

*Research Article*

## Immunohistochemical Study of Ghrelin and CD24 in Normal Cyclic Endometrium, Endometrial Hyperplasia and Endometrioid Carcinoma

Al Zahraa I. Khalil, Mariana F. Kamel, Nehad R. Abdel Maqsood, Heba M. Tawfik and Salwa G. Teleb

Department of Pathology, El-Minia Faculty of Medicine

### Abstract

Endometrial carcinoma (EC) is the sixth most common cancer in women worldwide. The distinction between atypical endometrial hyperplasia (EH) and endometrioid carcinoma, especially the well-differentiated grade, is particularly difficult with overlapping distinguishing criteria and small biopsy. Ghrelin is a 28 amino acid peptide that is synthesized by gastric mucosa and is expressed in a variety of normal and tumor tissues. CD24 is cell adhesion protein, which has been shown to be overexpressed in several carcinomas and to be associated with a poor clinical outcome. In the present study, immunohistochemical expression of Ghrelin and CD24 were evaluated using formalin-fixed and paraffin embedded tissue samples. Specimens included 20 cases cyclic endometrium, 30 cases EH without atypia, 25 cases atypical EH and 35 cases endometrioid carcinoma. Ghrelin loss and CD24 overexpression were significantly related to endometrioid carcinoma, compared with cyclic, EH without atypia and atypical EH ( $p < 0.001$ ). The combined Ghrelin negative and CD24 positive expression can help in the distinction of endometrioid carcinoma from atypical EH with high specificity and low sensitivity.

**Keywords:** Ghrelin, CD24, Endometrial carcinoma, Immunohistochemistry

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### Introduction

EC ranks the sixth most common cancer in women worldwide. In Egypt, it represents 14.72% of female genital tract malignancies<sup>1</sup> and 0.62% of all female cancers.<sup>2</sup> Type I EC constitutes about 80–90% of EC.<sup>3</sup> According to the Egyptian Cancer Institute Registry, endometrioid carcinoma constitutes three quarters of EC cases.<sup>1</sup> EH usually precedes or coexists with type I EC. The main problems encountered in EH and EC diagnosis is the difficulty of distinguishing EH with atypia from well-differentiated endometrioid carcinoma.<sup>4</sup>

Ghrelin is 28 amino acid peptide that is synthesized by gastric mucosa, but it is expressed in a variety of normal and tumor tissues.<sup>5</sup> In endometrial tissue, it is expressed during the menstrual cycle, involved in the cyclic growth.<sup>6</sup> It is involved in the growth and differentiation of hormone-dependent tumors as breast,<sup>7</sup> ovary,<sup>8</sup> and prostate.<sup>9</sup> Data regarding role of

Ghrelin in EC are contradictory. Its expression was absent or reduced in carcinoma of the endometrium.<sup>10</sup> In contrast, Fung et al.,<sup>11</sup> found that Ghrelin receptor down regulation is associated with EC development.<sup>11</sup>

CD24 is a sialoglycoprotein that is anchored to the cell surface by a glycosylphosphatidylinositol linkage. It was originally described as a B-cell specific marker, which is expressed in the early stages of B-cell development, as well as in several solid tumors, including breast, prostate and renal cell carcinomas.<sup>12-13</sup> In the current study, the expression of Ghrelin and CD24 by immunohistochemistry (IHC) is assessed in cyclic endometrium, EH and EC, aiming at evaluation of their role in the differential diagnosis between atypical EH and endometrioid carcinoma and exploring the presence of possible correlation between immunohistochemical expression of Ghrelin and CD24.

## Materials and Methods

Tissue specimens: Formalin-fixed and paraffin-embedded specimens were collected and prepared for this study from El-Minia University Hospital. Specimens included 20 cyclical endometria (10 cases were proliferative and 10 cases were secretory), 30 cases EH without atypia, 25 were atypical EH and 35 were endometrioid carcinoma specimens. Hyperplasia specimens were evaluated according to WHO classification 2003<sup>3</sup>, grouped into EH without atypia, atypical EH.

Endometrioid carcinoma was diagnosed based on evidence of stromal invasion, which is suggested by architectural complexity, extensive papillary formation, or desmoplastic stroma. Myometrial invasion were assessed as well for the malignant cases.

Immunohistochemistry: Tissues for IHC were fixed in 10% formalin, embedded in paraffin then was sectioned at 4µm, deparaffinized in Xylene, and hydrated with ethyl alcohol at decreasing concentrations. Antigen retrieval was done according to the citrate antigen retrieval protocols. Endogenous peroxidase activity was quenched by incubating the specimen for 5 min with 3% hydrogen peroxide. Sections were incubated with corresponding primary antibody. Ghrelin was provided as 0.1 concentrated polyclonal rabbit antibody, at 1:250 concentration (Biotin ab48285, Abcam). CD24 was provided as 0.1 concentrated monoclonal mouse antibody, at 1:100 concentration (clone SN3b, Biocare Medical). Then sections were incubated at 4C overnight, and then conjugation with the Streptavidine–Biotin–Peroxidase complex was done after application of secondary antibody. Negative control slides were prepared, by omitting the primary antibody from the staining procedure. Sections of normal human stomach were used as a positive control for Ghrelin, while for CD24; ovarian serous carcinoma was used as a positive control.

Immunohistochemistry assessment: For Ghrelin antibody, positive staining was detected as cytoplasmic staining and for

CD24 antibody, positive staining was detected as a membranous and/or cytoplasmic staining. Both Ghrelin antibody and CD24 antibody expression were grouped into positive and negative cases according to Kristiansen et al., and Younes et al.,<sup>14,15</sup>

## Statistical analysis:

Chi-square test has been used (SPSS 18 software). Results were considered significant when p-value < 0.05. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of Ghrelin and CD24 were estimated.

## Results

As shown in Table (1) and Figure (1), Ghrelin expression was statistically highly significant among the different histopathological groups (p<0.001). In cyclic endometrium, EH without atypia and atypical EH cases, most cases showed positive Ghrelin immunoreactivity (100%, 96.7% and 96%, respectively). Then a sudden decrease was seen in Ghrelin expression in endometrioid carcinoma cases (51.4%).

Statistically significant differences were seen between endometrioid carcinoma cases and each of cyclic endometrium, EH without atypia and atypical EH cases (p<0.001). A statistically significant difference between Ghrelin expression in atypical EH compared with well-differentiated endometrial carcinoma (p=0.001) was found.

Concerning CD24, CD24 expression was statistically highly significant among the histopathological groups (p<0.001). It was obviously increased with the progression from EH without atypia (13.4%), to atypical EH (24%), to endometrioid carcinoma (77.1%) (Table 1, Figure 1).

A statistically significant increase was found in CD24 endometrioid carcinoma cases as compared to its expression in EH without atypia and atypical EH cases (p<0.001 and p=0.002, respectively).

**Table (1): Ghrelin and CD24 immunostaining in different histopathological groups**

Histopathological groups	No.	Ghrelin		CD24	
		Negative (%)	Positive (%)	Negative (%)	Positive (%)
Cyclic endometrium	20	0	20 (100)	9 (45)	11(55)
EH without atypia	30	1 (3.3)	29 (96.7)	26 (86.6)	4 (13.4)
Atypical EH	25	1 (4)	24 (96)	19 (76)	6 (24)
Endometrioid carcinoma	35	17 (48.6)	18 (51.4)	8 (22.9)	27(77.1)

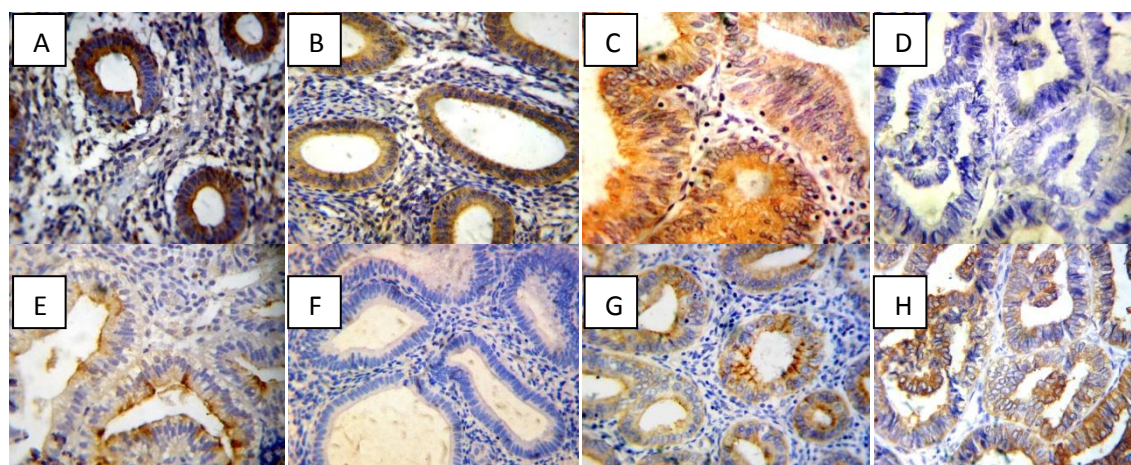


Figure 1. Ghrelin expression in cyclic endometrium (A), EH without atypia (B), Atypical EH (C) and Endometrioid carcinoma (D). CD24 expression in cyclic endometrium (E), EH without atypia (F), Atypical EH (G) and Endometrioid carcinoma (H).

As shown in Table (2), Ghrelin had high sensitivity (96%), but low specificity (48.6%) for distinction of endometrioid carcinoma from atypical EH and it had much lower specificity (26.7%) in distinction of well differentiated endometrioid carcinoma from atypical EH.

CD24 had 77.2% sensitivity and 76% specificity for distinction of atypical EH from endometrioid carcinoma and it had 52.9% sensitivity and 76% specificity in distinction of well differentiated endometrioid carcinoma from atypical EH (Table 2).

**Table (2): The diagnostic validity of Ghrelin immunoreactivity**

Diagnosis	Sensitivity		Specificity		PPV		NPV		Accuracy	
	Ghrelin	CD24	Ghrelin	CD24	Ghrelin	CD24	Ghrelin	CD24	Ghrelin	CD24
Atypical carcinoma vs. EH	96%	77.2%	48.6%	76%	57.2%	81.1%	94.4%	70.3%	68.4%	76.7%
Atypical EH vs. well differentiated carcinoma	96%	52.9%	26.7%	76%	68.6%	60%	80%	70.3%	70%	70%

There was statistically significant inverse correlation between Ghrelin and CD24 ( $r=-0.438$ ,  $p<0.001$ ) in all cases.

According to combined expression patterns of both markers, 4 immunophenotypes were identified including Ghrelin<sup>-ve</sup>/CD24<sup>-ve</sup>, Ghrelin<sup>+ve</sup>/CD24<sup>-ve</sup>, Ghrelin<sup>-ve</sup>/CD24<sup>+ve</sup> and Ghrelin<sup>+ve</sup>/CD24<sup>+ve</sup>. In cyclic endometrium, 55% cases showed Ghrelin<sup>+ve</sup>/CD24<sup>-ve</sup> and 45% showed Ghrelin<sup>+ve</sup>/CD24<sup>+ve</sup> expression patterns. In EH without atypia cases, Ghrelin<sup>+ve</sup>/CD24<sup>-ve</sup> combined expression was the most frequent being 86.6% of cases. In atypical EH, Ghrelin<sup>+ve</sup>/CD24<sup>-ve</sup> combined expression was the most frequent combined expression pattern seen in 64% of cases, followed by Ghrelin<sup>+ve</sup>/CD24<sup>+ve</sup> in 32% of cases. In endometrioid carcinoma

cases, the most frequent pattern was Ghrelin<sup>-ve</sup>/CD24<sup>+ve</sup> immunophenotype seen in 48.6% of cases, followed by Ghrelin<sup>+ve</sup>/CD24<sup>+ve</sup> in 34.2% of cases.

Table (3) demonstrates that the sensitivity and specificity of combined Ghrelin negative expression and CD24 positive expression were 48.5% and 100%, respectively, in distinction of endometrioid carcinoma from atypical EH. Moreover, the sensitivity and specificity of this combination were 46.7% and 100%, respectively, in distinction of well differentiated endometrioid carcinoma from atypical EH.

**Table (3): The diagnostic validity of combined Ghrelin negative expression and CD24 positive expression in diagnosis of endometrioid carcinoma in comparison to atypical EH**

<i>Diagnosis versus</i>	<i>Sensitivity</i>	<i>Specificity</i>	<i>PPV</i>	<i>NPV</i>	<i>Accuracy</i>
<b>Carcinoma vs. Atypical EH</b>	48.5%	100%	100%	58.1%	28.4%
<b>well differentiated carcinoma vs. Atypical EH</b>	46.7%	100%	100%	75.7%	17.5%

## Discussion

Distinction between atypical EH and well-differentiated endometrioid carcinoma is hard especially in small biopsy specimens. In this study, we sought to address whether the immunohistochemical expression of Ghrelin and CD24 are helpful in making the distinction between endometrioid carcinoma, especially the well differentiated carcinoma and EH with atypia.

In the current study, cyclic endometrium showed positive Ghrelin expression in 100%. This finding was consistent with Tawadros et al., Dagli et al., and Fung et al.,<sup>6,10,16</sup> There was gradual decrease in the positive expression rate of Ghrelin in hyperplastic lesions. These findings were in agreement with the findings of Dagli et al.,<sup>10</sup> However, Younes et al.,<sup>15</sup> reported positive Ghrelin immunoreactivity in 75% and 100% for EH without atypia cases and atypical EH cases, respectively.

In our study, Ghrelin immunoreactivity obviously decreased in endometrioid carcinoma cases with total depletion in 48.6% of cases. Our results were

comparable to Younes et al.,<sup>15</sup> who found that Ghrelin was totally absent in 36.36% of endometrioid carcinoma cases. Dagli et al.,<sup>10</sup> found no Ghrelin reactivity among their studied endometrioid carcinoma cases. Conversely, Fung et al.,<sup>16</sup> detected Ghrelin positivity in all cases of an immunohistochemical detection of Ghrelin in a tissue microarray of 70 endometrioid carcinoma cases. In addition, Fung et al.,<sup>16</sup> found that Ghrelin mRNA is highly expressed in a range of endometrial cancers and the expression of the Ghrelin axis was demonstrated in three EC cell lines by quantitative real-time RT-PCR and Western blot analyses.

Similarly, we detected a significantly higher Ghrelin expression in atypical EH than in endometrioid carcinoma and well differentiated endometrioid carcinoma as well. These results were similar to results reported by Dagli et al.,<sup>10</sup> and Younes et al.,<sup>15</sup> suggesting that Ghrelin loss may be implicated in the conversion from premalignant to malignant histopathology and suggests its diagnostic efficacy in

differentiating malignant from premalignant endometrial lesions.

In our study, Ghrelin was found to be a sensitive but not specific marker for the differentiation between atypical EH compared with endometrioid carcinoma and well-differentiated cases as well. These were comparable to results reported by Younes et al.,<sup>15</sup> who reported 100% sensitivity and 36.36% specificity for distinction of endometrioid carcinoma from atypical EH and it had much lower specificity (14.29%) and 85.6% sensitivity in distinction of well differentiated endometrioid carcinoma from atypical EH. Regarding CD24, A statistically significant association was observed between lesion progression and CD24 expression. This correlated with Kim et al.,<sup>17</sup> who reported that CD24 expression significantly enhanced along lesion progression. In addition, our study revealed the dynamic change of CD24 expression in hyperplasia and carcinoma, where a sharp down-regulation of CD24 in the hyperplastic lesions, followed by a remarkable upregulation in endometrioid carcinoma. These findings were in concordance with Kim et al.,<sup>17</sup> suggesting that CD24 expression may be useful for the differential diagnosis between endometrioid carcinoma and atypical EH.

To the best of our knowledge, this is the first study that evaluated the sensitivity and specificity of CD24 in distinction of endometrioid carcinoma from atypical EH. We found that CD24 had moderate sensitivity and specificity for distinction of atypical EH from endometrioid carcinoma and atypical EH from well-differentiated endometrioid carcinoma as well.

To the best of our knowledge, this is the first study that evaluated the correlation between Ghrelin and CD24 expression. The significant negative correlation was concluded to indicate that both loss of Ghrelin expression and CD24 overexpression may be implicated in the conversion from premalignant to malignant endometrial histopathology. Additionally, our study demonstrated that the combined Ghrelin negative and CD24 positive expression can help in the distinction of

endometrioid carcinoma from atypical EH with high specificity and low sensitivity. Moreover, in our study, the specificity of this combination was better than values obtained by the use of each marker singly in differentiating endometrioid carcinoma from atypical EH.

**In conclusion,** reduced Ghrelin expression and CD24 overexpression is considered to be in favor of the diagnosis of adenocarcinoma over atypical EH.

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