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ORIGINAL ARTICLE

Immunohistochemical Expression of GLUT-1 in Endometrial Hyperplasia and Carcinoma

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ABSTRACT

Keyword: Endometrial hyperplasia, endometrial carcinoma, IHC GLUT-1 expression.

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Background: Early detection of endometrial carcinoma (EC), especially its precursor lesions, is crucial for reducing mortality but remains challenging. Accurate diagnosis is essential, as misidentification can result in prolonged clinical follow-ups, and repeated biopsies. Immunohistochemistry (IHC), as a diagnostic or prognostic, method may be helpful in this conflict. Glucose transporter-1 (GLUT-1) has emerged as a promising biomarker for cancer development. This study aimed to distinguish between atypical endometrial hyperplasia/ endometrioid intraepithelial neoplasia (AEH/EIN) in comparable to endometrial hyperplasia (EH) without atypia based on the percentage of stained cells and staining intensity. Additionally, the study examined the relationship between GLUT-1 expression and clinicopathological factors linked to prognosis in EC cases. **Results:** A significant upward association was observed between GLUT-1 expression and disease progression from EH without atypia to EC. In EH without atypia, 33.3% of cases demonstrated weak GLUT-1 expression, whereas, among EH with atypia, 38.5% exhibited strong GLUT-1 expression, and 61.5% showed moderate expression. All cases of EC displayed strong GLUT-1 expression. Furthermore, GLUT-1 expression was positively correlated with increasing tumor grade and stage. **Conclusions:** GLUT-1 expression was increased progressively with disease progression from EH to EC and with higher grades and stages of EC. These findings suggest the potential role of GLUT-1 as a predictor for development and progression of EC**.**

INTRODUCTION:

According to the GLOBOCAN 2020 online database, EC ranked as the 15th most common malignancy in Egypt (1). EH includes two distinct conditions: EH without atypia; characterized by reactive proliferation due to unopposed estrogen effects, and AEH/ EIN, which is a precancerous lesion. Differentiating between these two conditions is critical for ensuring appropriate patient management (2).

The GLUT family of membrane transport proteins is responsible for most glucose absorption in mammalian cells, with GLUT-1 being one of the key transporters in tumor tissues. GLUT-1 is primarily responsible for glucose uptake and is overexpressed in many malignant tumors, where it is often linked to advanced tumor grade, lymphovascular invasion, and lymph node involvement. In contrast, benign tumors and non-tumorous tissues rarely show GLUT-1 expression when analyzed by IHC (3).

GLUT-1 expression has emerged as a promising indicator of malignant transformation. Its overexpression is thought to contribute significantly to the development of various neoplasms.

Previous researches has consistently associated GLUT-1 expression with increased malignancy, invasiveness, diagnosis, prognosis, and survival rates in a variety of tumors, including prostate, ovarian, colorectal, breast, lung, pancreatic, liver, esophageal, and cervical carcinomas (4-7).

Several studies have shown that GLUT-1 is significantly upregulated during the early stages of preneoplastic lesions, such as cervical intraepithelial neoplasia, borderline ovarian tumors, colonic adenoma, and prostatic intraepithelial neoplasia (8-11).

This study aimed to identify the optimal criteria for interpreting GLUT-1 immunostaining, assess AEH/EIN changes according to the percentage and intensity of stained cells, and explore the association between IHC expression of GLUT-1 and the clinicopathological variables of prognostic significance, including tumor size, grade, and pathological (p) stage.

MATERIAL AND METHOD:

This prospective cohort observational research was performed on 50 formalin-fixed paraffin embedded tissue blocks related to 50 cases of EC and EH with and without atypia obtained from the archived material of Pathology Lab, Aswan University Hospitals in the period from January 2022 to October 2023. The research was done after approval from the Ethical Committee of Aswan University Egypt (EC Ref NO.:Asw.U./ 630/5/22).

Histological examination:

Tissue blocks were utilized to produce 4µm thick tissue slices that were hematoxylin and eosin (H&E) stained. EH was classified according to WHO classification 2014 (12). Tumor histological type, grade and pathological stage was conducted in accordance with WHO classification 2020 (13).

Immunohistochemical staining:

Four micrometer (4µm)-thick sections were prepared and mounted on pre-labelled poly-L-lysine coated slides. Immunostaining was done as shown in the product data sheet.

Positive control: Positive control slides from colon carcinoma were included in each staining session. **Negative controls**: Additional tissue sections were stained simultaneously, but without utilizing the main antibody.

Immunohistochemical (IHC) detection and scoring of GLUT-1:

Tissue slices were analyzed histologically using a bright-field microscope. The immunoreaction was deemed positive when a brownish membranous staining was observed. The level of GLUT-1 expression was assessed utilizing a semi-quantitative technique. A histological scoring technique (H-score), which is a composite measure, takes into account both the proportion of positive cells and the intensity of their staining. The ultimate score varied from 0 and 300. The samples were classified based on a discriminatory threshold. Specimens with scores ranging from 0 to 29, 30 to 99, 100 to 199, in addition 200 to 300 were categorized as having negative, weak, moderate, and high positive, correspondingly (3).

Statistical analysis

The statistical analysis was performed utilizing SPSS v18. The quantitative data were represented using measures of central tendency such as means ± standard deviation (SD), median, and range. The qualitative data were represented as numerical values and percentages. The data underwent normality testing using the Shapiro-Wilk test. The statistical significance of various parameters was evaluated using the Chi-Square test and Fisher's Exact test. A p-value of 0.05 or lower was regarded as statistically significant.

Results:

Tissue specimens were obtained by total hysterectomy in 33 cases and by D & C in 17 cases (among these cases, 4 were EC). The age range of patients involved in this research was 35-73 years. The mean age of the patients with EH without atypia was 44.83 ± 5.98, and the mean of age of the

patients of EH with atypia was 52.08 ± 7.09, while the mean age of the patients with EC was 59.64 ± 6.80. Regarding tumor size, its range was 1-3 cm with a mean of 1.81 ± 0.622 **(Table 1).**

Among the studied parameters; no significant correlation between GLUT-1 and patients' age was detected while there were significant association between GLUT-1 and histological type, grade and stage ($p<0.01$, <0.01 and <0.05 respectively) as shown in **Table 2 and Table 3**.

The H&E-stained tissue sections of the 50 collected specimens were classified into: 12/50 (24%) cases of EH without atypia. 13/50 (26%) cases of EH with atypia, 25/50 (50%) cases of EC.

Regarding grading of the studied endometrial carcinoma: 13/25 (52%) cases were classified as Grade I, 10/25 (40%) cases were classified as Grade II, 2/25 (8%) cases were classified as Grade III tumor. Regarding tumor stage 10/21 (approximately 48%) of cases were T1A, 6/21 (approximately 29%) cases were T1B and 5/21 (approximately 24%) cases were T2.

GLUT-1 appeared as brownish membranous staining and was expressed in all cases of EC and EH with atypia with variation in its expression among cases of the later. GLUT-1 was strongly expressed in 100% of cases of EC **(figure 1).** In cases of EH with atypia only 38.5% showed strong expression and 61.5% showed moderate expression **(figure 2).** In cases of EH without atypia GLUT-1 was weakly expressed in 33.3% of cases **(figure 3A),** while 66.7% of cases showed negative expression **(figure 3B).**

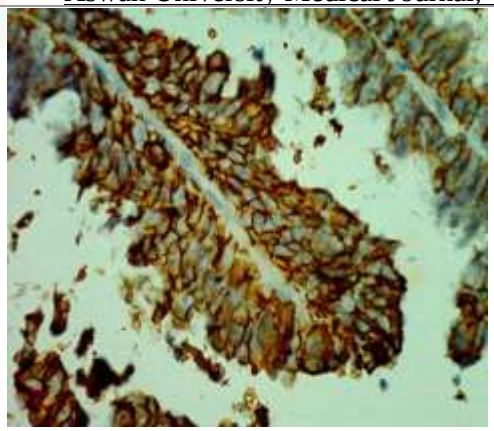
In case of EC, GLUT-1 expression (according to H score) showed positive association regarding tumor stage and grade.

Table 1: Clinico-pathological data of the examined cases

Studied parameter	**Results**
Age	
• Age range	35-73
Tumor size (cm)	
• Mean± SD	1.81 ± 0.622
• Range	1 -3
Histological diagnosis	
• EH without atypia	12/50 (24%)
• EH with atypia	13/50 (26%)
• EC	25/50 (50%)
Tumor grade	
• Grade I	13/25 (52%)
• Grade II	10/25 (40%)
• Grade III	2/25 (8%)
Tumor stage	
• T1A	10 (47.6%)
• T1B	6 (28.6%)
• T2	5 (23.8%)

Table 2: Correlation between GLUT-1 expression and studied clinico-pathological parameters

GLUT-1 expression	**EH without atypia**	**EH with atypia**	**EC**	**Test value**	**P-value <**	**Sig.**
	No. = 12	No. = 13	No. = 25			
• **Strong**	0 (0.0%)	5 (38.5%)	25 (100.0%)	75.641	0.000	HS
• **Moderate**	0 (0.0%)	8 (61.5%)	0 (0.0%)			
• **Weak**	4 (33.3%)	0 (0.0%)	0 (0.0%)			
• **Negative**	8 (66.7%)	0 (0.0%)	0 (0.0%)			



A

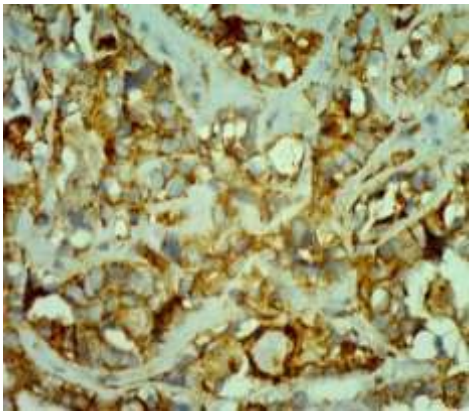


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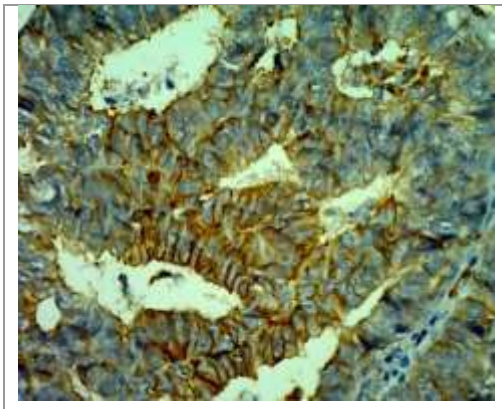
Figure 1: A, Strong GLUT-1 immuno-staining in endometrioid carcinoma (x400), **B,** Strong GLUT-1 immuno-staining in clear cell endometrial carcinoma (x400).

Table 3: Correlation between GLUT-1expression, grade and stage in EC cases

		GLUT-1 expression		**P-value <**	**Sig.**
		Mean ± SD	Range		
Grade	**I**	243.54 ± 14.56	220 – 268	0.000	HS
	II	276.00 ± 9.94	260 – 295		
	III	271.50 ± 23.33	255 – 288		
Stage	**T1A**	252.40 ± 20.34	220 – 288	0.025	S
	T1B	258.33 ± 16.63	230 – 275		
	T2	281.00 ± 9.62	270 – 295		



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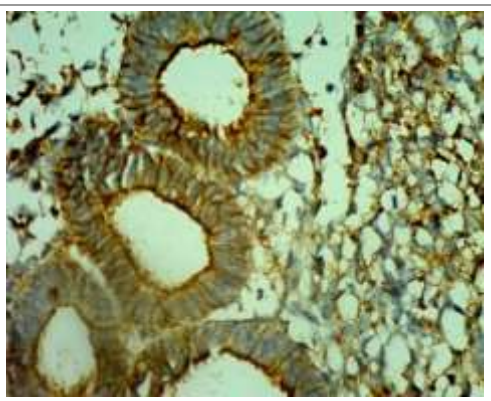

B

Figure 2: Endometrial hyperplasia with atypia (x400), **A,** moderate staining of GLUT-1, **B,** strong staining of GLUT-1

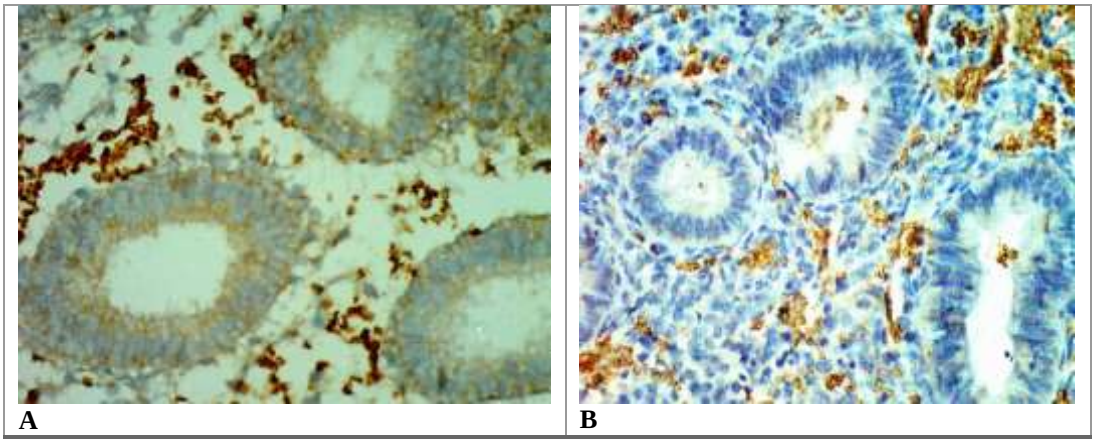

Figure 3: Endometrial hyperplasia without atypia (x400), **A,** weak staining of of GLUT-1, **B,** negative staining of GLUT-1

DISCUSSION

The expression of GLUT-1 in EH and EC has been supported by various studies (14), with IHC expression potentially serving as a diagnostic marker to differentiate EC from other endometrial conditions (15). In this study, the mean age of the patients with the EH without atypia group was 44.83 ± 5.98, while EH with atypia group had a mean age of 52.08 ± 7.09, and EC had a mean age of 59.64 ± 6.80. This age variation was significant in the progression from EH to EC, aligning with Al-Sharaky et al.'s findings on the IHC expression of GLUT-1 in atypical EH and EC, where the mean ages 51.88 ± 7.89 for were atypical EH and 60.46 ± 6.98 for EC (16).

There was a strong association existed between GLUT-1 expression and histopathological parameters, showing a progressive increase from EH without atypia to EC. In EH without atypia, 33.3% of cases exhibited weak GLUT-1 expression, with a mean H-score of 28.83 ± 21.30, while 66.7% showed no expression. In contrast, 38.5% of cases of EH with atypia had strong GLUT-1 expression, and 61.5% had moderate expression, with a mean H-score of 174.62 ± 46.21. All EC cases had strong GLUT-1 expression, with a mean H-score of 258.76 ± 20.70. These findings suggest a strong association between GLUT-1 expression and the progression from hyperplasia to cancer. This aligns with Al-Sharaky et al.'s research, who demonstrated a gradual increase in GLUT-1 H-scores from hyperplasia (65) to type I EC (150) and type II EC (207.5), with statistically significant difference($p=0.008$) between type I EC and hyperplasia, as was the disparity among type I EC as well as type II EC ($p=0.002$). (16). Němejcová et al. also reported increased GLUT-1 expression with atypia or malignancy, detecting GLUT-1 in 87% of endometrioid carcinomas, 100% of serous and clear cell carcinomas, 50% of polyps with atypical hyperplasia, 12.5% of polyps with non-atypical hyperplasia, 77% of hyperplasias with atypia, 9 % of hyperplasias without atypia (3).

Ma et al. found GLUT-1 expression in 25% of EH cases and 70% of EC cases (17), while Canpolat et al. reported that 79.3% of hyperplasia with atypia and 20.7% of hyperplasia without atypia cases and all EC cases were expressing GLUT-1 (18). GLUT-1 may also indicate early neoplastic transformation in endometrial tissues, as suggested by Ashton-Sager et al. (19) and further supported by Khabaz et al., who demonstrated significantly higher GLUT-1 expression in ECs than in normal endometrium (20).

In this study, there were 13/25 (52.0%) grade I, 10/25 (40.0%) grade II, and 2/25 (8.0%) grade III EC cases. GLUT-1 expression increased significantly with increasing tumor grade, with mean H-scores of 243.54 ± 14.56 for grade I, 276.00 ± 9.94 for grade II, and 271.50 ± 23.33 for grade III EC ($p<0.01$). This was consistent with Al-Sharaky et al.'s findings of a gradual rise in GLUT-1 H-scores from grade I (173 ± 53.31) to grade III (201.94 ± 51.85) EC (16). Similarly,

Němejcová et al. reported GLUT-1 positivity in 89% of well-to-moderately differentiated ECs, and in 96.5 % of grade III EC, while 100% positivity was found in serous and clear cell carcinomas, with high grade carcinoma exhibiting stronger staining (3).

Regarding tumor stage, the study included 10/21 cases of T1A, 6/21 cases of T1B, and 5/21 cases of T2 EC. GLUT-1 expression showed a statistically significant association with increasing tumor stage ($p<0.05$). With mean H-scores increasing from 252.40 ± 20.34 in T1A to 258.33 ± 16.63 in T1B and 281.00 ± 9.62 in T2 EC. Ma et al. found a significant increase in GLUT-1 expression with advancing EC stages, reduced differentiation, and increased lymphatic metastasis (18). Canpolat et al. investigated endometrioid cancer across stages I to IV, and reporting similar findings (19). Kawamura et al. also noted associations between GLUT-1 expression and various gastric cancer progression factors, including lymph node metastasis, liver metastasis and tumor stage (5). The study of Ma et al., reinforces the strong link between GLUT-1 expression and clinicopathological features like advancing tumor stage, decreased differentiation, and lymphatic spread in endometrial cancers (18).

CONCLUSIONS:

GLUT-1 had an ascending manner of expression regarding progression of disease from EH to EC. GLUT-1 also had a stepwise increase in its expression with increasing grade and stage of EC. These findings support the potential utility of GLUT-1 as a predictor of development and progression of EC.

Recommendations

We recommended to study GLUT-1 expression on a larger number of cases of different endometrial lesions and a larger number of cases regarding each histological type, grade and stage of EC.

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Conflict of Interest: Nil

Abbreviations: EC: Endometrial hyperplasia, GLUT-1: Glucose transporter-1. IHC: immunohistochemistry, immunohistochemical, AEH/EIN: atypical endometrial hyperplasia/ endometrioid intraepithelial neoplasia, H&E: hematoxylin and eosin, SD: standard deviation.

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