RESEARCH ARTICLE

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Moesin and Ezrin as New Promising Markers for Early Detection of Endometrial Carcinoma: An Immunohistochemical Study

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Abstract

Objective: the aim of this study is to evaluate immunohistochemically the expression of ezrin, and moesin, in endometrial lesion cases in order to detect EC at early stages which will have an important implication on the patients' outcome. **Method:** 100 stored, formalin-fixed, paraffin-embedded tissue blocks of endometrial curettage obtained due to abnormal uterine bleeding or postmenopausal bleeding were collected. Each paraffin block was re-cut by rotatory microtome at 4 μm thickness then mounted on a glass slide and stained by hematoxylin and eosin for routine histopathological examination and on charged slides for immunohistochemistry using an automated staining system (Dako autostainer link 48) with antibodies against Moesin and Ezrin. Cytoplasmic staining was evaluated for both Moesin and Ezrin based on the intensity and extent of staining and scored for each sample. **Result:** Both Moesin and Ezrin were significantly higher in atypical endometrial hyperplasia compared to benign hyperplasia and significantly higher in endometrial carcinoma compared to atypical hyperplasia. Moesin also significantly correlated with higher tumor grades while Ezrin was significantly higher in postmenopausal women denoting their role in tumor progression and poor prognosis. **Conclusion:** Both Moesin and Ezrin could be potentially used as predictive markers for endometrial carcinoma screening programmes as well as indicators for cancer progression.

Keywords: Moesin – ezrin – endometrial – carcinoma

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Introduction

Endometrial carcinoma (EC) is the commonest cancer of female genital tract, it is rated that 3% of women will develop EC in their lifetime (Santoro et al., 2021). Although Egypt has significantly lower incidence of EC than other Middle East countries, but it showed a significant increase in incidence over the past 12 years especially among premenopausal women (Sung et al., 2021). While early stages ECs are surgically curable, however patients with advanced or recurrent cancer are mostly unresectable with very limited treatment options and low 5 year survival rate (Tanaka et al., 2022). Thus emerges the utmost importance of early detection of EC when surgical and medical treatment options are variable and promising. Therefore a predictive marker needs to be identified for early detection of EC and its progression.

Moesin and Ezrin are members of a protein family which are responsible for the connection between plasma membrane proteins and the actin cytoskeleton. These proteins are present in actin-rich surfaces such as microvilli, filopodia, and membrane ruffles. They play a role in cell shapes, cell attachments, and interactions as well as supportive role during cytokinesis (Agacayak et al., 2022). They are also involved in cell motility as well as tumor metastasis (Mhawech-Fauceglia et al., 2012). Although Moesin and Ezrin are widely expressed in normal tissues, but their expression is only specific during human development as in early embryo stages but should not be expressed during later development or postnatal stages (Lipreri da Silva et al., 2023). However recent researches show that they are upregulated in several human tumors such as thyroid tumors, pancreatic tumors and glioblastoma (Yu et al., 2019). That is why, we think that these markers are crucial for endometrial carcinoma development hence could be used for early diagnosis of endometrial cancer.

Consequently, in this research we tried to evaluate immunohistochemically the expression of moesin and ezrin, in endometrial lesions in order to detect EC at early stages which will have an important implication on the patients' outcome.

Materials and Methods

This study included 100 stored, formalin-fixed, paraffin-embedded tissue blocks of endometrial curettage obtained due to abnormal uterine bleeding

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or postmenopausal bleeding. The specimens were anonymous for confidentiality and replaced by numbers. The study was approved by the Medical Ethics Committee.

Staining procedures

Each paraffin block was re-cut by rotatory microtome at 4 µm thickness then mounted on a glass slide and stained by hematoxylin and eosin (H&E) for routine histopathological examination and on charged slides for immunohistochemistry (IHC). IHC was performed using an automated staining system (Dako autostainer link 48) with antibodies against Moesin (monoclonal mouse antibody 1:200 dilution; 38/87 clone; cat. no. sc-58806; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and Ezrin (monoclonal mouse antibody, clone MS-661-R7, 7 ml ready to use; Lab Vision Laboratories, Fremont, CA, United States).

Immunohistochemical evaluation

Immunohistochemical cytoplasmic staining was evaluated for both Moesin and Ezrin based on the intensity and extent of staining and scored for each sample.

The immunoreactivity was scored, depending on the intensity of staining and the percentage of positive cells on a scale of 0–3. The intensity of Ezrin staining was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive cells was scored as 0 (<1%), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The two scores were multiplied and then the total immunohistochemical score values (0–12) were classified as follows: score 0 as negative, score 1 (multiplication values 1 and 2) as weakly positive, score 2 (multiplication values 3, 4, and 6) as moderately positive, and score 3 (multiplication values 8, 9, and 12) as strongly positive (Agacayak et al., 2022).

Statistical analysis

All data was transferred to the Statistical Package of Social Science (SPSS) Software program, version 25 to be statistically analyzed. Data was summarized using mean, and standard deviation (for age) and frequency and percentages (for other variables). Comparison between groups was then performed using Chi square test. A P value of ≤ 0.05 was considered statistically significant. All slides were screened using a Leica DM500 microscope. Microscopic photos were captured using Leica EC4 camera using The Leica LAS EZ software.

Results

Our study included 100 paraffin embedded blocks of endometrial lesions of which 21% of cases were benign endometrial hyperplasia, 23% of cases were atypical endometrial hyperplasia and 56% of cases were endometrial carcinoma all of which were of the conventional endometrioid type showing focal squamous differentiation in 12.5% of cases (7/56). In endometrial carcinoma cases, grades 1, 2 and 3 were diagnosed in (16/56, 30/56 and 10/56 respectively). The age of patients ranged from 40 to 80 years with mean of 56.4 ± 9.4. Postmenopausal patients represented about two thirds of cases (65/100).

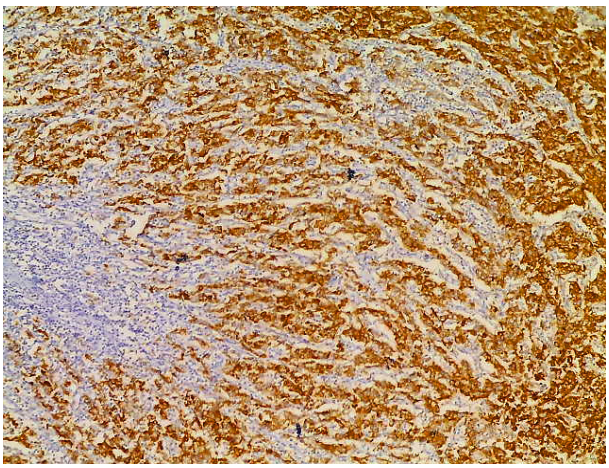

Figure 1. A Case of Endometrial Carcinoma High Grade Showing Positive Moesin Expression in more than 75% of Tumor Cells (4) and Strong Staining (3) with Histoscore (12) Representing Strong Positivity.

Moesin immunohistochemical expression

Moesin was positively expressed in all cases of endometrial carcinoma (100%), most of which were of histoscore 3 (70%) as shown in Figure 1. As for endometrial hyperplasia with atypia 80% of cases showed positive Moesin expression mostly of histoscore 2 (90%) while benign endometrial hyperplasia showed positive Moesin expression in 50% of cases all showing histoscore 1 (100%) as shown in Figure 2. Therefore by comparing Moesin expression between endometrial carcinoma and atypical endometrial hyperplasia, Moesin was found to be higher in the former with a highly statistically significant value (0.001) (Table 1). Similarly by comparing Moesin expression between atypical endometrial hyperplasia and benign endometrial hyperplasia, Moesin was found to be higher in the former with a highly statistically significant value (0.0001). Further study of endometrial carcinoma cases, Moesin expression showed a highly statistically significant correlation with the tumor grade (0.0001) as

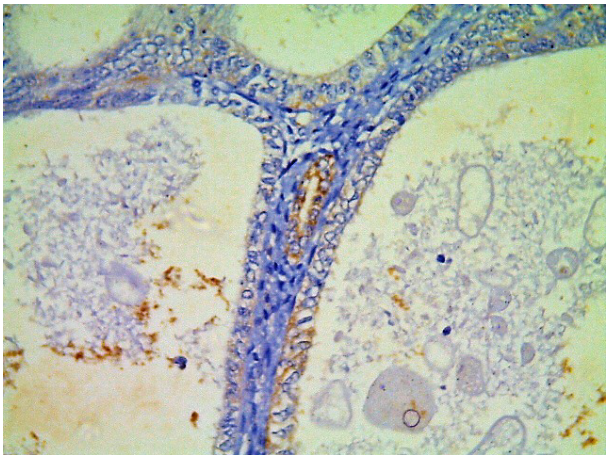

Figure 2. A Case of Benign Endometrial Hyperplasia Showing Positive Moesin Expression in 26-50% of Tumor Cells (2) and Weak Staining (1) with Histoscore (2) Representing Weak Positivity.

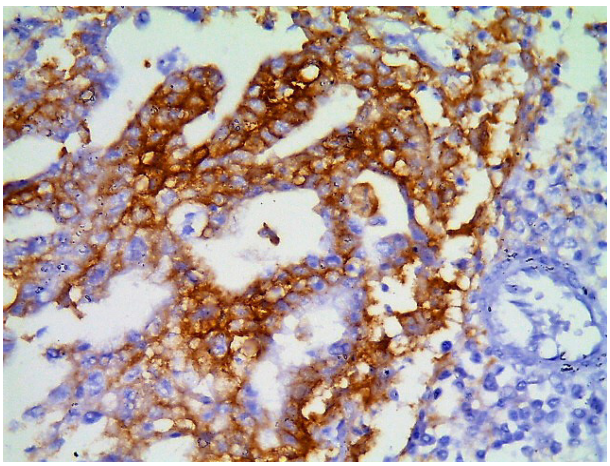

Figure 3. A Case of Endometrial Carcinoma Showing Positive Ezrin Expression in more than 75% of Tumor Cells (4) and Strong Staining (3) with Histoscore (12) Representing Strong Positivity.

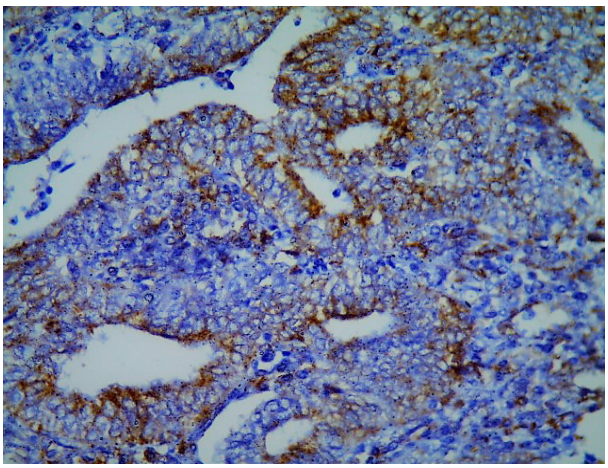

Figure 4. A Case of Atypical Endometrial Hyperplasia Showing Positive Ezrin Expression in 25-50% of Tumor Cells (2) and Moderate Staining (2) with Histoscore (4) Representing Moderate Positivity.

Table 1. Relation between Moesin Expression and Tumor Grade in Endometrial Carcinoma Cases

Moesin expression histoscore	Endometrial carcinoma grade 1	Endometrial carcinoma grade 2	Endometrial carcinoma grade 3	Total	P value
1(weak)	16 (100%)	0	0		
2 (moderate)	0	30 (100%)	0	56	0.0001
3 (strong)	0	0	10 (100%)		

shown in table 1. No statistically significant correlation was observed between Moesin expression and age of patients. Although Moesin expression histoscore 2 and 3 were seen more in postmenopausal women but no statistically significant relation was noticed.

Moesin expression was found to be statistically significantly reduced in endometrial carcinoma cases showing squamous differentiation (p value 0.003).

Ezrin immunohistochemical expression

Ezrin was positively expressed in all cases of endometrial carcinoma (100%), most of which were of histoscore 3 (80%) as shown in figure3. As for endometrial hyperplasia with atypia 85% of cases showed positive Ezrin expression mostly of histoscore 2 (75%) as shown in figure 4, while benign endometrial hyperplasia showed positive Ezrin expression in 55% of cases all showing histoscore 1 (100%). Therefore by comparing Ezrin expression between endometrial carcinoma and atypical endometrial hyperplasia, Ezrin was found to be higher in the former with a highly statistically significant value (0.002). Similarly by comparing Ezrin expression between atypical endometrial hyperplasia and benign endometrial hyperplasia, Ezrin was found to be higher in the former with a highly statistically significant value (0.001). All of the 65 postmenopausal cases showed Ezrin histoscore 3 which revealed a statistically significant relation. Study of endometrial carcinoma cases, Ezrin expression showed no statistically significant correlation with either patients' age, tumor grade or squamous differentiation.

Discussion

Cell-cell interactions are essential to ensure normal function and morphology of cells. Ezrin and Moesin proteins have been mainly concerned with epithelial cell morphogenesis and attachments, the disruption of which participates in induction and progression of malignant tumors (Ahmed et al., 2020).

Our concern in the current study was to achieve early diagnosis of endometrial carcinoma in patients with endometrial hyperplasia. We tried to find out whether increased Moesin and Ezrin overexpression in patients with hyperplasia may eventually lead to endometrial carcinoma which should alert the surgeon in terms of malignancy.

In our study, Moesin was positively expressed in all endometrial carcinoma cases, mostly of histoscore 3, and in 80% of atypical endometrial hyperplasia mostly of histoscore 2 and in 50% of benign endometrial hyperplasia mostly of histoscore 1. These results show that Moesin is overexpressed in endometrial cancer compared to atypical hyperplasia and overexpressed in atypical hyperplasia when compared to benign hyperplasia. These results were statistically highly significant. Similar results were observed by Agacayak et al., (2022). We also observed a highly statistically significant correlation between Moesin expression and tumor grade. Likewise Mhawech-Fauceglia et al., (2012) stated the same observation, suggesting that Moesin might play a role in tumorigenesis of endometrial adenocarcinomas. On the contrary Agacayak et al., (2022) found no significant difference between Moesin expression and tumor grade. Our study

and the study done by Agacayak et al., (2022) could not find any significant relation between Moesin expression and the age of patients. Our study stated a significant correlation between Moesin expression and squamous differentiation in endometrial carcinoma cases. To our knowledge this parameter has not been investigated before. Adding to this that studies about the relation between endometrial carcinoma and Moesin expression are very limited.

Concerning Ezrin, our study stated that it was positively expressed in all endometrial carcinoma cases, mostly of histoscore 3, and in 85% of atypical endometrial hyperplasia mostly of histoscore 2 and in 55% of benign endometrial hyperplasia mostly of histoscore 1. These results show that Ezrin is overexpressed in endometrial cancer compared to atypical hyperplasia and overexpressed in atypical hyperplasia when compared to benign hyperplasia. These results were statistically highly significant. Same results were stated by Ahmed et al., (2020) who also linked Ezrin overexpression with tumor invasiveness and poor prognosis. Similarly the study done by Ohtani et al., (2002) concluded the same results as ours and also linked its expression to metastasis. Moreover the three studies could not find any significant relation between Ezrin overexpression and tumor grade. However the study done by Ahmed et al., (2020) observed a statistically significant correlation with squamous differentiation which we couldn't, moreover we observed that Ezrin was statistically significantly overexpressed in postmenopausal unlike their study.

The significant role of both Moesin and Ezrin in tumor progression could be attributed to their function in organizing cytoskeleton and cell morphology besides their crucial role in coordinating cell-cell signaling therefore, cells showing overexpression lose their cell-cell contacts hence lead to the development and progression of malignancy.

To sum up increased Moesin and/or Ezrin expression in the histopathological evaluation of precancerous endometrial lesions should alert the surgeon in terms of malignant potentiality and that the patient should be followed closely in terms of progression in the postoperative period. Moreover, their overexpression in cancerous lesions should be considered as being a poor prognostic factor. However, more comprehensive studies with longer follow-up periods are needed.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

The authors declare that there is no conflict of interest and received no financial support for this research. The authors solely developed the theory, verified the analytical methods and wrote the manuscript. The material obtained in this study was collected in the form of archived paraffin blocks and clinical data were taken from pathology request sheets designated by numbers, therefore no consent from patients was required. All steps of this research were approved by the ethical committee.

Ethics approval

All steps are approved by the ethical committee.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Agacayak E, Keles A, Deger U, et al (2022). Could Moesin Be a New Marker for Indicating Progression in Endometrial Cancer?. *Cancer Manag Res*, **4**, 1247-7.

Ahmed R, Ghada M, Mohamed S, et al (2020). A Potential Invasion-Promoting Role for Ezrin in Endometrial Carcinoma. *Res Oncol*, **16**, 48-5.

Lipreri da Silva J, Vicari H, Machado-Neto J (2023). Perspectives for Targeting Ezrin in Cancer Development and Progression. *Future Pharmacol*, **3**, 61-9.

Mhawech-Fauceglia P, Wang D, Lele S, et al (2012). Claudin7 and moesin in endometrial Adenocarcinoma; a retrospective study of 265 patients. *BMC Res Notes*, **5**, 65.

Ohtani K, Sakamoto H, Rutherford T, et al (2002). Ezrin, a membrane-cytoskeletal linking protein, is highly expressed in atypical endometrial hyperplasia and uterine endometrioid adenocarcinoma. *Cancer Lett*, **179**, 79-6.

Santoro A, Angelico G, Inzani F, et al (2021). Pathological features, immunoprofile and mismatch repair protein expression status in uterine endometrioid carcinoma: focus on MELF pattern of myoinvasion. *Eur J Surg Oncol*, **47**, 338-5.

Sung H, Ferlay J, Siegel RL, et al (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, **71**, 209-9.

Tanaka C, Kobori T, Okada R, et al (2022). Ezrin Regulates the Cell Surface Localization of PD-L1 in HEC-151 Cells. *J Clin Med*, **11**, 2226-9.

Yu L, Zhao L, Wu H, et al (2019). Moesin is an independent prognostic marker for ER-positive breast cancer. *Oncol Lett*, **17**, 1921-3.