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ORIGINAL ARTICLE

Combined expressions of ALEX1 and HNF-1β could improve the ability of differentiation between squamous cell carcinoma and adenocarcinoma of the uterine-cervix

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ABSTRACT

Objective: Differentiation between poorly differentiated cervical squamous cell carcinoma (SCC) & adenocarcinoma which is essential for proper therapy, by histopathology alone can be confusing. Immunohistochemistry can aid important data to diagnosis. The transcription factor hepatocyte-nuclear-factor-1-beta (HNF-1β) essentially participates in the visceral-endoderm differentiation from the primitive-endoderm. Arm-protein-lost-in-epithelial-cancers-on-chromosome X (ALEX) is a recent subtype of armadillo- families that had arm-repeat-domains, detected on chromosome-X.

The goal of our research: was to explore the diagnostic roles of HNF-β and ALEX-1 protein expressions, in differentiation between adenocarcinoma and squamous-cell-carcinoma of the cervix using immunehistochemical method.

Methods: HNF-1-β and ALEX-1 protein expressions were evaluated in 32 cases of SCC and 18 cases of adenocarcinoma of the cervix. The relationships between their levels of expression and the ability of panel of both markers in differentiation between both types of carcinomas were analyzed.

Results: We observed positive staining for HNF-1β in 83.4% (15/18) of adenocarcinoma cases. The difference of HNF-1β expression between cervical SCC and adenocarcinoma was statistically significant ($p < 0.001$). Positive ALEX-1 expression was observed in all cases of SCC of the cervix. The difference of ALEX-1expression between cervical SCC and adenocarcinoma was statistically significant ($p < 0.001$). This methodology for distinguishing cervical squamous cell carcinoma and adenocarcinoma had a sensitivity of 93.8% and a specificity of 100% ($p < 0.001$).

Conclusion: The panel of both HNF-1β and ALEX-1 expressions can help in proper subtyping of cervical carcinoma with high sensitivity and specificity.

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KEYWORDS

ALEX1; uterine cervix, adenocarcinoma; HNF-1β; immunohistochemistry; squamous cell carcinoma

Introduction

Carcinoma of the uterine cervix is the 3rd commonest female-cancer globally and considered a major cause of mortality due to cancer in developing countries [1]. In Egypt; it is the 13th between women cancers and the 10th among cause of malignancy related mortality in females between 16-45 years old [2]. Squamous cell carcinoma (SCC) is the commonest cervical malignancy that formed seventy five percent of all cases of cancer-cervix. In Poorly differentiated SCC the tumor cells are immature, with small amount of cytoplasm, nuclear pleomorphism and show few features of differentiation [3]. Cervical adenocarcinoma of the classic type is the commonest subtype of adenocarcinoma of the cervix. Differentiation between poorly differentiated cervical SCC& adenocarcinoma, which is essential for proper therapy, by histopathology alone can be confusing, so in that situation immunohistochemistry can add important-data to diagnosis [4]. There are many markers that found to differentiate between both types but with low sensitivity and specificity. The transcription

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Table 1. Clinicopathological features and immunohistochemical markers expression in our patients.

Characteristics	Number	%	Characteristics	Number	%
Age (year)			Stage		
Mean ± SD	55.92 ± 8.15		Stage I	10	20%
Median (Range)	56 (39–72)		Stage II	25	50%
≤55 years	23	46%	Stage III	9	18%
>55 years	27	54%	Stage IV	6	12%
Histopathology			Lymph node		
SCC	32	64%	Negative	35	70%
Adenocarcinoma	18	36%	Positive	15	30%
Size			Distant metastasis		
<4 cm	10	20%	Negative	44	88%
>4 cm	40	80%	Positive	6	12%
Grade			ALEX1		
Grade I	14	28%	Negative	16	32%
Grade II	28	56%	Low positive	6	12%
Grade III	8	16%	High positive	28	56%
LVSI			HNF-1β		
Absent	43	86%	Negative	33	66%
Present	7	14%	Low positive	6	12%
			High positive	11	22%

Continuous variables were expressed as mean ± SD & median (range); categorical variables were expressed as number (percentage)

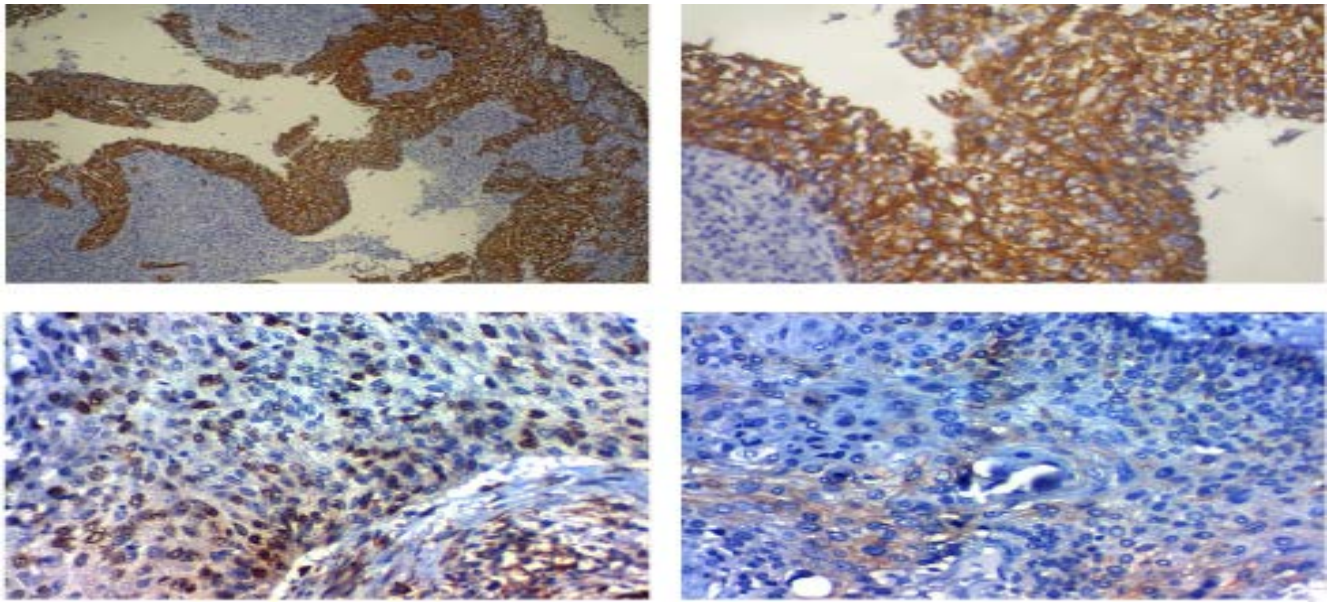

Fig 1. Squamous cell carcinoma of the uterine cervix showed. A; Diffuse cytoplasmic ALEX1 expression, B; Diffuse cytoplasmic ALEX1 expression, C; Focal nuclear HNF-1β expression D; Negative nuclear HNF-1β Magnification; A, original magnification X 100, B, C& D; original magnification X 400.

Table 2. Correlation between clinicopathological features and HNF-1β expression in our patients.

Characteristics	All (N = 50)	HNF-1β			p-value
		Negative (N = 33)	Low positive (N = 6)	High positive (N = 11)	
	No. (%)	No. (%)	No. (%)	No. (%)	
Age (years)					
Mean ± SD	55.92 ± 8.15	57.21 ± 8.65	56 ± 3.22	52 ± 7.65	0.187*
Median (Range)	56 (39-72)	59 (39-72)	56 (51-61)	51 (39-65)	
≤55 years	23 (46%)	13 (56.5%)	2 (8.7%)	8 (34.8%)	0.127‡
>55 years	27 (54%)	20 (74.1%)	4 (14.8%)	3 (11.1%)	
Histopathology					
SCC	32 (64%)	30 (93.8%)	1 (3.1%)	1 (3.1%)	<0.001‡
Adenocarcinoma	18 (36%)	3 (16.7%)	5 (27.8%)	10 (55.6%)	
Size					
<4 cm	10 (20%)	9 (90%)	1 (10%)	0 (0%)	0.144‡
>4 cm	40 (80%)	24 (60%)	5 (12.5%)	11 (27.5%)	
Grade					
Grade I	14 (28%)	12 (85.7%)	2 (14.3%)	0 (0%)	0.007§
Grade II	28 (56%)	18 (64.3%)	3 (10.7%)	7 (25%)	
Grade III	8 (16%)	3 (37.5%)	1 (12.5%)	4 (50%)	
LVSI					
Absent	43 (86%)	31 (72.1%)	5 (11.6%)	7 (16.3%)	0.042‡
Present	7 (14%)	2 (28.6%)	1 (14.3%)	4 (57.1%)	
Lymph node					
Negative	35 (70%)	25 (71.4%)	5 (14.3%)	5 (14.3%)	0.123‡
Positive	15 (30%)	8 (53.3%)	1 (6.7%)	6 (40%)	
Distant metastasis					
Negative	44 (88%)	31 (70.5%)	5 (11.4%)	8 (18.2%)	0.161‡
Positive	6 (12%)	2 (33.3%)	1 (16.7%)	3 (50%)	
Stage					
Stage I	10 (20%)	9 (90%)	1 (10%)	0 (0%)	0.016§
Stage II	25 (50%)	16 (64%)	4 (16%)	5 (20%)	
Stage III	9 (18%)	6 (66.7%)	0 (0%)	3 (33.3%)	
Stage IV	6 (12%)	2 (33.3%)	1 (16.7%)	3 (50%)	
ALEX1					
Negative	16 (32%)	3 (18.8%)	5 (31.3%)	8 (50%)	<0.001§
Low positive	6 (12%)	5 (83.3%)	0 (0%)	1 (16.7%)	
High positive	28 (56%)	25 (89.3%)	1 (3.6%)	2 (7.1%)	

Categorical variables were expressed as number (percentage). Continuous variables were expressed as mean ± SD & median (range). *One Way ANOVA test. ‡Chi-square test. §Chi-square test for trend. $p < 0.05$ is significant

factor hepatocyte-nuclear-factor-one-beta (HNF-1β), participated in the visceral-endoderm differentiation from the primitive-endoderm and it is present in the epithelium of all human-tissues [5]. HNF-1β is expressed in epithelial cells of the liver, urogenital tract, pancreas, gut and lung in normal tissues [6], and it is expressed in carcinomas of many organs [7]. Arm-protein-lost-in epithelial-cancers-on-chromosome-X (ALE-X) is a recent subtype of armadillo families which contains many members like ALEX-1, 2 & 3 and had arm-repeats domains, placed on chromosome-X and is expressed in plethora of malignant and normal tissues [8]. ALEX-1 which contained two armadillo-repeats domains is lost in lots epithelial malignancies [9] Armadillo-repeat-proteins are proteins that are

Table 3. Correlation between clinicopathological features and ALEX1 expression in our patients.

Characteristics	All (N = 50)	ALEX1			p-value
		Negative (N = 16)	Low positive (N = 6)	High positive (N = 28)	
	No. (%)	No. (%)	No. (%)	No. (%)	
Age (years)					
Mean ± SD	55.92 ± 8.15	51.50 ± 7.69	49.50 ± 3.93	59.82 ± 6.95	<0.001*
Median (Range)	56 (39-72)	51 (39-65)	48.50 (44-55)	60 (44-72)	
≤55 years	23 (46%)	11 (47.8%)	6 (26.1%)	6 (26.1%)	<0.001‡
>55 years	27 (54%)	5 (18.5%)	0 (0%)	22 (81.5%)	
Histopathology					
SCC	32 (64%)	0 (0%)	5 (15.6%)	27 (84.4%)	<0.001‡
Adenocarcinoma	18 (36%)	16 (88.9%)	1 (5.6%)	1 (5.6%)	
Size					
<4 cm	10 (20%)	4 (40%)	5 (50%)	1 (10%)	<0.001‡
>4 cm	40 (80%)	12 (30%)	1 (2.5%)	27 (67.5%)	
Grade					
Grade I	14 (28%)	5 (35.7%)	5 (35.7%)	4 (28.6%)	0.078§
Grade II	28 (56%)	10 (35.7%)	0 (0%)	18 (64.3%)	
Grade III	8 (16%)	1 (12.5%)	1 (12.5%)	6 (75%)	
LVSI					
Absent	43 (86%)	15 (34.9%)	5 (11.6%)	23 (53.5%)	0.554‡
Present	7 (14%)	1 (14.3%)	1 (14.3%)	5 (71.4%)	
Lymph node					
Negative	35 (70%)	13 (37.1%)	5 (14.3%)	17 (48.6%)	0.270‡
Positive	15 (30%)	3 (20%)	1 (6.7%)	11 (73.3%)	
Distant metastasis					
Negative	44 (88%)	16 (36.4%)	5 (11.4%)	23 (52.3%)	0.200‡
Positive	6 (12%)	0 (0%)	1 (16.7%)	5 (83.3%)	
Stage					
Stage I	10 (20%)	4 (40%)	5 (50%)	1 (10%)	0.022§
Stage II	25 (50%)	9 (36%)	0 (0%)	16 (64%)	
Stage III	9 (18%)	3 (33.3%)	0 (0%)	6 (66.7%)	
Stage IV	6 (12%)	0 (0%)	1 (16.7%)	5 (83.3%)	
HNF-1β					
Negative	33 (66%)	3 (9.1%)	5 (15.2%)	25 (75.8%)	<0.001§
Low positive	6 (12%)	5 (83.3%)	0 (0%)	1 (16.7%)	
High positive	11 (22%)	8 (72.7%)	1 (9.1%)	2 (18.2%)	

Categorical variables were expressed as number (percentage). Continuous variables were expressed as mean ± SD & median (range). *One Way ANOVA test. ‡Chi-square test. §Chi-square test for trend. $p < 0.05$ is significant

eukaryotic with different positions& functions in tissues, like nuclear-transport, cell junctions-assembly and transcription-activations, by interactions of its armadillo-domains with molecules such as adenomatous-polyposis-coli (APC) and β-catenin [10], also they are important components of the Wnt-signaling pathways [11]. Up to our knowledge no previous researches used both of HNF-1β and ALEX-1 protein expressions by using immune-histochemistry to differentiate between adenocarcinoma and SCC of the uterine cervix which may form a problem in diagnosis when based on morphology alone.

So we aimed in our work to evaluate the expression of both HNF-1β and ALEX1 proteins in both SCC and adenocarcinoma of the uterine cervix by immunohistochemistry then assessed the diagnostic roles of the panel of both markers in differentiation between both types of cancers.

Table 4. Diagnostic performance of HNF-1β & ALEX1 in differentiation between squamous cell carcinoma and adenocarcinoma of cervix uteri.

Markers	SN % (95% CI)	SP % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy (95% CI)
ALEX1 (positive)	100%	88.9% (74.4-100)	94.1% (86.2-100)	100%	96% (90.6-100)
HNF-1B (negative)	93.8% (85.4-100)	83.3% (66.1-100)	90.9% (81.1-100)	88.2% (72.9-100)	90% (81.7-98.3)
ALEX1 (positive) & HNF-1B (negative)	93.8% (85.4-100)	100%	100%	90% (76.9-100)	96% (90.6-100)
ALEX1 (positive) or HNF-1B (negative)	100%	72.2% (51.5-92.9)	86.5% (75.5-97.5)	100%	90% (81.7-98.3)

SN: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value, 95% CI: 95% Confidence Interval; $p < 0.05$ is significant.

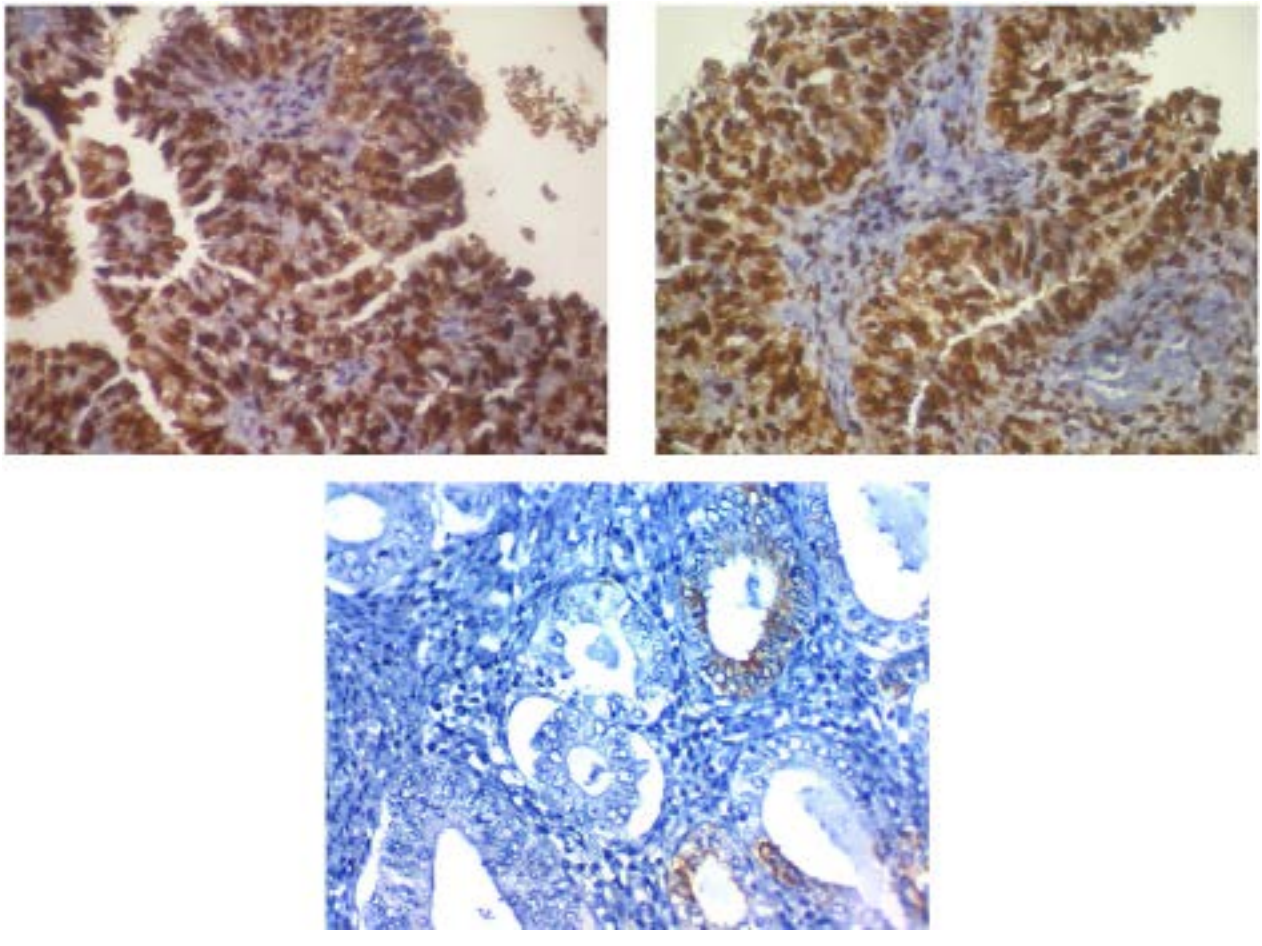

Fig 2. Adenocarcinoma of the cervix showed. A; Diffuse nuclear HNF-1β expression, B; Diffuse nuclear HNF-1β expression, D; Negative cytoplasmic ALEX1expression, Magnification; A, B&C the original magnification X400.

Materials and Methods

We obtained paraffin-embedded tissue thirty-two blocks of SCC and eighteen blocks of adenocarcinomas of the cervix from the archives of the department-of-Pathology, Faculty of Medicine, Zagazig University; retrospectively from the period between 2009 and 2014. Immunohistochemical staining was done for all blocks with primary anti-ALEX-1and HNF-1-β antibodies.

Immunohistochemical staining

We used the technique stated by Hsu et al. [12] for staining with primary rabbit polyclonal anti- HNF-1β

antibody (H-205): (sc-8986) and primary mouse monoclonal anti-ALEX antibody (G-5): sc-376291 (Santa Cruz biotechnology), diluted 1/200 at 4˚C overnight, then evaluated all the stained sections without identification of the clinical or pathological parameters in a coded manner.

Evaluation of HNF-1β protein expression

We considered only nuclear staining as positive. We assessed and graded the stained slides based on the percentage of stained cells as; one = 5-30 percent; two = 31-60 percent; three > 60 percent & zero < 5 percent of positive cells, the intensity of HNF-1β stain as weak one, moderate two or strong three [13]. We calculate the total final from zero to nine by multiplying the intensity values by the percentages of positive cells; we consider the final cut-off point is four above which considered high-expression and below which was considered low-expression of the marker.

Evaluation of ALEX-1 protein expression

We considered only cytoplasmic staining as positive. We assessed and graded the staining intensity and the extent of positively stained cells. The intensity was scored as; one (pale yellow), two (yellow), three (brown), zero (no staining). The extent of positive stains was scored as one (<25% of staining), two (25%-50% of staining), three (50%-80% staining), four (80%-100% staining), zero (no staining) [14]. We calculate the final staining scores (zero to seven) by summation of the intensity and percentage of positive scores. The final staining scores of less than three were named as low expressions and those with scores of more than or equal three were named as high expression.

Statistical Analysis

All statistics were performed using SPSS 22.0 for windows (Chicago, SPSS Inc., IL, and USA) and windows of Med-Calc. Validity of immunohistochemical markers in diagnosis of histo-pathological types was calculated using diagnostic performances depending on sample 2 x 2 contingency tables generation. Sensitivity, specificity, positive predictive values, negative predicative values and accuracy were calculated. All tests were two sided. *P*-values of less than 0.05 were considered significant.

Results

Clinicopathological results

- A total of 50 cases of carcinoma of the uterine cervix were studied in this work; with age range from (39–72) years.
- The cases included: 32 (64%) cases of squamous cell carcinoma and 18 (36%) cases of adenocarcinoma. Table 1.

Immunohistochemical results

- Positive staining for HNF-1β was observed in 83.4% (15/18) of cervical adenocarcinoma cases, 10 was high positive expression, while the remaining 5 cases showed low positive expression.
- Regarding squamous cell carcinoma; 30 (93.8%) cases were negative for HNF-1β; high positive expression was found in one case (3.1%) and high expression in one case.
- The difference of HNF-1β expression between cervical squamous cell carcinoma and adenocarcinoma was statistically significant ($p < 0.001$).
- Positive staining for ALEX1 was observed in all cases of squamous cell carcinoma of the cervix 27/30 (84.4%) was diffuse expression, while 5 (15.6%) showed focal expression.
- Regarding cervical adenocarcinoma; 16 (88.9%) of cases were negative for ALEX1 focal positivity was found in 1 case (5.6%) and 1 cases (5.6%) showed diffuse positivity.
- The difference of ALEX1 expression between cervical squamous cell carcinoma and adenocarcinoma was statistically significant ($p < 0.001$).
- This methodology for distinguishing cervical squamous cell carcinoma and adenocarcinoma had a sensitivity of 93.8% and a specificity of 100%.

Discussion

HNF-1β is found in the epithelium of many normal human organs as; the urinary tract, pancreas, liver, intestine and lung. Epigenetic mutations & inactivation of the HNF-1β gene was incriminated in the occurrence of many malignancies [15].

Few studies have evaluated HNF-1β expression in normal tissues and malignancies of the female genital tract as; endometriosis and cancer cervix, ovary and endometrium [16].

Expression of HNF-1β in uterine-cervix carcinoma had been assessed in previous researches. Park et al. [17] concentrated on the HPV status and immunohistochemical HNF-1β expression in endocervical adenocarcinoma.

Differentiation between poorly differentiated SCC and adenocarcinoma of the uterine cervix can be conflicting when based only on histo-pathological features and immunohistochemistry can be of help in that situation.

A sum-up of data concerning expression of both markers was carried out in our study to shed light on their importance both together in the diagnosis of many controversial cases.

In our study, we found expressions of HNF-1β in 83.4% (15/18) of endocervical adenocarcinoma cases and negative expression in 93.8% of SCC cases. The difference of HNF-1β expression between cervical SCC and adenocarcinoma was statistically significant ($p < 0.001$). This is close to study by Němejcová et al. [13].

Regarding correlation with clinic-pathological parameters, HNF-1βexpression was related significantly to pathological sub-type and age of the patient ($p < 0.05$). But, there were no correlations with pathological grading or stage significantly.

One of the essential components of the Wnt signaling pathway is members of armadillo family [9]. B-catenin played vital roles in developmental and cellular functions like adhesion& signaling proteins [18].

Armadillo proteins had many different functions, e.g. cell adhesions, signal transductions, development and carcinogenesis by interactions of their armadillo-domains with many binding-molecules [19]. Iseki et al. [8] found that overexpression of ALEX-1 plays a role in carcinogenesis suppression. ALEX-1 protein expressions in cervical cancers are markedly more than non-neoplastic cervical tissues. Zeng et al. [14] stated that ALEX-1 protein expression is related to tumorogenesis in cervical carcinoma.

In this study ALEX1 expression was observed in all of our cases of squamous cell carcinoma of the cervix. The differences of ALEX-1expressions between cervical SCC and adenocarcinoma was statistically significant ($p < 0.001$). In the current study ALEX-1 expression was correlated significantly with pathological cancer sub-type ($p < 0.05$). But there were no significant correlations with patient age, cancer pathological grading or staging. This is in consistent with Zeng et al. [14]. ALEX-1, in normal tissues, shows nuclear expression. However, ALEX-1 show cytoplasmic expression in tumor tissues that may explain that ALEX-1 protein transported to and accumulated in the cytoplasm. The use of HNF-1-β and ALEX-1 immunohistochemical markers expression for distinguishing cervical squamous cell carcinoma and adenocarcinoma had a sensitivity of 93.8% and a specificity of 100% ($p < 0.001$). Up to date, a statistical analysis of both markers expression was not carried out in previous researches, and we found that assessment of the expression of HNF-1β and ALEX-1 can be a significant diagnostic tool.

Summary

Differentiation between poorly differentiated adenocarcinoma and SCC of the uterine-cervix can be difficult when based only on histological features. In our study we prove the benefit of the use of HNF-1-β and ALEX-1 protein expressions for distinguishing cervical SCC and adenocarcinoma with high sensitivity a specificity.

Conclusion

The panel of both HNF-1β and ALEX-1 expressions can help in proper sub-typing of cervical cancer into adenocarcinoma and squamous cell carcinoma with high sensitivity and specificity.

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