

Comparison of LPA1 and LPA2 receptor expression with proliferative and prognostic factors in endometroid carcinomas and endometrial hyperplasias

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To cite this article:

Ayşegül Kaynar, Serdar Yanık, Ayşe Neslin Akkoca, Raziye Kurt, Ozan Turgut, Zeynep Tuba Özdemir, Nurdan Tatar, Ufuk Usta. Comparison of LPA1 and LPA2 Receptor Expression with Proliferative and Prognostic Factors in Endometroid Carcinomas and Endometrial Hyperplasias. *American Journal of Clinical and Experimental Medicine*. Vol. 2, No. 4, 2014, pp. 64-69. doi: 10.11648/j.ajcem.20140204.12

Abstract: Objective: In this study we aimed to evaluate the staining patterns of lysophosphatidic acid in endometrial carcinomas(EC) and endometrial hyperplasias(EH). Materials and Method: Sixty diagnostic cases were included in this study in order to evaluate the staining patterns of lysophosphatidic acid in EC and EH. EC was diagnosed in 20 of the cases, EH with atypia was present in 20 and EH without atypia was evident in 20 of the cases. Patients staged according to FIGO(International Federation of Gynecology and Obstetrics,2014). After performing the new sections on each of these cases, 55 routine Hematoxylin and eosin staining was repeated, sections of chosen diagnostic blocks were stained immune histologically with LPA1(Lysophosphatidic acid 1), LPA2(Lysophosphatidic acid 2), MMP-2(Matrix metalloproteinase 2) and Ki-67 antibodies. Results: According to the data obtained, LPA1 showed most intense staining in cases with EH without atypia, however endometrioid type endometrial carcinoma (EEC) cases had the levels very close to this. Furthermore, it was found that there was a reverse correlation between LPA1 staining and histological grade in cases with EEC. It was noted that highest level of LPA2 staining was in cases that had EH with atypia where as lowest level was seen in cases with EEC cases. No relationship between LPA2 and the grade in cases with EEC. It was found that MMP-2 increased linearly with the histological grade in cases with EEC. Correlation tests done among LPA1, LPA2 and MMP-2 antibodies revealed moderate degree of relation only between LPA1 and MMP-2 scores in cases with EEC. No significant relation could be shown in correlation test done between LPA1 and LPA2 and Ki-67, a marker for proliferation index. Conclusion: When correlation of LPA1, LPA2 and MMP-2 H scores with stages of EEC cases was taken into account, the average LPA1 H score was higher in stage 1, while H score averages of LPA2 and MMP-2 H scores were higher in stage 2+3 tumors, however these differences were not statistically significant.

Keywords: LPA1-2, MMP-2 Immunohistochemistry, Endometrial Carcinoma, Endometrial Hyperplasia, FIGO, Endometrioid Carcinoma

1. Introduction

Two types of precursor lesion were described for two pathways of endometrial carcinogenesis. Precursor lesion

for endometrioid type endometrial carcinoma (EEC) is atypical hyperplasia [1-4] . Endometrial intraepithelial carcinoma, on the other hand, is precursor of non endometrioid endometrial carcinomas and serous

carcinoma, which is the most common prototype of these carcinomas [1,4,5]. Endometrial hyperplasias (EH) are divided into two major groups as simple and complex hyperplasia according to structural changes; they are also classified in terms of cytology and nuclear atypia as with atypia and without atypia. Therefore both types of hyperplasias are roughly classified as atypical and non-atypical, as well as simple and complex hyperplasia based on glandular crowding and complexity [6]. EEC is the primary adenocarcinoma of the endometrium that forms glandular structures resembling normal endometrium [7]. They make up three fourth of all EC's [1,3,8,9].

Thought to have a close relationship with prognosis parameters are histopathological type, grade, myometrial invasion, lymph node metastasis, vascular invasion, age and metaplasia.

According to FIGO:

Stage I: Tumor confined to ovaries

Stage II: Tumor involves 1 or both ovaries with pelvic extension (below the pelvic brim) or primary peritoneal cancer

Stage III: Tumor involves 1 or both ovaries with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes

Stage IV: Distant metastasis excluding peritoneal metastasis

Matrix metalloproteinase 2 (MMP-2, gelatinase A, 72-kd type IV collagenase) and Matrix metalloproteinase 9 (MMP-9, gelatinase B, 92-kd type IV collagenase) are responsible for breaking up type IV collagen, a component for all basal membranes, and possibly they stimulate stromal and vascular invasion of the tumor cells [10]. Ki-67 is widely used for measuring cell proliferation in normal and neoplastic tissues [11,12]. Expression of Ki-67 is found higher in proliferation phase compared to secretion and menstruation phases [13,14]. It is found that Ki-67 proliferation index in ECs is related with the histological type, grade and stage of the tumor [15,16]. Although LPA and related lipids are active components of the serum, they are also present in significant amount in ascites fluids of the patients with intraperitoneal tumor, especially ovarian carcinoma [17,18]. It is found that LAP1 is widely expressed in testicles, brain, lungs, heart, spleen and bowels and similar tissues in humans, and contrary this LPA2 and LPA3 distribution is limited [19]. In many studies on cancer tissues such as ovary, thyroid, colon, stomach and breast, it was reported that LPA2 is expressed highly in tissues with tumors and LPA2/LPA1 mRNA ratio is increased in cancer tissue compared to normal tissue [20].

2. Materials and Method

Sixty cases that were sent from Trakya University, Medical Faculty, Department of Pathology were included in this study. Twenty of these cases were total abdominal hysterectomy plus bilateral salpingo-oophorectomy

(TAH+BSO) material that were diagnosed as EEC and 40 cases were TAH+BSO material that had the diagnosis of EH (20 with atypia and 20 without atypia). Sections with 5 mm-thickness taken from paraffin blocks that were obtained from our laboratory archive were stained with hematoxylin eosin (HE). Ages of the cases were obtained from pathology report sample and FIGO stages and follow up information of the patients with tumor were abstracted from clinical records. Cases were stained immunohistochemically with LPA1, LPA2, MMP-2 and Ki-67 antibodies. Paraffin blocks belonging two cases with ovarian serous carcinoma were used as positive control for all antibodies. In evaluating LPA1, LPA2 and MMP-2, extension and strength of staining were taken into account. According to this: degree 0: no staining; degree 1: staining in 1-11% of the cells; degree 2: staining in 12-33% of the cells; degree 3: staining in 34-66% of the cells; degree 4: staining in 67-100% of the cells [21]. Also, strength of staining was scored as weak, moderate and strong. H score values of LPA1, LPA2 and MMP-2 antibodies were calculated for each case in order to merge strength and extent of staining under single value [21]. According to this, highest H-score value was recorded as 4 and lowest value as 0.

$$\text{H-Score} = \frac{\text{Percentage of the stained cells} \times (\text{severity of staining} + 1)}{100}$$

To evaluate reactivity of Ki-67, positive nuclei were counted in 150 adjacent epithelial cells and this procedure was repeated under 5x image magnification; total number of stained cells was calculated as percentage of 750.

Statistical analyses were done with S0064 Minitab Release 13 (License No WCP 1331.00197) program in Trakya University, Medical Faculty, Center for Data Processing. Mann-Whitney U test was used for detecting average and median age of the cases and assessing relation between LPA1, LPA2 and MMP-2 antibodies with the grade and stage in cases with tumor. Kruskal-Wallis test was used for detecting the distribution pattern in all three groups, finding average H scores and comparing them with each other. Pearson correlation test was performed in order to compare LPA1, LPA2 staining patterns with MMP-2 and Ki-67 antibodies.

3. Results

The mean age of all cases was 55,7, of EEC was 61,2 and of EH with atypia was 51,8 and 53,6 in EH without atypia.

Eight of the EEC cases were histologic grade 1 (40%), 8 were grade 2 (40%) 4 were grade 3 (20%). Ten of the cases were stage 1 (50%), 3 were stage 2 (14%), 7 were stage 3 (35%) (Table 1). When the histologic grade was compared with myometrial invasion, four of the cases with infiltration of half of the myometrium or more was grade 1 and 9 was grade 2+3. Four of the cases who had infiltration of less than half of the myometrium were grade 1, 3 were 2+3. As histologic grade increased, myometrial invasion rate also enhanced (Table 1).

Table 1. Clinical and pathological characteristics of the study population according to histological grade

Clinicopathological characteristics		Histological grade	
		Degree I(n=8)	Degree 2+3 (n=12)
Myometrial invasion	< ½	4 (%50)	3 (%25)
	≥ ½	4 (%50)	9 (%75)
Angiolymphatic invasion	Available	2 (%25)	8 (%66,7)
	Unavailable	6 (%75)	4 (%33,3)
Lymph node metastasis	Available	0 (%0)	3 (%42,9)
	Unavailable	6 (%100)	4 (%57,1)
Tumor cells in washing fluid	Available	0 (%0)	3 (%33,4)
	Unavailable	4 (%100)	6 (%66,6)
FIGO staging	I	6 (%75)	4 (%33,3)
	II	2 (%25)	1 (%8,3)
	III	0 (%0)	7 (%58,4)

FIGO: International Federation of Gynecology and Obstetrics.

When histologic grade is compared with angiolymphatic invasion, in 2 cases with grade 1 angiolymphatic invasion was observed, while in 8 cases with grade 2+3 angiolymphatic invasion was evident. All three cases who had tumor cells in abdominal washing fluid were histologic type 2+3 (Table 1).

Lowest H-score value for LPA1 was 0 and highest value was 3,8 for EEC and the average H-score value was 2,7 for EEC. In cases with and without atypia, the average H-score values for LPA1 were 2,2 and 2,8, respectively (Table 2).

Table 2. Average H-scores of LPA1, LPA2 and MMP-2 Ki-67 Index in EEC, EH with or without atypia

Gruplar	LPA1/H-score	LPA2/H-score	MMP-2/H-score	Ki-67 %
EEC	2,7	1	2,6	38,8
Atypia EH	2,2	2,04	2,1	18,1
Nonatypia EH	2,8	1,8	2,4	17,8

*Kruskal-Wallis test.

When EEC cases were evaluated according to grades, the average H-score in grade 1 EEC is 3,4 and in grade 2+3 EEC this value was calculated as 2,3 (Table 3).

Table 3. LPA 1, LPA 2 ve MMP-2/ h-score measurements in EEC according to the severity

H. degree	n	LPA1/H-score		LPA2/H-score		MMP-2/H-score	
Degree 1 EEC	8	3,4	P=0,044	0,9	p>0,05	2,1	P=0,049
Degree 2+3 EEC	12 (8+4)	2,3		1		2,9	

H.degree: Histologic degree, n: Number of cases, *Mann-Whitney U test.

Percentage of LPA1 staining-positive (H-score value above 1) cases were calculated as 100% in EH without atypia, 95% in EH with atypia, 100% in grade 1 EEC, and 82% in grade 2+3 EEC (Table 4).

Table 4. Percentage of LPA 1, LPA 2 and MMP-2 staining observed in EH with atypia-nonatypia and EECs according to degree

Gruplar	LPA1	LPA2	MMP-2
Nonatypia EH	%100	%95	%95
Atypia EH	%95	%95	%85
Degree 1 EEC	%100	%38	%95
Degree 2+3 EEC	%82	%34	%100

Lowest H-score value for LPA2 was 0.2 and highest value was 2,7 for EEC and the average H-score value was 1. In cases with and without atypia, the average H-score values for LPA2 were 2.04 and 1.8, respectively (Table 2). When EEC cases were evaluated according to grades, the average H-score in grade 1 EEC is 0.9 and in grade 2+3 EEC this value was calculated as 1 (Table 3). Percentage of LPA2 staining-positive cases were calculated as 95% in EH without atypia, 95% in EH with atypia, 38% in grade 1 EEC, and 34% in grade 2+3 EEC (Table 4).

Lowest H-score value for MMP-2 was 0.8 and highest value was 3.6 for EEC and the average MMP-2 H-score value was 2.6. In cases with and without atypia, the average H-score values for MMP-2 were 2.1 and 2.4, respectively (Table 2). When EEC cases were evaluated according to grades, the average H-score in grade 1 EEC is 2.1 and in grade 2+3 EEC this value was calculated as 2.9 (Table 3). Percentage of MMP-2 staining-positive cases were calculated as 95% in EH without atypia, 85% in EH with atypia, 95% in grade 1 EEC, and 100% in grade 2+3 EEC (Table 4).

When Ki-67 Expression was investigated, one case in EEC cases had positive staining in maximum 435 (58%) cells and in one case with the least staining 75 (10%) positive nuclear stained cells were counted. The average number of cells that were stained in all EEC cases was 291 (38,8%). These averages were 135 (18,1%) and 133 (17,8%) in EH with and without atypia, respectively (Table 2).

4. Discussion

EEC is the most common type of endometrial adenocarcinomas, occur in postmenopausal women in 80% of the cases and the average age is 59, only 1-8% of the patients are under 40 years of age [1,8,9]. The average age of EEC cases in our study group was 61,2 and this complies with the literature.

Most of the EEC arise as histologic grade 1 [21]. In our study group, 40% of the EEC cases were grade 1. FIGO staging, used in planning of treatment, is the single most important prognostic predictor [22]. Maneschi et al. reported that five year disease-free survival rate is 90% in stage 1, 83% in stage 2, and 43% in stage 3 [23]. In our study, 2-year disease free survival rate is 56% in stage 1, 40% in stage 2 and 25% in stage 3. These data partially comply with the literature.

It is well known that histologic grading has a prognostic value in ECC [24]. Zaino et al. reported that five-year relative survival is 94% in grade 1, 84% in grade 2, 72% in grade 3 [25]. According to our data, 2-year survival rate is 75% in grade 1, and 45% in grade 2+3. These data comply with the literature.

Dai et al. investigated LPA1 and LPA2 mRNA levels with RT-PCR method in 26 cases with colorectal carcinoma and 16 cases with normal colon mucosa; and they found that LPA1 mRNA level was lower in cancer tissue compared to normal tissue [26]. On the contrary, they found that LPA2 mRNA level was markedly high in carcinoma cases. Schulte et al. reported that in thyroid cancers, LPA2 mRNA level increased three-fold compared to normal thyroid or goiter [27]. In our study LPA1 positive staining was in all groups with a nearly ratios and all ratios was over 80%.

Highest LPA1 H-score value was obtained in EH without atypia, followed by EEC with a slight difference. When LPA2 H-score values were compared in EH with and without atypia, there was a statistically significant difference between these two groups ($p=0,009$). LPA1 H-score value demonstrated a reverse relation with grade in EEC cases. According to this, LPA1 H-score value was higher in grade 1 EEC compared to grade 2+3 EEC ($p=0,044$). we found high staining-positiveness with LPA2 in EH with or without atypia but staining in EEC was low (under 50%).

Highest LPA2 H-score value was obtained in EH with atypia, followed by EH with atypia and EEC.

In this study we investigated the distribution pattern of LPA1 and LPA2 receptors in EEC and precursor lesions of endometrium. We found that LPA1 receptor is expressed in cancer tissue similar to study done by Joji et al. in mammary tissue with RT-PCR method [28]. However contrary to other studies done in several organ tumors, in our study LPA2 receptor expression did not display a

marked increase in transition from hyperplasia to carcinoma, on the contrary, it showed a decrease. In studies done with immunohistochemical and in situ hybridization methods in ECs, Aglund et al. and Guo et al. found that MMP-2 and/or MMP-9 correlate with histologic grade and stage of the disease [29,30]. In a study done on 39 cases with hyperplastic endometrium (17 with atypia and 22 without atypia) and 38 cases with EEC using immunohistochemical method, Graesslin et al. reported that EH with atypia tended to be stained higher with MMP-2 antibody compared to EH without atypia [31]. EEC cases, however tended to stain higher compared to hyperplasia with atypia. Furthermore, Graesslin et al. found that MMP-2 expression increased linearly with the histologic grade in cases with EEC, however they did not find any relation between MMP-2 expression and FIGO stage, vascular or lymphatic invasion or disease-free survival. In our study staining positiveness with MMP-2 was higher in EEC and this result correlates with literature [32].

However, when EEC and EH with and without atypia MMP-2 H-score values are compared, difference was not statistically significant. Again, similar to literature, MMP-2 H-score values in grade 2+3 EEC cases were considerably high compared to grade 1 EEC cases, and this difference was statistically significant ($p=0,049$). In correlation test that was done for comparing LPA1 and LPA2 H-score levels with MMP-2 H-score levels, there was a moderate relation in only EEC cases between LPA1 and MMP-2 H-score levels and there was not any relation with hyperplasias with and without atypia.

Ki-67 reactivity in ECs shows a rise with grade of the carcinoma and nuclear Ki-67 expression is accepted as an independent prognostic factor [32,33,34]. In our study Ki-67 index is significantly higher in EC cases compared to EH cases with and without atypia ($p=0,000$). In correlation test done for comparison of H-score values of LPA1 and LPA2 antibodies with stain pattern of Ki-67, a predictor for proliferation index, weak correlation was found between LPA1 and LPA2 and Ki-67 antibodies in all three case groups and this difference was not statistically significant ($p>0.05$).

It seems that LPA1 and LPA2 antibodies reacts less in endometrial premalignant and malignant lesions compared to other organ malignancies and there is not any significant correlation between MMP-2 and prognostic predictors. Therefore results of the study do not support the relationship of ECs with LPA2, and they also probably do not support treatment of these cancers with LPA antagonists, and further studies are needed. When correlation of LPA1, LPA2 and MMP-2 H scores with stages of EEC cases was taken into account, the average LPA1 H score was higher in stage 1, while H score averages of LPA2 and MMP-2 H scores were higher in stage 2+3 tumors, however these differences were not statistically significant.

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