Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2023 Mar 20; 11(B):1-6.
https://doi.org/10.3889/oamjms.2023.11565
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Oncology*

Expression of Salivary Immediate Early Response Gene X-1 as a Predictor of Malignancy in Epithelial Ovarian Tumor

Hiro Nasution[1], Gatot Purwoto[1]*, Hariyono Winarto[1], Andri Andrijono[1], Andi Darma Putra[1], Tofan Widya Utami[1], Aria Kekalih[2]

[1]Department of Obstetrics and Gynecology, Division of Gynecology Oncology, Faculty of Medicine, University of Indonesia, Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia; [2]Department of Public Health, Faculty of Medicine, University of Indonesia, Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Nasution H, Purwoto G, Winarto H, Andrijono A, Putra AD, Utami TW, Kekalih A. Expression of Salivary Immediate Early Response Gene X-1 as a Predictor of Malignancy in Epithelial Ovarian Tumor. Open Access Maced J Med Sci. 2023 Mar 20; 11(B):1-6. https://doi.org/10.3889/oamjms.2023.11565
Keywords: Expression of IEX-1; Malignant epithelial ovarian tumor; Salivary IEX-1; Tumor Marker
***Correspondence:** Gatot Purwoto, MD, PhD, Oncology-Gynecologist. Oncology Gynecology Division, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Indonesia - Cipto Mangunkusumo National General Hospital, Indonesia. Email: gatotpurwoto@gmail.com
Received: 21-Feb-2023
Revised: 01-Mar-2023
Accepted: 10-Mar-2023

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: This diagnostic study aims to determine the expression of salivary IEX-1 as a predictor of malignancy in epithelial ovarian tumors.

METHODS: Samples were obtained from ovarian cancer patients who were scheduled for elective surgery. Patients' saliva was collected before surgery and used as the study's research material. Research subjects who met the inclusion and exclusion criteria were divided into two groups based on the post-operative histopathological results, benign, and malignant epithelial ovarian tumors. The salivary IEX-1 expression was examined using the real-time qPCR method. We compared and analyzed the salivary IEX-1 expression in benign and malignant epithelial ovarian tumors.

RESULTS: The results of this study were obtained from 47 epithelial ovarian tumor subjects, 22 malignant tumors, and 27 benign tumors. The mean salivary IEX-1 expression in benign epithelial ovarian tumors was higher (1.976) than the malignant tumors (0.554) ($p < 0.001$). The AUC value of IEX-1 expression was 0.949 (95% CI: 0.894–1,000), and cut-off point of salivary IEX-1 is ≥ 0,9115 with sensitivity 84%, specificity 86,4%, positive predictive value 82.6%, and negative predictive value 87.5%. There was a significant correlation between salivary IEX-1 expression and malignant epithelial ovarian tumors with an OR 5.031 (95% CI: 2.039-12.4; $p < 0,001$).

CONCLUSION: Salivary IEX-1 expression declines in tandem with the development of malignant epithelial ovarian tumors, providing a very sensitive and specific indicator of the presence of these malignant tumors.

Introduction

One of the malignancies with a high mortality rate globally is ovarian cancer. There were around 313,959 ovarian cancer diagnoses worldwide, with 207,252 cases of mortality. With an incidence of 3.4% and a mortality rate of 4.7%, ovarian cancer ranks among the top 10 malignancies in women with the highest incidence and mortality rate [1].

Due to the nonspecific nature of the disease, the difficulty in detecting tumor growth, the delayed onset of symptoms, and the lack of screening procedures, ovarian cancer diagnosis might be delayed until it has progressed to an advanced stage [2].

Only about 15.7% of ovarian cancers were detected at an early stage and about 58% were identified at a late stage, resulting in a 5-year survival rate to decrease to 30.2% from 92.6% when detected at an early stage with local deployment [3].

Patients diagnosed with epithelial ovarian cancer or primary serous peritoneal cancer are often evaluated with cancer antigen 125 (CA-125) as one of the serologic indicators now in use. It is not suggested that asymptomatic women have their CA-125 levels checked because of the test's poor sensitivity (50–62% for identifying early-stage epithelial ovarian cancer) [4]. Moreover, routine pelvic examinations every year are also considered ineffective and give false-positive results. CA-125 and transvaginal ultrasonography, especially in premenopausal women, are considered not cost-effective and cannot detect ovarian cancer at an early stage [5].

Accordingly, it is critical to identify suitable biomarkers with higher sensitivity and specificity for the detection of ovarian cancer. Saliva provides a huge possibility for improving the diagnosis and monitoring of health and illness in general, and this has been the focus of numerous recent research. Saliva offers numerous advantages over blood as a liquid biopsy because collection is simple, safe, non-invasive, and affordable. Numerous molecular markers for local and systemic illnesses, including cancer, have been found in salivary diagnostic research [6].

Rapid initial response cellular stress has been linked to the activities of gene X-1 (IEX-1) or IER3, which has been shown to have a role in cell differentiation, proliferation, and death. Using RT-qPCR in blood and saliva, the therapeutic efficacy of IEX-1 was confirmed in a variety of epithelial ovarian cancers and in healthy control women [7].

Because IEX-1 is often downregulated in epithelial ovarian cancers, it is safe to assume that it functions as a tumor suppressor gene in this kind of cancer [8].

From the above background, the authors are interested in conducting research on salivary IEX-1 expression as a predictor of malignancy in epithelial ovarian tumors by finding the cut-off, sensitivity, and specificity values so that IEX-1 expression can be an alternative non-invasive examination and diagnostic of an epithelial ovarian tumor.

Methods

Study population

This was a cross-sectional study with consecutive sampling method. The data were taken from ovarian tumor patients who were treated at Obstetrics and Gynecology Department of Cipto Mangunkusumo National Hospital, Jakarta, between September 2021 and June 2022. The study involved 55 ovarian tumor patients who had complete informed consent, who had been planned to undergo an elective surgery, had no concomitant non-gynecological tumor disease, no history of other malignant tumors, and no history of chemotherapy treatment. Research subjects were divided into two groups based on the post-operative histopathological results, benign, and malignant epithelial ovarian tumors. Patients suffering from ovarian tumors with histopathological results of non-epithelial ovarian tumors, borderline ovarian tumor or with damaged saliva samples which cannot be examined were excluded from the study.

Sample collection and RNA isolation

After having the mouth wiped out with distilled water before to the elective operation, samples ranging from 3 to 5 milliliters of unstimulated saliva were expectorated into sterile tubes and then sent directly to the Human Cancer Research Laboratory at Indonesian Medical Education and Research Institute (IMERI), Jakarta. The RNA isolation from saliva samples was carried out on the same day with Quick RNA Miniprep Plus (Zymo Research Kit, USA). After the RNA was extracted, the levels and purity of the RNA were measured using a Nano Spectrophotometer. The RNA samples were then stored at −80°C until it is used for PCR examination collectively. The patient then underwent surgery and tumor mass removed was subjected to histopathological examination at the Department of Anatomical Pathology, Cipto Mangunkusumo National Hospital, Jakarta.

IEX-1 expression quantification with real-time PCR

The expression of IEX-1 saliva was examined with the real-time qPCR (polymerase chain reaction) method using the primer IEX-1 5′-GCCGCCTTCTAACTGTGACTC-3′ and reverse 5′-GTCTCCGCTGTAGTGTTCTGAG-3′. These genes were compared using an endogenous control actin, with primers 5′-TGACGTGGACATCCGCAAAG-3′ and 5′-CTGGAAGGTGGACAGCGAGG-3′. The PCR process was carried out using the ABI 7500 Fast real-time PCR machine with PCR conditions of 45°C for 10 min for the reverse transcription process for 1 cycle, 95°C for 2 minutes to activate polymerization and 40 PCR cycles covering 95°C for 5 s for denaturation process, and 60°C for 30 seconds for the annealing and extension process. The IEX-1 expression is then represented as cycle threshold data. RNA-actin gene was used as an endogenous control. IEX-1 expression was normalized by β-aktin expression and expressed as ΔCt (delta Ct). The relative expression of IEX-1 is ΔCt IEX-1 = Ct IEX-1– Ct β-aktin. IEX-1 expression levels are expressed in fold changes (FC). FC was

Table 1: Baseline characteristics and immediate early response gene X-1 expression

Characteristics	Total, n (%)	Epithelial ovarian tumor		P	IEX-1 expression		p
		Malignant (n = 22), n (%)	Benign (n = 25), n (%)		Mean ± SD	Median (minimum–maximum)	
Age (years old)							
<20	2 (4.3)	0	2 (100.0)	0.068	4.97 ± 0.14	4.97 (4.89–5.06)	0.031
20–50	25 (53.2)	10 (40.0)	15 (60.0)		1.26 ± 0.87	1.08 (0.11–3.49)	
>50	20 (42.6)	12 (60.0)	8 (40.0)		1.03 ± 1.05	0.83 (0.16–5.02)	
Menopausal state							
Menopause	22 (46.8)	12 (54.5)	10 (45.5)	0.387	1.04 ± 1.01	0.84 (0.11–5.02)	0.125
Premenopause	25 (53.2)	10 (40.0)	15 (60.0)		1.56 ± 1.34	1.23 (0.15–5.06)	
Parity							
P0	23 (48.9)	12 (52.2)	11 (47.8)	0.738	1.39 ± 1.22	1.13 (0.15–5.06)	0.690
P1-2	14 (29.8)	5 (35.7)	9 (64.3)		1.51 ± 1.54	0.86 (0.26–5.02)	
≥3	10 (21.3)	5 (50.0)	5 (50.0)		0.89 ± 0.47	0.91 (0.11–1.51)	
Menarche (tahun)							
≤12	16 (34.0)	11 (68.8)	5 (31.3)	0.030	1.07 ± 1.21	0.79 (0.15–4.89)	0.197
>12	31 (66.0)	11 (35.5)	20 (64.5)		1.50 ± 1.25	1.08 (0.11–5.06)	
FIGO stage							
Stage I	13 (27.7)	13 (100.0)	0	<0.001	0.65 ± 0.35	0.59 (0.15–1.23)	<0.001
Stage II	1 (2.1)	1 (100.0)	0				
Stage III	8 (17.0)	8 (100.0)	0		0.38 ± 0.21	0.35 (0.11–0.75)	

SD: Standard deviation, IEX-1: Immediate early response gene X-1.

Table 2: Distribution of epithelial ovarian tumor and salivary immediate early response gene X-1 expression based on histopathological results

Types of histopathology	Salivary IEX-1 expression (fc)				p
	n (%)	Mean ± SD	Median	Minimum–maximum	
Malignant					
High-grade serous adenocarcinoma	5 (10.6)	0.730 ± 0.33	0.75	0.27–1.17	<0.001
Mucinous adenocarcinoma	6 (12.8)	0.577 ± 0.39	0.51	0.15–1.23	
Clear-cell adenocarcinoma	8 (17.0)	0.398 ± 0.19	0.45	0.11–0.70	
Endometrioid adenocarcinoma	3 (6.4)	0.629 ± 0.47	0.62	0.16–1.10	
Benign					
Mucinous cystadenoma	10 (21.3)	2.305 ± 1.51	1.61	0.83–5.02	
Serous cystadenoma	6 (12.8)	2.163 ± 1.74	1.43	0.72–5.06	
Endometriosis cyst	9 (19.1)	1.486 ± 0.52	1.29	0.86–2.31	

SD: Standard deviation, IEX-1: Immediate early response gene X-1.

calculated by comparing the mean expression of the test and comparison groups ($\Delta\Delta Ct = \Delta Ct$ test $- \Delta Ct$ Comparison) and calculated by the formula $FC = 2\text{-}\Delta\Delta Ct$. Data from laboratory results of IEX-1 examination and histopathology were collected, tabulated, and analyzed.

Statistical analysis

We performed data analysis using SPSS Statistics Version 26 and used descriptive and analytical methods to analyze the data. The analysis used to determine differences in salivary IEX-1 expression in patients with benign and malignant epithelial ovarian tumors was the Mann–Whitney alternative test with data that were not normally distributed. To determine the relationship between increased salivary IEX-1 expression and the incidence of epithelial ovarian tumors, Fisher's exact analysis (X2) was performed. Analysis with ROC curve was used to determine the diagnostic discrimination ability of IEX-1. The optimal cut-off point value was determined by the ROC curve and presented in terms of sensitivity, specificity, positive predictive value, and negative predictive value compared to the gold-standard examination determined by the histopathological report after surgery. $p < 0.05$ is said to be statistically significant.

Results

55 patients had participated in this study with details of 50 patients who had epithelial ovarian tumors, 3 patients with non-epithelial ovarian tumors, and 2 saliva samples could not be examined because they were damaged. Of the 50 people with epithelial ovarian tumors, 3 people with borderline ovarian tumors were not included in the study. This study was followed by 47 subjects analyzed, 22 patients (46.8%) with malignant epithelial ovarian tumors and 25 patients (53.2%) with benign epithelial ovarian tumors. IEX-1 expression based on the age of the research subjects, it was found that with increasing age, the mean expression of IEX-1 decreased, the mean expression of IEX-1 in patients <20 years old was 4.97; age 20–50 years 1.26; and age >50 years 1.03 ($p < 0.05$). Based on FIGO stage, the mean expression of IEX-1 was higher in stage I–II (0.65) compared to stage III (0.38) and statistically significant ($p < 0.001$; Table 1).

Table 3: *Post hoc* analysis of saliva immediate early response gene X-1 expression based on histopathological examination results of epithelial ovarian tumors

Histopathology type	p
Epithelial ovarium malignancy	
High-grade serous and mucinous	0.429
High-grade serous and clear cell	0.045*
High-grade serous and endometrioid	0.786
Mucinous and clear cell	0.414
Mucinous and endometrioid	0.903
Clear cell and endometrioid	0.497

Salivary IEX-1 expression in patients with epithelial ovarian tumors

Based on the histopathological examination of epithelial ovarian tumors, there were 4 histopathological types of malignant epithelial ovarian tumors and 3 types of benign epithelial ovarian tumor. Among the four types of malignant epithelial ovarian tumors, the most common types were clear-cell adenocarcinoma in 8 patients (17%), mucinous adenocarcinoma in 6 patients (12.8%), high-grade serous adenocarcinoma in 5 patients (10.6%), and endometrioid adenocarsinoma in 3 patients (6.4%). Salivary IEX-1 expression was significantly different ($p = 0.001$) between benign and malignant epithelial ovarian cancers (Table 2).

After *post hoc* follow-up analysis, it was found only high-grade serous adenocarcinoma and clear-cell adenocarcinoma ($p = 0.045$) that statistically significant difference in the mean salivary IEX-1 expression (Table 3).

The mean salivary IEX-1 expression in benign epithelial ovarian tumors was higher (1.976) than the malignant epithelial ovarian tumors (0.554) and based on the analysis, there was a statistically significant difference with $p < 0.001$(Table 4).

Table 4: Differences in immediate early response gene X-1 saliva expression in benign and malignant ovarian epithelial tumors

Epithelial ovarium tumor	Saliva IEX-1 expression (fc)				p*
	n	Mean ± SD	Median	Minimum–maximum	
Malignant	22	0.554 ± 0.331	0.514	0.11–1.23	<0.001
Benign	25	1.976 ± 1.313	1.516	0.72–5.06	

*Mann–Whitney test. SD: Standard deviation, IEX-1: Immediate early response gene X-1.

The relationship between increased salivary IEX-1 expression with the occurrence of malignant epithelial ovarian tumors

Based on ROC analysis, the AUC IEX-1 expression value or AUC IEX-1 0.949 (CI 95% 0.894–1,000) has a strong power of determination (AUC 0.8–1.0) as a marker to distinguish benign and malignant tumors of the epithelial ovary (Figure 1a). Plotting the ROC curve on IEX-1 expression values (Table 5;

Figure 1b) shows that the optimal cut-off point for IEX-1 expression is >0.9115 as a marker for benign epithelial ovarian tumors (sensitivity 84%; specificity 86.4%). It means that the higher expression value of IEX-1 (>0.9115) can be a marker of a relative tumor, including benign epithelial ovarian tumors. Based on statistical analysis, there was a significant relationship between salivary IEX-1 expression and histopathological results of the tumors (p < 0.001; OR: 5.301 (95% CI: 2.039–12.41) (Table 6). Our IEX-1 expression examination had a sensitivity < 0.9115 of 86.4% (95% CI: 66.66 – 95.25), specificity 84% (95% CI: 65.35–93.6), positive predictive value 82.6 % (CI: 95% 62.86–93.02), negative predictive value 87.5% (CI: 95% 69.00–95.66), and an accuracy value of 85% (95% CI: 72.31–92.59).

Table 5: Sensitivity and specificity of saliva immediate early response gene X-1 expression

Number	Positive if greater than or equal to[a]	Sensitivity	Specificity
15	0.6015	1.000	0.636
16	0.6610	1.000	0.682
17	0.7070	1.000	0.727
18	0.7335	0.960	0.727
19	0.7700	0.960	0.773
20	0.8105	0.960	0.818
21	0.8360	0.920	0.818
22	0.8505	0.880	0.818
23	0.8685	0.840	0.818
24	0.9115	0.840	0.864
25	1.0065	0.800	0.864
26	1.0770	0.760	0.864
27	1.0950	0.720	0.864
28	1.1380	0.720	0.909
29	1.1750	0.720	0.955
30	1.2055	0.680	0.955
31	1.2625	0.680	1.000
32	1.3220	0.640	1.000
33	1.3580	0.600	1.000
34	1.4365	0.560	1.000
35	1.5130	0.520	1.000

Discussion

The highest incidence of malignant epithelial ovarian tumors was found in the age group >50 years old. This is consistent with several other studies that the highest incidence of ovarian cancer was found in women aged between 55 and 64 years old (mean age 63 years old) with the highest mortality rate in women aged 75 and 84 years old (mean age 71 years old) [4]. This study found that the average expression of IEX-1 decreased with increasing age, where the highest mean expression of IEX-1 was 4.97 in <20-year-old group and the lowest expression of IEX-1 1.03 was in the > 50-year-old group (p < 0.05). This increased risk is found in patients with a higher number of ovulatory cycles such as in patients with younger menarche age and older menopause age, where the release of follicular fluid which is rich in pro-inflammatory cytokines and inflammatory factors and genetic stress can cause DNA damage in the secretory epithelium of the fallopian tubes. In addition, repeated ovulation causes repetitive trauma to the epithelium. Trauma can damage the cellular DNA and lead it to change so that facilitate the occurrence of cortical stromal invagination and cortical inclusion cysts from epithelial cells, and they turn into cancer cells [9]. Research by Han *et al*. (2011) found that age had no correlation with IEX-1 expression [10].

Table 6: The relationship of increased saliva immediate early response gene X-1 expression with the occurrence of malignant epithelial ovarian tumors

IEX-1 expression (fc)	Histopathology of epithelial ovarium tumor		p	OR	IK 95%
	Malignant, n (%)	Benign, n (%)			
<0.9115	19 (82.6)	4 (17.4)	<0.001	5.031	2.039–12.41
≥0.9115	3 (12.5)	21 (87.5)			

IEX-1: Immediate early response gene X-1, OR: Odds ratio.

Ovarian cancer risk has been investigated in connection to parity. Ovarian cancer risk is inversely related to the number of children a woman has, thus having even one kid reduces that risk by a factor of 0.3 to 0.4 [5]. As the number of pregnancies increased, the expression of IEX-1 decreased relatively, at 1.39 nulliparas and >3 parity at 0.89. This is also supported by Noela *et al*. study, that the highest incidence of ovarian cancer in Cipto Mangunkusumo hospital is higher in nulliparous patients [11], [12]. Based on the parity status of the subjects in this study, the most parity 0 (P0) were 23 patients (48.9%) and among 23 nulliparous patients, 12 patients had malignant epithelial ovarian tumors (52.2%) and 11 patients (47.8%) found benign epithelial ovarian tumors.

Women who have more than a few ovulatory cycles every year, such as those with a younger menarche and longer menopausal age, were shown to have an elevated risk of ovarian cancer, however, the association between menarche age and the incidence of ovarian cancer was only established in a few studies. Having more ovulatory cycles raises the risk of cellular division leading to tumor formation [12]. In this study, based on age at menarche, the highest menarche age was >12 years old as many as 31 subjects (66%) and in epithelial ovarian tumors, 11 subjects (35.5%) with menarche age <12 years old.

In this study, the majority of the malignant epithelial tumors patients were found in FIGO stage I (13 subjects; 27.7%) and stage III (8 subjects; 17%). Han *et al*. investigated that FIGO stage and IEX-1 expression have prognostic properties for survival in ovarian cancer patients [10]. The distribution found by Li *et al*. (2018), out of 26 subjects with malignant epithelial ovarian cancer, 17 were classified at stage III/IV [7].

It was seen that the expression of salivary IEX-1 was statistically significantly higher in the benign ovarian tumor group (mean 1.976) compared to the malignant epithelial ovarian cancer group (mean 0.554) (p < 0.001), and the optimal cut-off point of IEX-1 expression was >0.9115 as a marker value for benign epithelial ovarian tumors with a sensitivity of 84% and specificity of 86.4% (OR 5.031 (95% CI 2.039–12.41). If a patient has an IEX-1 expression <0,

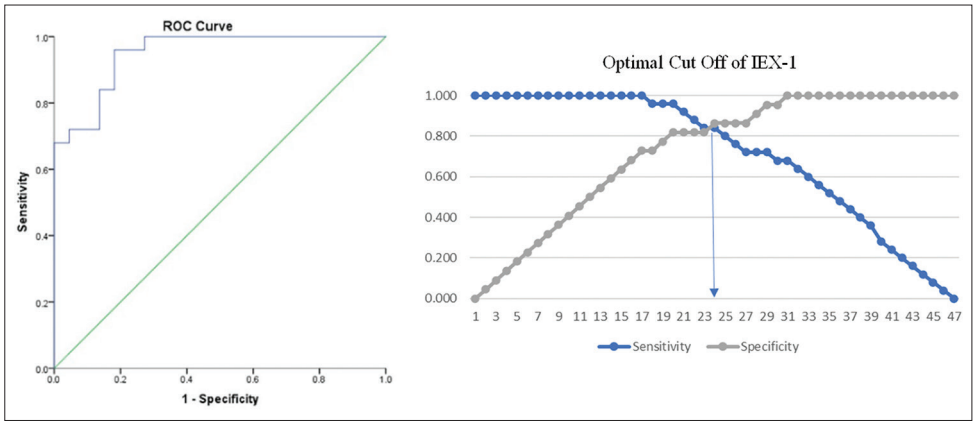

Figure 1: (a) IEX-1 saliva expression ROC curve; (b) Saliva IEX-1 Expression cut-off value

9115 then the probability of suffering from malignant epithelial ovarian tumors is 5 times. This result is in line with the findings of Li *et al*. (2018) discovered that, in contrast to the cancer group, IEX-1 was well and abundantly expressed in the blood and saliva of women with benign ovarian tumors and healthy women. The ROC-area under the curve (AUC) values of 0.947 and 0.929, respectively, compare the expression of IEX-1 in the ovarian cancer group to that of benign ovarian tumors and healthy women. Using IEX-1 blood to differentiate between malignant and benign groups had a sensitivity of 84.6% and a specificity of 94.60% and was able to differentiate the ovarian carcinoma group from healthy women with a sensitivity of 84.6% and a specificity of 90.9%. The cutoff value of IEX-1 expression in determining ovarian cancer among the benign ovarian tumor group was 1.118, and the cutoff value in determining ovarian cancer among the healthy female group was 1.554. Li *et al*. reported that there was no significant difference in the expression of IEX-1 in blood and saliva. From this study, it can be concluded that IEX-1 is expressed equally well in blood and saliva samples, thus supporting the proposed clinical potential of salivary IEX-1 as a diagnostic tool [7].

IEX-1 is responsible for both anti-apoptotic and pro-apoptotic activity [13]. The ability of IEX-1 to regulate the formation of reactive oxygen species (ROS) in the mitochondria appears to be linked to both its pro-apoptotic activity and nuclear localization [13]. The complex I–IV and the functional components F1 and Fo of the ATP synthase/ATPase make up the mitochondrial respiratory chain. Proton flow will be delayed by ATP synthase inhibition brought on by high membrane potential related to stress, a high ATP to ADP ratio, or an accumulation of IF1, a natural F1 complex inhibitor. As a defense mechanism against reactive oxygen species (ROS) production in stressed cells, F1Fo-ATP synthase changes to ATPase, hydrolyzes ATP, increases proton flow through Fo channels, and reduces mitochondrial inner membrane potential from a stressed state to a phosphorylated one [14]. By binding to the F1 complex and inhibiting its ATPase activity, IF1 contributes to the regulation of the switch from F1F0-ATP synthase to ATPase activity. Because IF1 expression is elevated in cancer, it is crucial for boosting aerobic glycolysis in cells. Through its control of IF1 protein levels in cancer cells, IEX-1 may be a crucial link between the environment and the regulation of energy metabolism in mitochondria [15], [16].

Han *et al*.'s (2011) results from the first research to examine IEX-1 expression in ovarian cancer tissue were published, IEX-1 expression was shown to be lower in ovarian cancer than in benign ovarian tumors, with a $p < 0.05$, and this difference was associated with improved prognosis for ovarian cancer patients' survival. Ovarian epithelial tumor progression from benign to malignant was accompanied by a concomitant reduction in IEX-1 expression, which was likewise linked to the pro-apoptotic impact of IEX-1. Therefore, IEX-1 promotes cell death in epithelial ovarian cancer [10].

Investigating the mechanisms behind the downregulation of IEX-1 expression in certain cases of ovarian cancer and developing a more precise link between IEX-1 expression levels and the progression to malignancy in these cases are important directions for future study. These studies should be conducted with a bigger sample size.

There is still no effective ovarian cancer screening approach that has been established to date. It is concluded that screening for ovarian cancer with ultrasound and CA-125 is not recommended for general population-based screening because routine pelvic examinations are thought to be ineffective and give false-positive results, and CA-125 examination and transvaginal ultrasound, especially in premenopausal women, are thought to be not cost-effective and unable to detect ovarian cancer at an early stage [5]. Moreover, by including the salivary IEX-1 examination as a component of diagnostic tool for malignant ovarian tumors, it is hoped that it can increase the accuracy of diagnostic that is non-invasive and easy to perform.

Conclusion

There was a significant relationship between decreased salivary IEX-1 expression and the incidence of malignant epithelial ovarian tumors. The mean salivary IEX-1 expression in malignant epithelial ovarian tumors was lower than benign epithelial ovarian tumors with statistically significant differences. The salivary AUC IEX-1 expression was found with a strong determination and a cut-off point at 0.9115, which express a good sensitivity and specificity so that they could be used as predictors of malignancy in epithelial ovarian tumors.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2020;71(3):209-49. https://doi.org/10.3322/caac.21660
   PMid:33538338
2. Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: Epidemiology and risk factors. Int J Womens Health. 2019;11:287-99. https://doi.org/10.2147/IJWH.S197604
   PMid:31118829
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30. https://doi.org/10.3322/caac.21590
   PMid:31912902
4. Sölétormos G, Duffy MJ, Abu Hassan SO, Verheijen RH, Tholander B, Bast RC Jr., *et al*. Clinical use of cancer biomarkers in epithelial ovarian cancer: Updated guidelines from the European group on tumor markers. Int J Gynecol Cancer. 2016;26(1):43-51. https://doi.org/10.1097/IGC.0000000000000586
   PMid:26588231
5. Berek JS. Berek and Hacker's Gynecologic Oncology. 7th ed. United States: Wolters Kluwer Health; 2021. p. 2254.
6. Rapado-González Ó, Majem B, Muinelo-Romay L, Álvarez-Castro A, Santamaría A, Gil-Moreno A, *et al*. Human salivary microRNAs in cancer. J Cancer. 2018;9(4):638-49. https://doi.org/10.7150/jca.21180
   PMid:29556321
7. Li Y, Tan C, Liu L, Han L. Significance of blood and salivary IEX-1 expression in diagnosis of epithelial ovarian carcinoma. Obstet Gynecol Res. 2018;44(4):764-71. https://doi.org/10.1111/jog.13576
   PMid:29431239
8. Chandra K, Yadav S. Biomarkers in ovarian cancer and saliva: An update. J Reprod Healthc Med. 2021;2(1):1-11. https://doi.org/10.25259/JRHM_22_2020
9. Budiana IN, Angelina M, Pemayun TG. Ovarian cancer: Pathogenesis and current recommendations for prophylactic surgery. J Turk Ger Gynecol Assoc. 2019;20(1):47-54. https://doi.org/10.4274/jtgga.galenos.2018.2018.0119
   PMid:30362670
10. Han L, Geng L, Liu X, Shi H, He W, Wu MX. Clinical significance of IEX-1 expression in ovarian carcinoma. Ultrastruct Pathol. 2011;35(6):260-6. https://doi.org/10.3109/01913123.2011.608916
    PMid:22085302
11. Noela F, Nuryanto KH. Epidemiology data of ovarian cancer in Dr. Cipto Mangunkusumo hospital, Jakarta. Indones J Obstet Gynecol. 2016;4(2):101. https://doi.org/10.32771/inajog.v4i2.84
12. Gaona-Luviano P, Medina-Gaona LA, Magaña-Pérez K. Epidemiology of ovarian cancer. Chin Clin Oncol. 2020;9(4):47. https://doi.org/10.21037/cco-20-34
    PMid:32648448
13. Wu MX, Ustyugova IV, Han L, Akilov OE. Immediate early response gene X-1, a potential prognostic biomarker in cancers. Expert Opin Ther Targets. 2013;17(5)593-606. https://doi.org/10.1517/14728222.2013.768234
    PMid:23379921
14. García-Bermúdez J, Cuezva JM. The ATPase inhibitory Factor 1 (IF1): A master regulator of energy metabolism and of cell survival. Biochim Biophys Acta. 2016;1857(8):1167-82. https://doi.org/10.1016/j.bbabio.2016.02.004
    PMid:26876430
15. Wu MX. Roles of the stress-induced gene IEX-1 in regulation of cell death and oncogenesis. Apoptosis. 2003;8(1):11-8. https://doi.org/10.1023/a:1021688600370
    PMid:12510147
16. Formentini L, Sanches-Arago M, Sanches-Cenizo L, Cuezva JM. The mitochondrial ATPase inhibitory factor 1 triggers a ROS-mediated retrograde prosurvival and proliferative response. Mol Cell. 2012;45(6):731-42. https://doi.org/10.1016/j.molcel.2012.01.008
    PMid:22342343