Original Article

DOI: 10.7860/JCDR/2016/19576.7805

Pathology Section

HIF-1α and GLUT-1 Expression in Atypical Endometrial Hyperplasia, Type I and II Endometrial Carcinoma: A Potential Role in Pathogenesis

DALIA RIFAAT AL-SHARAKY[1], ASMAA GABER ABDOU[2], MOSHIRA MOHAMMED ABDEL WAHED[3], HEND ABDOU KASSEM[4]

ABSTRACT

Introduction: Hypoxia-Inducible Factor 1α (HIF-1α) is one of the major adaptive responses to hypoxia, regulating the activity of glucose transporter -1 (GLUT-1), responsible for glucose uptake.

Aim: To evaluate the immunohistochemical expression of both HIF-1α and GLUT-1 in type I and II endometrial carcinoma and their correlation with the available clinicopathologic variables in each type.

Materials and Methods: A retrospective study was conducted on archival blocks diagnosed from pathology department between April 2010 and August 2014 included 9 cases of atypical hyperplasia and 67 cases of endometrial carcinoma. Evaluation of both HIF-1α and GLUT-1 expression using standard immunohistochemical techniques performed on cut sections from selected paraffin embedded blocks.

Statistical Analysis: Descriptive analysis of the variables and statistical significances were calculated by non-parametric chi-square test using the Statistical Package for the Social Sciences version 12.0 (SPSS).

Results: HIF-1α was expressed in epithelial (88.9%, 52.2%, 61.2% and 50%) and stromal (33.3%, 74.6%. 71.4% and 83.3%) components of hyperplasia, total cases of EC, type I and II EC, respectively. GLUT-1 was expressed in the epithelial component of 88.9%, 98.5%, 98% and 100% of hyperplasia, total EC cases, type I and II EC, respectively. The necrosis related pattern of epithelial HIF-1α expression was in favour of type II (p=0.018) and grade III (p=0.038). HIF-1α H-score was associated with high apoptosis in both type I and total cases of EC (p=0.04). GLUT-1 H-score was negatively correlated with apoptotic count (p=0.04) and associated with high grade (p=0.003) and advanced stage in total EC (p=0.004). GLUT-1 H-score was correlated with the pattern of HIF-1α staining in all cases of EC (p= 0.04).

Conclusion: The role of HIF-1α in epithelial cells may differ from that of stromal cells in EC; however they augment the expression of each other supporting the crosstalk between them. The stepwise increase in H- score of GLUT-1 in the studied cases implies its potential role in carcinogenesis of EC. HIF-1α may promote GLUT-1 expression in EC especially surrounding areas of necrosis. The differences between type I and type II EC regarding HIF-1α and GLUT-1 expression may confirm the differences in their aetiopathogenesis.

Keywords: Hypoxic markers, Immunohistochemistry, Necrosis

INTRODUCTION

In Egypt, Endometrial Carcinoma (EC) represents 1.6% of total female cancers ranking the 13th. It is the third most common gynecological cancer after ovary and cervix constituting about 23% [1]. Adaptation of cancer cells to their microenvironment is an important driving force that leads to invasive and metastatic diseases. Oxygen concentration is one of the microenvironmental factors that affects growth and invasion of malignant cells [2]. Hypoxia contributes to local and systemic tumour progression as well as potentially compromising radiotherapy and chemotherapy [3].

Regulation of transcription by hypoxia-inducible factor 1α (HIF-1α) is one of the major mechanisms mediating adaptive responses to hypoxia [2]. HIF-1α plays an important role in tumour angiogenesis, promoting tumour cell proliferation and helps in metastases [4]. More than 60 genes, involved in many cancer biological processes as angiogenesis and glucose metabolism, are targeted by HIF-1α [3]. HIF-1α regulates the activity of glucose transporters (GLUTs) including GLUT-1 and GLUT-3, which are responsible for glucose uptake [5-7].

The metabolism of glucose in tumour microenvironment is changed from oxygen mitochondrial process to glycolysis (the Warburg effect) [8]. Expression of GLUT-1 under hypoxemic conditions induces a shift in glucose metabolism towards glycolysis. The expression of GLUT-1 was regulated by hypoxia in a HIF-1α dependent manner [9], which may represent a key mechanism by which malignant cells may achieve increased glucose uptake and compensate the lack of energy caused by inefficient anaerobic glycolysis [10].

Despite the well-established separate role of HIF-1α [11,12] and GLUT-1 in endometrial carcinoma [10,13], yet their expression pattern in subtypes of endometrial carcinoma and the relationship between them are not sufficiently studied.

AIM

Therefore, the present study aimed to evaluate the immunohistochemical expression of both HIF-1α and GLUT-1 in type I and II EC and their correlation with the established clinicopathologic variables in each type.

MATERIALS AND METHODS

This retrospective study was conducted on 76 archival cases of Egyptian patients. The study was approved by the Research Ethics Committee at Faculty of Medicine, Menofia University. Cases were diagnosed in Pathology Department, Faculty of Medicine, Menofia University, Egypt spanning the period between April 2010 and August 2014. Inclusion criteria included; the availability of

paraffin-embedded blocks for serial cutting and examination and the availability of most of the clinical data required for this study. The exclusion criteria included; absence of any one of previously mentioned inclusion criteria and endometrial carcinoma cases subjected to chemotherapy or radiotherapy prior to the surgery. The study included 9 cases of atypical hyperplasia and 67 cases of EC, which was classified into 49 type I and 18 type II EC.

Clinical Features

Clinical features were collected from the patients' medical records.

Histopathological Features

Histological types were assessed according to the 2014 WHO classification of the tumours of EC [14]. Grading of endometrioid adenocarcinoma was performed according to FIGO (International Federation of Gynecology and Obstetrics) system [15]. Classification of EC into type I and II was performed accordinly [16]. Staging was based on 2009 FIGO staging system [17]. Myometrial invasion was designated as present or absent, positive cases were further divided into more than one half and less than one half [18]. Necrosis and lymphovascular invasion were assessed whether present or absent. Scoring of mitosis and apoptosis was carried out using an Olympus CH2 light microscope with wide angle (field size: 0.274 mm^2, field diameter: 0.59 mm) and were assessed as mean±SD, median and range.

Immunohistochemical Procedure

Immunohistochemical staining of both HIF-1α and GLUT-1 was performed on cut sections from selected paraffin embedded blocks. Four µm thick sections were cut from selected blocks, which were mounted onto positively charged glass slides. The sections were dewaxed, rehydrated and boiled in citrate buffer solution (pH 6) for 20 minutes in order to allow antigen retrieval. Endogenous peroxidase activity was blocked by treating with 0.5% hydrogen peroxide in methanol for 15 minutes. The slides were incubated overnight with the primary antibodies, rabbit polyclonal antibody, 7.0 ml ready to use (Cat. #RB-9052-R7) rose against Glut-1(Lab Vision Corporation. Fremont, CA, USA) and rabbit monoclonal antibody (Cat. # CME349 A,B) raised against HIF-1 alpha, which was received as 0.1ml concentrated (Biocare Medical, LLC. Pike Lane Concord, CA, USA) and diluted by Phosphate Buffered Saline (PBS) in a dilution 1:150. In this system, two reagents were utilized, the biotinylated secondary anti-immunoglobulin which was a purified bovine monoclonal anti-mouse IgG (Thermo scientific, NOS-3F7-B11 B5) capable of binding to the primary antibody and the streptvidin-biotin enzyme complex. A 0.1% solution of Diaminobenzidine (DAB) was used for 5 minutes as a chromogen. Slides were counter-stained with Mayer's haematoxylin for 5–10 minutes. Negative control slides were prepared, by omitting the primary antibodies from the staining procedure. Tissue sections prepared from cutaneous squamous cell carcinoma and breast carcinoma were used as positive controls for GLUT-1 and HIF-1 alpha, respectively.

Assessment of HIF-1α and GLUT-1 immunostaining

HIF-1α and GLUT-1 expression were evaluated in both endometrial glands (epithelial) and stroma (stromal). In positive cells, nuclear, cytoplasmic or both staining in any number of cells were required to assign HIF-1α positive expression [19]. The positive staining of GLUT-1 was considered when any number of cells exhibited membranous, cytoplasmic or both staining [20].

H-score was applied to evaluate positive cases for both markers either in epithelial or stromal components according [21,22], where both intensity (scored 1-3 as 1= mild, 2=moderate and 3=strong) and percentage of positive cells was considered according to the following formula:

H-score = strong intensity (3) × percentage + moderate intensity (2) × percentage + mild intensity (1) × percentage. H-score ranged from 0 to 300. The pattern of epithelial HIF-1α and GLUT-1 expression were divided into necrosis related (the expression is related to necrotic areas) and necrosis not related (the marker is expressed throughout the tumour irrespective to necrotic areas).

Variables		Hyperplasia (No=9)		Total endometrial Carcinoma group (no=67)		Type I EC (no=49)		Type II EC (no=18)	
Age (years)	Mean±SD Median Range	51.88±7.89 50 39-62		60.46±6.98 60 46-79		59.93±6.94 59 46-79		61.88±7.11 64 49-71	
Menopausal status		No	%	No	%	No	%	No	%
	Premenopausal Postmenopausal	3 6	33.3 66.7	5 62	7.5 92.5	4 45	8.2 91.8	1 17	5.6 94.4
FIGO stage	I II III IV	***	***	39 10 16 2	58.2 14.9 23.9 3.00	32 9 6 2	65.3 18.4 12.2 4.1	7 1 10 0	38.9 5.6 55.6 0
Stage grouping	Early Advanced	***	***	49 18	73.1 26.9	41 8	83.7 16.3	8 10	44.4 55.6
Myoinvasion With myoinvasion Without myoinvasion	Inner 1/2 Outer 1/2	***	***	 21 41 5	 31.3 61.2 7.5	 18 28 3	 36.7 57.1 6.1	 3 13 2	 16.7 72.2 11.1
LVI	Negative Positive	***	***	53 14	79.1 20.9	41 8	83.7 16.3	12 6	66.7 33.3
Necrosis	Absent Present	8 1	88.8% 11.1%	18 49	26.9 73.1	14 35	28.6 71.4	4 14	22.2 77.8
Stromal reaction	Inflammatory Desmoplastic Both	***	***	15 11 41	22.4 16.4 61.2	12 5 32	24.5 10.2 65.3	3 6 9	16.7 33.3 50
Mitosis	Mean±SD Median Range	1.33±0.71 1 0-2		5.55±2.21 5 1-13		5.12±1.95 5 1-9		6.72±2.49 7 3-13	
Apoptosis	Mean±SD Median Range	1.89±0.78 2 1-3		23.91±16.06 25 3-56		29.65±13.88 30 7-56		8.28±10.18 4 3-40	

[Table/Fig-1]: Clinico-pathologic data of studied cases.

STATISTICAL ANALYSIS

Data were collected, tabulated, and statistically analyzed using a personal computer with the "Statistical Package for the Social Sciences" (SPSS) version 20. The Chi-square and the Fisher-exact tests were used for comparisons between qualitative variables. The Mann-Whitney (U), Kruskal-Wallis (K) tests and Pearson correlation were used for comparisons between quantitative variables. The p-value ≤ 0.05 was considered significant.

RESULTS

Clinical and Histopathological Features of the Studied Groups

Clinical and histopathological features of hyperplasia, total EC, type I and II EC cases were summarized in [Table/Fig-1].

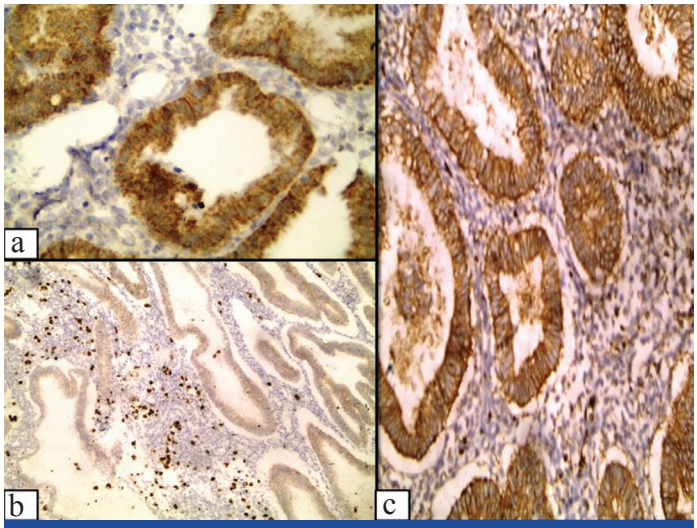
Immunohistochemical Profile of HIF-1α and GLUT-1 in Hyperplasia

HIF-1α was expressed in both the epithelial and the stromal components of the hyperplasia group. HIF-1α was expressed in the epithelial component of eight out of nine cases (88.9%) [Table/Fig-2a]. Seven out of 8 positive cases (87.5%) displayed necrosis not related pattern of staining while only one case displayed necrosis related pattern. With regard to stromal HIF-1 α-expression, three out of nine cases (33.3%) showed positive expression [Table/Fig-2b]. The H-score ranged between 0 and 30 with a mean±SD of 8.89±13.64 and a median of 0 [Table/Fig-3].

GLUT-1 was expressed in the epithelial part of the hyperplasia group and not in the stroma [Table/Fig-2c]. GLUT-1 was expressed in eight cases out of nine (88.9%). Regarding GLUT-1 pattern of staining; the entire cases displayed necrosis not related pattern [Table/Fig-4].

Immunohistochemical profile of HIF-1α and GLUT-1 in Endometrial Carcinoma (EC) and its subtypes

HIF-1α was expressed in both the epithelial and the stromal components of the carcinoma group and its subtypes. In the total EC cases, HIF-1α was expressed in the epithelial component of 39 out of 67 cases (58.2%). Fifty out of 67 cases (74.6%) showed stromal HIF-1α expression [Table/Fig-3].



[Table/Fig-2]: Atypical hyperplasia showing strong diffuse cytoplasmic epithelial HIF-1α with negative stroma (a) (IHC×400), (b) strong stromal nuclear HIF-1α (b) (IHC×100) and (c) strong cytoplasmic GLUT-1 (c) (IHC×200).

Epithelial and stromal HIF-1α		Hyperplasia group (n=9)		Type I EC (n=49)		p-value Between type I and hyperplasia	Type II EC (n=18)		p-value Between type I and type II EC	Total EC (n=67)	
		No	%	No	%		No	%		No	%
Epithelial HIF-1α expression	Positive Negative	8 1	88.9 11.1	30 19	61.2 38.8	0.11	9 9	50 50	0.41	39 28	58.2 41.8
Epithelial HIF-1α H-score	Mean±SD Median Range	108.33±78.34 100 0-230		50.51±58.91 20.00 0-180		0.02*	46.19±59.41 15 0-225		0.25	46.19±59.41 15 0-225	
		Positive cases (n = 8)		Positive cases (n= 30)			Positive cases (n = 9)			Positive cases (n = 39)	
Epithelial HIF-1α Pattern of staining	Necrosis not related Necrosis related	7 1	87.5 12.5	20 10	66.7 33.3	0.24	2 7	22.2 77.8	0.018*	22 17	56.4 43.6
		No	%	No	%		No	%			
Stromal HIF-1α expression	Positive Negative	3 6	33.3 66.7	35 14	71.4 28.6	0.02*	15 3	83.3 16.7	0.32	50 17	74.6 25.4
Stromal HIF-1α H-score	Mean±SD Median Range	8.89±13.64 0 0-30		25.2±34.73 15.00 0-180		0.06	28.13±37.04 20 0-180		0.22	28.13±37.04 20 0-180	

[Table/Fig-3]: HIF-1α expressionin the studied cases (Hyperplasia, EC and its subtypes). *Statistically significant

		Hyperplasia group (n=9)		Type I EC (n=49)		p-value Between type I and hyperplasia	Type II EC (n=18)		p-value Between type I and type II EC	Total EC (n=67)	
		No	%	No	%		No	%		No	%
GLUT-1 Expression	*Positive* *Negative*	8 1	88.9 11.1	48 1	98 2	0.17	18 0	100 0	0.54	66 1	98.5 1.5
GLUT-1H-score	*Mean±SD* *Median* *Range*	80.00±61.13 65.00 0-170		146.53±59.56 150.00 0-250		0.008*	*201.94±51.5* *207.5* *110-285*		0.002*	*161.42±62.3* *165* *0-285*	
		No. of positive cases (8)		No of positive cases (48)			*No of positive cases (18)*			*No. of positive cases (66)*	
GLUT-1 Pattern of staining of positive cases	*Necrosis not related* *Necrosis related*	8 0	100 0	21 27	43.8 56.3	0.003*	9 9	50 50	0.65	30 36	45.4 54.5

[Table/Fig-4]: GLUT-1 expression in the studied cases (Hyperplasia, EC and its subtypes). *Statistically significant

In type I EC, epithelial HIF-1α was expressed in 30 out of 49 cases (61.2%) [Table/Fig-5a&b]. Stromal HIF-1α was expressed in 35 out of 49 cases (71.4%) [Table/Fig-3].

In type II EC, epithelial HIF-1α was expressed in half of the cases (9/18) [Table/Fig-5c]. Seven cases showed HIF-1α expression related to necrosis [Table/Fig-5d] and 2 cases showed diffuse expression irrespective to necrosis. Stromal HIF-1α was expressed in15 out of 18 cases (83.3%) [Table/Fig-3,6].

GLUT-1 was expressed in the epithelial and not in the stromal part of the carcinoma group . In the total number of endometrial carcinoma cases, 66 out of 67 cases (98.5%) expressed GLUT-1. Regarding the staining pattern, 45.4% (30/66) displayed necrosis not related pattern while 54.5% (36/66) displayed necrosis related pattern [Table/Fig-4].

Regarding the expression of GLUT-1 in type I EC, 48 cases out of 49 (98%) expressed GLUT-1 [Table/Fig-7a&b]. H -score values of GLUT-1 expression ranged between 0 and 250 with a mean±SD of 146.53±59.56 and a median of 150. Regarding the staining pattern, 43.8% (21/49) displayed non necrosis related pattern while 56.3% (27/49) displayed necrosis related pattern [Table/Fig-4].

Regarding the expression of GLUT-1 in type II EC, all cases (100%) expressed GLUT-1 [Table/Fig-7c]. H-score ranged between 110 and 285 with a mean±SD of 201.94±51.5. The cases equally displayed both patterns, nine necrosis not related pattern and 9 necrosis related patterns [Table/Fig-4,7d].

Comparison between Hyperplasia and Type I EC Regarding the HIF-1α and GLUT-1

Regarding epithelial HIF-1α expression, the hyperplasia group displayed higher median H-score in comparison with type I EC (p=0.02). HIF-1α expression by stromal cells was significantly in favour of type I EC in comparison to hyperplasia group (p=0.02) [Table/Fig-8a]. Meanwhile, the median value of H-score of HIF-1α in the stroma was higher in type I EC than in the hyperplasia group (p=0.06) [Table/Fig-3].

Regarding the GLUT-1, higher H-score of GLUT-1 was statistically in favour of type I EC group in comparison with hyperplasia group (p=0.008). Necrosis related pattern of GLUT-1 was in favour of type I EC since all positive cases of hyperplasia displayed necrosis not related pattern (p=0.003) [Table/Fig-4].

Comparison between Type I and Type II EC regarding the HIF-1α and GLUT-1

Regarding epithelial HIF-1α, the necrosis related pattern of expression was significantly in favour of type II (77.8%) compared to type I (33.3%) [Table/Fig-3,5b].

Regarding GLUT-1, a higher H-score of GLUT-1 was statistically in favour of the type II EC group in comparison with type I EC group (p=0.002) [Table/Fig-4].

Relationship between HIF-1α and the Studied Parameters in EC and Its Subtypes

In total EC cases, epithelial HIF-1α expression was significantly in favour of grade II(p=0.05). The necrosis related pattern was significantly in favour of type II EC (p=0.018) and grade III (p=0.038) [Table/Fig-8c]. Regarding the H-Score of epithelial HIF-1α, apoptotic count was positively correlated with H-score of HIF-1α (p=0.04) [Table/Fig-8d].

In type I EC, epithelial HIF-1α expression was significantly in favour of grade II (p=0.02). The apoptotic count was positively correlated with H-score of HIF-1α; i.e., the higher the apoptotic count, the higher the H-score of HIF-1α (p=0.04).

In type II EC, epithelial HIF-1α expression and H-score evaluation didn't statistically correlate with any of the studied parameters.

There was no significant association between stromal HIF-1α expression and the studied parameters in total EC, type I and type II.

Relationship between GLUT-1 and the Studied Parameters in EC and Its Subtypes

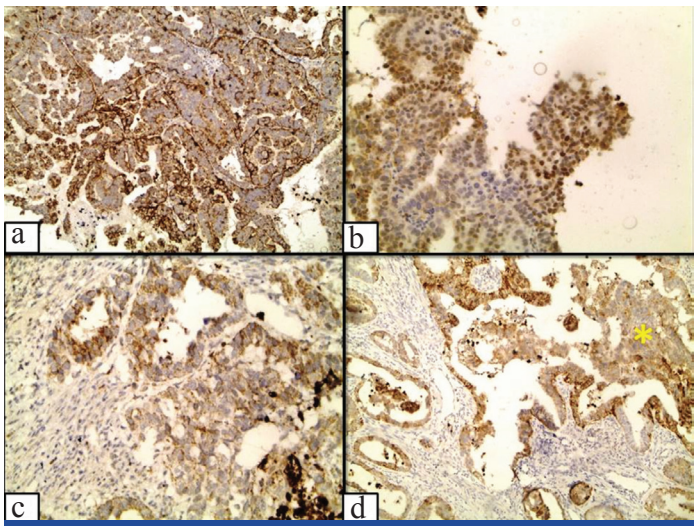
In total EC cases, GLUT-1 H-score was positively correlated with age (p=0.03) and negatively correlated with apoptotic count (p=0.04) and was significantly higher in grade III (p=0.003) and advanced stage (p=0.004). There was an observed progressive increase in GLUT-1 mean H-score values from grade I (173±53.31) to grade III (201.94±51.85). The pattern of GLUT-1 staining did not correlate with the studied parameters.

In type I EC, advanced stage grouping was associated with high mean values of GLUT-1 H-score compared to early stage grouping (p=0.02). On the other hand there was no statistical significant relationship between pattern of GLUT-1 staining and the studied parameters in type I EC.

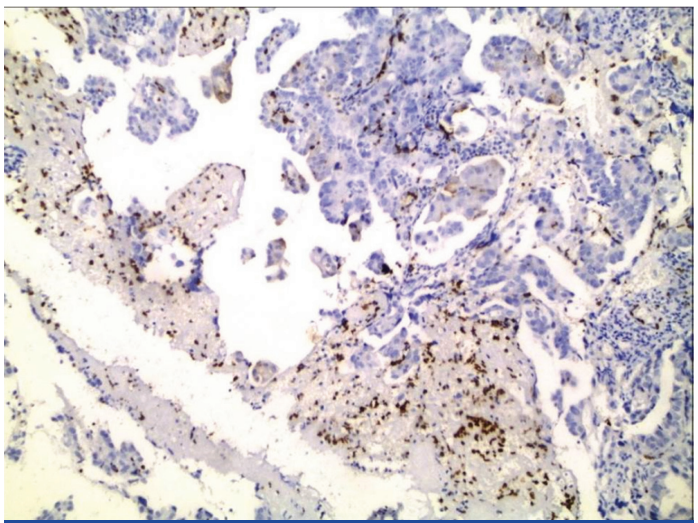
In type II EC, GLUT-1 H-score and pattern of GLUT-1 staining failed to show statistical association with the studied clinicopathological parameters.

Correlation between Epithelial and Stromal HIF-1α Expression in EC Cases and Its Subtypes

Thirty one out of 39 (79.5%) EC displayed both epithelial and stromal HIF-1α reaching a statistical significance (p=0.03). On the



[Table/Fig-5]: (a): moderate to strong cytoplasmic epithelial HIF-1α in grade II endometriod adenocarcinoma (IHC, 100X) and in perinecrotic tumour cells in a grade II endometroid adenocarcinoma (D: yellow asterisk :necrosis) (IHC 200X), (b): nuclear epithelial HIF-1 α in grade II endometriod adenocarcinoma (IHC, 200X),(c); and cytoplasmic epithelial HIF-1α in clear cell adenocarcinoma (IHC, 200X).

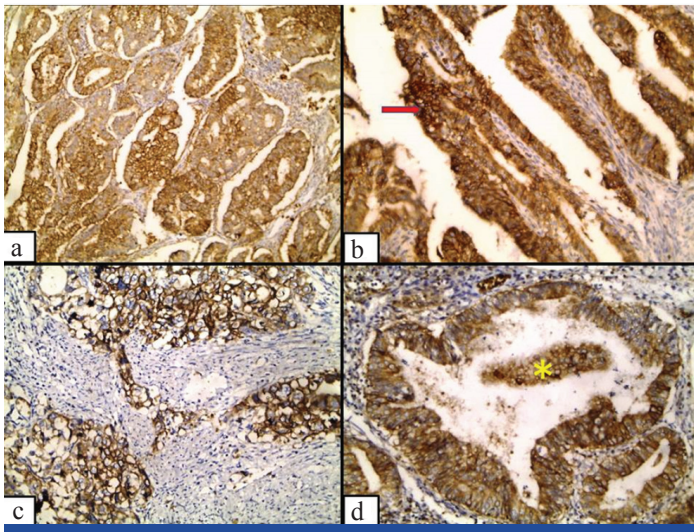


[Table/Fig-6]: Strong nuclear stromal HIF-1α with absence of epithelial expression in a case of papillary serous adenocarcinoma (IHC 100x).

other hand, no correlation was observed in either subtypes (type I and type II EC) [Table/Fig-9].

Correlation between GLUT-1 and HIF-1α Expression in EC Cases and Its Subtypes

Higher GLUT -1 H-score was noticed in cases showing necrosis related pattern of HIF-1α staining (p=0.04) compared to cases showing non necrosis related pattern. On the other hand, no correlation was observed in either subtypes (type I and type II EC) [Table/Fig-10].



[Table/Fig-7]: (a): Moderate cytoplasmic and (b): Strong diffuse cytoplasmic with occasional membranous (red arrow) GLUT-1 in grade II endometroid adenocarcinoma (A, B: IHC 100X). (c): Strong membranous epithelial GLUT-1 in a case of clear cell carcinoma (IHC 200x). (d): Moderate to strong cytoplasmic GLUT-1 expression in perinecrotic tumour cells in a grade II endometroid adenocarcinoma (yellow asterisk :necrosis) (IHC 400X).

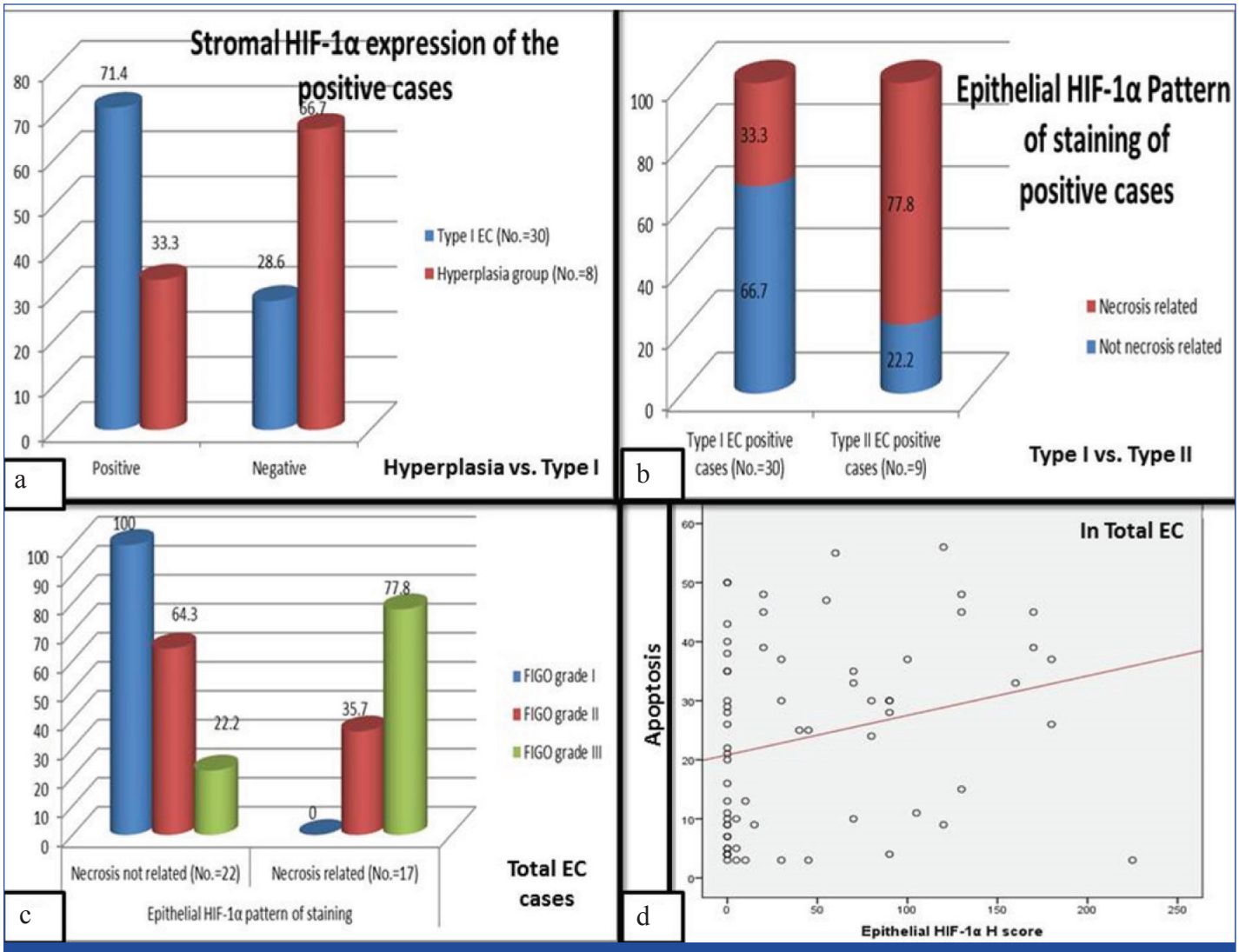
DISCUSSION

In the current study, high expression of epithelial HIF-1α was seen in hyperplasia agreeing with previous studies with a variable percentage, such as 60.9% in Horree et al., study and 41.6% in Feng et al., study [11,23]. The percentage of HIF-1α epithelial expression in EC (58.2%) of the present study was close to Sivridis et al., who reported HIF-1α expression in 49% (41/80) of the cases [24]. On the other hand, a higher expression was reported by Sadlecki et al., who demonstrated 97% positive expression [25].

Over-expression of HIF-1α in the hyperplasia group than in the EC group could be attributed to the evident hypoxic conditions in cases of hyperplasia. Although there is only one case of endometrial hyperplasia showing necrosis in the current study, however this is not against the presence of hypoxic microenvironment. According

	Positive epithelial HIF-1α (no. =39)		Negative epithelial HIF-1α (no. =28)		Test of significance	p-value
Positive stromal HIF-1α	No	%	No	%	FE=5.29	0.03*
	31	79.5	19	67.9		
Negative stromal HIF-1α	8	20.5	9	32.1		

[Table/Fig-9]: Correlation between epithelial and stromal HIF-1α expression in total cases of endometrial carcinoma (N0=67).
*Statistically significant



[Table/Fig-8]: (a) Stromal HIF-1α expression in hyperplasia and type I EC. (b): Epithelial HIF-1α staining patterns in types I and II EC. (c): Epithelial HIF-1α staining pattern and grade in total EC cases (d): Epithelial HIF-1α H-score and apoptosis in total EC cases

	Glut -1 expression					Glut -1 H-score		Glut -1pattern of staining				
	Positive		Negative		p value	Mean ± SD	p-value	Diffuse		Necrosis related		p-value
	No	%	No	%				No	%	No	%	
Epithelial HIF-1 α expression Positive Negative	38 28	97.4 100	1 0	2.6 0	0.39*	162.56±59.5 159.82±67.15	0.76#	17 13	44.7 46.4	21 15	55.3 53.6	0.89*
Epithelial HIF-1α Mean ± SD	46.59±59.77		20.00		—		0.71	46.33±55.61		46.81±63.82		0.98#
Epithelial HIF-1α Pattern of staining of the positive cases Necrosis no related Necrosis related	21 17	95.5 100	1 0	4.5 0	0.37*	144.32±62.53 186.18±47.15	0.04#*	8 9	38.1 52.9	13 8	61.9 47.1	0.36*
Stromal HIF-1α α expression Positive Negative	49 17	98 100	1 0	2 0	0.56*	159.7±63.12 166.47±61.53	0.64#	7 23	41.2 46.9	10 26	58.8 53.1	0.63*
Stromal HIF-1α Mean ± SD	27.65±37.11		60.00		—		0.54	30.00±34.71		25.69±39.37		0.16#

[Table/Fig-10]: Correlation between GLUT-1 and epithelial/stromal HIF-1α expression in all cases of EC cases (n=67)
*Fisher Exact test
Mann Whitney test% is being calculated by *Row*
*Statistically significant

to Chianeh et al., endometrial hyperplasia is characterized by presence of large thin walled tortuous, superficial endometrial vessels, which eventually causes loss of blood due to fragility of the blood vessels [26]. The loss of blood could cause hypoxic microenvironment which mediates the release of HIF-1α. This could explain the HIF-1α over-expression in hyperplasia than in endometrial carcinoma.

In the current study, epithelial HIF-1α expression showed high percentage in type I (61.2%) compared to type II (50%) but with absence of significant difference (p>0.05). Meanwhile, Pansare et al., reported predilection of HIF-1α to type II EC (86%) than type I EC (26%) [12]. On the other hand, necrosis related pattern of HIF-1α was statistically in favour of type II EC (77.8%, p=0.018) compared to type I EC. We agreed with Seeber et al., who demonstrated the association of perinecrotic HIF-1α expression with high-grade endometrial carcinoma represented by type II EC in the present study [27]. Furthermore, Daponte et al., reported an association between epithelial HIF-1α expression and high grade ovarian carcinoma [28].

The expression of HIF-1α in the stroma was higher in type I EC than hyperplasia group (p=0.02). Stromal HIF-1α expression was noted occasionally in the stromal fibroblast and endothelial cells in a case of endometrial carcinoma, as reported by Sivridis et al., [24]. In the current study, this stepwise increase in the level of HIF-1α in the stroma implies a potential role of HIF-1α in the early carcinogenesis of endometrial carcinoma.

Most of the cancer studies focused on cancer cell metabolism, however, crosstalk between cancer metabolism and the metabolism of non-malignant stroma is also crucial for understanding malignant progression [29]. Activation of HIF-1α in stromal cells up-regulates the ability of stromal cells to produce CXCL12, which is required for proliferation and survival of the malignant cells [30]. Thus, over-expression of stromal HIF-1α in the EC group than in the hyperplasia group and the significant correlation between epithelial and stromal HIF-1α expression in the total endometrial carcinoma (p=0.03) support the crosstalk between stroma and the malignant endometrial glands, suggesting that the initial expression of HIF-1α may come from the stroma in response to hypoxia.

In the current study, the epithelial HIF-1α expression and stromal HIF-1α H-score are in favour of grade II. Some studies reported a correlation between HIF-1α and the grade in EC [24] and urothelial carcinoma [31] while others didn't find such correlation such as Sadlecki et al., in endometrial carcinoma, Dos Santos et al., in squamous cell carcinoma of the oral cavity and Kim et al., in cervical carcinoma [25,32,33].

High epithelial H-score of HIF-1α was positively correlated with apoptotic count in total EC (p=0.04) and in type I EC (p=0.04), agreeing with Theodoropoulos et al., in urothelial carcinoma [31]. Previous studies connected HIF-1α accumulation directly with the mutant p53 and inversely with bcl-2. HIF-1α acts by enhancing apoptosis of neoplastic cells by stabilization of wild type P53 which induce apoptosis, and down regulation of anti-apoptotic factor bcl-2 [34,35].

GLUT-1 is a glucose transporter which is also responsible for the uptake of sugar. The expression of GLUT-1 increased under non aerobic conditions, inducing a metabolic shift towards glycolysis. Previous studies failed to document the expression of GLUT-1 in most normal epithelial cells. In contrast, over-expression of GLUT-1 was found in various neoplasms, including colorectal carcinoma [36], cancer cervix [33], laryngeal carcinoma [37], ovarian tumours [38], and in oral squamous cell carcinoma [39].

In the current study, GLUT-1 was expressed only in the epithelial part sparing the stromal one. In contrast to epithelial HIF-1α, GLUT-1 was noticed to be higher in the EC group compared to the hyperplasia group. Our results agreed with Horree et al., Sadlecki et al., and Xiong et al., who reported GLUT-1 over expression in endometrial cancer than in the precancerous lesions (CAH), suggesting that this molecule can be involved in early stages of endometrial carcinogenesis [11,25,40]. The percentage of GLUT-1 expression in EC cases in the current study (98.5%) was close to that reported by Sadlecki et al., (100%) and Wang et al., (100%) [25,41]. On the other hand, Krzeslak et al., reported that GLUT-1 was only expressed in 67.1 % (51/76) of the studied EC cases [13].

A stepwise increase in the median H- score of GLUT-1 was noticed between the hyperplasia group (65) and type I EC (150) and type II EC (207.5), reaching a high statistical significance between type I and hyperplasia (p=0.008), and between type I and II EC (p=0.002). This stepwise increase supports a potential role of GLUT-1 in early carcinogenesis of EC, which was previously reported by others [11,25,40]. Necrosis related pattern of GLUT-1 expression was seen mainly in endometrial carcinoma group, a pattern which has not been mentioned before for GLUT-1 staining.

GLUT-1 H-score (p=0.003) was significantly associated with grade III of total EC agreeing with others [10,13]. Moreover, GLUT-1 H-score was significantly associated with advanced stage of type I EC (p=0.004). Other studies have demonstrated that GLUT-1 expression was increased linearly with FIGO stage in cancer cervix [42], while others failed to find an association between GLUT-1 expression and stage of EC [13,25]. This poor prognostic impact

of GLUT-1 was explained by Mayer et al., who suggested that the environmental factors (e.g., glucose deprivation) are important for the expression of the protein [42]. Activated oncogenes may have an effect on the degree of activation by these factors. This influence is likely to become more relevant in higher stages and larger tumours. Because oncogenic mutations, accumulated during malignant progression may become more prevalent.

Opposite to the HIF-1α, high GLUT-1 H-score was associated with low apoptotic count in total EC (p=0.04). We agreed with Vesely et al., who stated that enhanced expression of the facilitative glucose transporter, GLUT-1, has been shown to inhibit apoptosis in several cell systems including vascular smooth muscle cells (VSMCs) [43]. The antiapoptotic and prosurvival genes that increase VSMC, are proposed to be induced by GLUT-1 [43].

In the current study, high GLUT-1 H-score was correlated with necrosis related HIF-1α staining pattern in total EC. This correlation binds hypoxia with stimulation of both HIF-1α and GLUT-1, expression inducing a metabolic shift towards glycolysis and lead to malignant progression. This scenario was previously reported by Wu et al., in laryngeal carcinoma, Xiong et al., in endometrial carcinoma, Ishikawa et al., in oral squamous cell carcinoma, and Li et al., in lung cancer, [37,40,44,45]. Meanwhile, absence of a significant correlation between HIF-1α and GLUT-1 has been demonstrated by Koukourakis et al., in colorectal carcinoma and Schrijvers et al., in laryngeal carcinoma [36,46].

LIMITATION

Small sample size of atypical endometrial hyperplasia cases and confinement of studying both HIF-1α and GLUT-1 at the protein level only not the gene level contributed to the limitation in the present study.

CONCLUSION

Necrosis related pattern of HIF-1α and GLUT-1 expression in EC may refer to the role of hypoxia in their up-regulation. The role of HIF-1α in epithelial cells may differ from that of stromal cells in EC. Although of this apparent difference in distribution, they augment the expression of each other in EC supporting the crosstalk between the epithelial and stromal components of cancer. Stromal expression of HIF-1α is the one that could be incriminated in endometrial carcinoma pathogenesis because it is highly expressed in EC compared to atypical hyperplasia group.

The stepwise increase in the median H-score of GLUT-1 in the studied cases implies its potential role in carcinogenesis of EC. GLUT-1 may carry poor prognosis in EC because of its association with advanced stage, high grade and low apoptosis. HIF-1α may promote GLUT-1 expression in endometrial carcinoma especially surrounding areas of necrosis. The differences between type I and type II EC regarding HIF-1α and GLUT-1 expression may confirm the differences in their aetiopathogenesis

REFERENCES

[1] EL Bolkainy MN, Nouh MA, Farahat IG, et al., (eds); Pathology of cancer 2013b. Chapter 17 4th edition. Cairo press, Cairo, Egypt. 277-97.
[2] Semenza GL. Oxygen homeostasis. *Wiley Interdiscip Rev Syst Biol Med*. 2010;2(3):336-61.
[3] Quintero M, Mackenzie N, Brennan PA. Hypoxia inducible factors 1 (HIF- 1) in cancer. *Eur J Surg Oncol*. 2004;30(5):465-68.
[4] Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci USA*.1995;92(12):5510–14.
[5] Hayashi M, Sakata M, Takeda T, Yamamoto T, Okamoto Y, Sawada K, et al. Induction of glucose transporter 1 expression through hypoxia-inducible factor 1α under hypoxic conditions in trophoblast-derived cells. *J Endoc Rinol*. 2004;183(1):145–54.
[6] Calvert JW, Cahill J, Yamaguchi-Okada M, Zhang JH. Oxygen treatment after experimental hypoxia-ischemia in neonatal rats alters the expression of HIF-1α and its downstream target genes. *J Appl Physiol*. 2006;101(3):853–65.
[7] Liu Y, Li YM, Tian RF, Liu WP, Fei Z, Long QF, et al. The expression and significance of HIF-1α and GLUT-3 in glioma. *Brain Res*. 2009;1304:149–54.
[8] Warburg O. On the origin of cancer cells. *Science*. 1956;123(3191):309–14.
[9] Chen C, Pore N, Behrooz A, et al. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. *J Biol Chem*. 2001;276 (12):9519–25.
[10] Goldman NA, Katz EB, Glenn AS, Weldon RH, Jones JG, Lynch U et al. GLUT1 and GLUT8 in endometrium and endometrial adenocarcinoma. *Mod Pathol*. 2006;19(11):1429–36.
[11] Horrée N, van Diest PJ, van der Groep P, Sie-Go DM, Heintz AP. Hypoxia and angiogenesis in endometrioid endometrial carcinogenesis. *Cell Oncol*. 2007; 29(3):219–27.
[12] Pansare V, Munkarah AR, Schimp V, Haitham Arabi M, Saed GM, Morris RT, et al. Increased expression of hypoxia-inducible factor 1 in type I and type II endometrial carcinomas. *Mod Pathol*. 2007;20(1):35-43.
[13] Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A, et al. Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers. *Pathol Oncol Res*. 2012;18(3):721-28.
[14] Kurman RJ, Carcaniu ML, Herrinton CS, et al. editors of WHO classification of tumours of female reproductive organs, international agency for research on cancer(IARC), Lyon 2014, 4th edition.
[15] Lax SF1, Kurman RJ, Pizer ES, Wu L, Ronnett BM. A binary architectural grading system for uterine endometrial endometrioid carcinoma has superior reproducibility compared with FIGO grading and identifies subsets of advance-stage tumours with favourable and unfavourable prognosis. *Am J Surg Pathol*. 2000;24(9):1201–08.
[16] Kurman RJ, Lora HE and Brigitte MR. Endometrial carcinoma in: Blaustein's Pathology of the Female Genital Tract, 6th edn. Springer: New York 2011, pp 394-441.
[17] Lewin SN, Herzog TJ, Barrena Medel NI, Deutsch I, Burke WM, Sun X, et al. Comparative performance of the new versus old FIGO staging system for endometrial cancer. *Gynecol Oncol*. 2010;116:SG- 7.
[18] Soyi L, Kwang BL, Chan YP. The prognostic significance of Lymphovascular space involvement in patients with uterine confined endometroid endometrial cancer. *Korean J Obstet Gynecol*. 2012;55(2):76-82.
[19] Wu XH, Lu YF, Hu XD, et al. Expression of hypoxia inducible factor-1alpha and its significance in laryngeal carcinoma. *J Int Med Res*. 2010;38(6):2040-46.
[20] Monaco SE, Dabbs DJ. editors in "diagnostic immunohistochemistry: theranostic and genomic applications" 4th edition, chapter 21 entitled immunocytology 2014; pp 829-853.
[21] Khan J, Saal LH, Bittner ML, Chen Y, Trent JM, Meltzer PS. Expression profiling in cancer using cDNA microarrays. *Electrophoresis*. 1999;20(2):223-29.
[22] Bilalovic N, Sandstad B, Golouh R, Nesland JM, Selak I, Torlakovic EE. CD10 protein expression in tumour and stromal cells of malignant melanomas associated with tumour progression. *Mod Pathol*. 2004;17(10):1251-58.
[23] Feng Z, Gan H, Cai Z, Li N, Yang Z, Lu G, Chen J. Aberrant Expression of Hypoxia-inducible Factor 1α, TWIST and E-cadherin is associated with aggressive tumour phenotypes in endometrioid endometrial carcinoma. *Jpn J Clin Oncol*. 2013;43(4):396-403.
[24] Sivridis E, Giatromanolaki A, Gatter KC, Harris AL, Koukourakis MI. Tumour and angiogenesis research group tumour and angiogenesis research group; Association of hypoxia-inducible factors 1α and 2α with activated angiogenic pathways and prognosis in patients with endometrial carcinoma. *Cancer*. 2002;95(5):1055–63.
[25] Sadlecki P, Bodnar M, Grabiec M, Marszalek A, Walentowicz P, Sokup A, et al. The role of hypoxia inducible factore-1 alpha, glucose transporter- (glut-1) and carbon anhydrase ix in endometrial cancer patients1. *Biomed Res Int*. 2014;2014:616850.
[26] Chianeh YR, Rao P. Molecular and hormonal regulation of angiogenesis in proliferative endometrium. *Int J Res Med Sci*. 2014;2(1):1-9.
[27] Seeber LM, Horrée N, van der Groep P, van der Wall E, Verheijen RH, van Diest PJ. Necrosis related HIF-1α expression predicts prognosis in patients with endometrioid endometrial carcinoma. *BMC Cancer*. 2010;19:10-307.
[28] Daponte A, Ioannou M, Mylonis I, Simos G, Minas M, Messinis IE, et al. Prognostic significance of Hypoxia-Inducible Factor 1 alpha (HIF-1α) expression in serous ovarian cancer: an immunohistochemical study. *BMC Cancer*. 2008;8:335.
[29] Mucaj V, Shay J, Simson MC. Effects of hypoxia and HIFs on cancer metabolism. *Int J Haematol*. 2012;95(5):464-70.
[30] Kojima K, McQueen T, Chen Y, Jacamo R, Konopleva M, Shinojima N, et al. p53 activation of mesenchymal stromal cells partially abrogates microenvironment-mediated resistance to FLT3 inhibition in AML through HIF-1α–mediated down-regulation of CXCL12. *Blood*. 2011;118(16):4431-39.
[31] Theodoropoulos VE, Lazaris AC, Kastriotis I, Spiliadi C, Theodoropoulos GE, Tsoukala V, et al. Evaluation of hypoxia-inducible factor 1α overexpression as a predictor of tumour recurrence and progression in superficial urothelial bladder carcinoma. *BJU int*. 2004;95(3):425-31.
[32] Dos Santos M, Mercante AM, Louro ID, Gonçalves AJ, de Carvalho MB, da Silva EH, da Silva AM. HIF1-alpha expression predicts survival of patients with squamous cell Carcinoma of the Oral Cavity. *Plos one*. 2012;7(9):e45228.
[33] Kim BW, Cho H, Chung JY, Conway C, Ylaya K, Kim JH, et al. Prognostic assessment of hypoxia and metabolic markers in cervical cancer using automated digital image analysis of immunohistochemistry. *J Transl Med*. 2013;11:185.
[34] Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, et al. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. *Cancer Research*. 1999;59(22):5830–85.
[35] Giatromanolaki A, Koukourakis MI, Sivridis E, Turley H, Talks K, Pezzella F, et al. Relation of hypoxia inducible factor 1α and 2α in operable non-small cell lung cancer to angiogenic/molecular profile of tumours and survival. *Br J Cancer*. 2001;85(6):881–90.

[36] Koukourakis MI, Giatromanolaki A, Harris AL, Sivridis E. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumour-associated stroma. *Cancer Res*. 2006;66(2):632-37.
[37] Wu XH, Chen SP, Mao JY, Ji XX, Yao HT, ZHOU SH. Expression and significance of hypoxia-inducible factor-1α and glucose transporter-1 in laryngeal carcinoma. *Oncol Lett*. 2013;5(1):261–66.
[38] Kurokawa T, Yoshida Y, Kawahara K, Tsuchida T, Okazawa H, Fujibayashi Y, et al. Expression of GLUT-1 glucose transfer, cellular proliferation activity and grade of tumour correlate with [F-18]-fluorodeoxyglucose uptake by positron emission tomography in epithelial tumours of the ovary. *Int J Cancer*. 2004;109(6):926-32.
[39] Eckert AW, Lautner MH, Taubert H, Schubert J, Bilkenroth U. Expression of Glut-1 is a prognostic marker for oral squamous cell carcinoma patients. *Oncol Rep*. 2008;20(6):1381-85.
[40] Xiong. Y, Xiong YY, Zhou YF. Expression and significance of βeta-catenin, Glut-1 and PTEN in proliferative endometrium, endometrial intraepithelial neoplasia and endometrioid adenocarcinoma. *Eur J Gynaecol Oncol*. 2010;31(2):160–64.
[41] Wang BY, Kalir T, Sabo E, Sherman DE, Cohen C. Immunohistochemical staining of glut1 in benign, hyperplastic, and malignant endometrial epithelia. *Cancer*. 2000;88(12):2774–81.
[42] Mayer A, Hockel M, Wree A, et al. Microregional Expression of glucose transporter-1 and oxygenation status: lack of correlation in locally advanced cervical cancers. *Clin Cancer Res*. 2005;11(7):2768-73.
[43] Vesely ED, Heilig CW, Brosius FC. GLUT1-induced cFLIP expression promotes proliferation and prevents apoptosis in vascular smooth muscle cells. *Am J Physiol Cell Physio*. 2009;297(3):759-65.
[44] Ishikawa T, Nakashiro K, Klosek SK, Goda H, Hara S, Uchida D, et al. Hypoxia enhances CXCR4 expression by activating HIF-1 in oral squamous cell carcinoma. *Oncol Rep*. 2009;21(3):707–12.
[45] Li Y1, Qiu X, Zhang S, Zhang Q, Wang E. Hypoxia induced CCR7 expression via HIF-1α and HIF-2α correlates with migration and invasion in lung cancer cells. *Cancer Biol Ther*. 2009;8(4):322–30.
[46] Schrijvers ML, van der Laan BF, de bock GH, Pattje WJ, Mastik MF, Menkema L, et al. Overexpression of intrinsic hypoxia markers hif1α and ca-ix predict for local recurrence in stage t1-t2 glottic laryngeal carcinoma treated with radiotherapy. *Int J of radiat oncolo Biol Phys*. 2008;72(1):161-69.

PARTICULARS OF CONTRIBUTORS:
1. Lecturer, Department of Pathology, Faculty of Medicine, Menoufia University, Egypt.
2. Professor, Department of Pathology, Faculty of Medicine, Menoufia University, Egypt.
3. Professor, Department of Pathology, Faculty of Medicine, Menoufia University, Egypt.
4. Assistant Lecturer, Department of Pathology, Faculty of Medicine, Menoufia University, Egypt.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Dalia Rifaat Al-Sharaky,
Lecturer, Department of Pathology Faculty of Medicine, Menoufia University Shibeen El Koom,
32817 Menoufiya Governorate, Egypt.
E-mail: daliah_alsharaky@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 16, 2016**
Date of Peer Review: **Mar 07, 2016**
Date of Acceptance: **Mar 18, 2016**
Date of Publishing: **May 01, 2016**