahlaut and Arora (2016) in their work on breast cancer found that there was a significant correlation between MNA and tumor grade ($P<0.01$) as the MNA was higher in G III tumors than G I and G II tumors.
According to the present study, it was found that the MNA, but not other nuclear morphometric parameters, was correlated with deep myometrial invasion ($P<0.05$), lymph node metastasis ($P<0.01$), and FIGO stage ($P<0.01$) of endometrioid adenocarcinoma cases.

This was in line with Mutter et al. (2008) who found that aggressive, deeply myoinvasive adenocarcinomas are associated with nuclear pleomorphism as measured by an increase in the standard deviation of the nuclear diameter. Moreover, Natarajan et al. (2010) found a significant correlation between MNA and lymph node metastasis in oral squamous cell carcinoma. In addition, Veltri et al. (2012) found that MNA increases with the progression of cancer prostate, so it is correlated with advanced tumor stage.

Regarding survival, the present study stated that through 5-year follow-up obtained from archive, 54.3% of cases survived free of the tumor, 17.1% of cases showed tumor recurrence, and 28.6% died.

In our study, there was a significant statistical correlation between the score of EMMPRIN expression and the 2-year survival of the patients ($P<0.01$). Its overexpression was correlated with clinically aggressive behavior and poor patient survival, which is in agreement with Ueda et al. (2007) and Nakamura et al. (2012) who reported that high levels of EMMPRIN expression were associated with tumor recurrence.

On the contrary, Bovenzi et al. (2015) and Xin et al. (2016) reported that increased EMMPRIN expression in patients with endometrial carcinoma was associated with increase in disease-free survival. This may be explained by differences in the interpretation system of EMMPRIN, variations in the used techniques, and subjective variability.

Moreover, Tsai et al. (2013) reported no statistical difference between survival of high grades of astrocytoma and EMMPRIN expression, suggesting that EMMPRIN was not the only factor that played a role in determining outcome.

In our study, there was a significant statistical correlation between MNA and the 2-year survival of the studied cases ($P<0.01$), which is in agreement with the study carried out by Miller et al. (2004) on endometrial carcinoma. Similarly, Nakazato et al. (2010) found that MNA is a useful independent marker for evaluating the prognosis of lung adenocarcinoma.

Moreover, this agrees with Carleton et al. (2016) who found that automated quantification of tissue nuclear morphometry can be used to accurately predict breast cancer aggressiveness and recurrence.

Upon using ROC curve for statistical analysis to evaluate the role of EMMPRIN and the nuclear morphometric parameters (MNA, Mmnd, MMND, and MNE), it was found that the MNA was excellent, but EMMPRIN score was very good in endometrial cancer prognosis.

Upon correlating the EMMPRIN expression score and the four nuclear morphometric parameters in our study, it was found that there was a statistically significant correlation between score of EMMPRIN expression and only the MNA among the endometrioid adenocarcinoma cases ($P<0.001$) ($r=0.531$), denoting that both EMMPRIN and MNA are better than other nuclear morphometric parameters to predict the outcome of the endometrioid carcinoma cases and presence of complementary relationship between them.

**Conclusion**

EMMPRIN expression score and MNA may be used as prognostic markers to predict poor outcome in patients with endometrioid carcinoma.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**


