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Original Research Article

Study of leucine-rich alpha-2-glycoprotein-1 marker serum level in cases of malignant epithelial ovarian tumors

Abd Elfatah Agameia[1], Rania Shafik Swelem[2], Hossam Hassan El Sokkary[1]*, Ghada Shehata Elsayed[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Alexandria University, Egypt
[2]Department of Clinical and Chemical pathology, Faculty of medicine, Alexandria University, Egypt

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***Correspondence:**
Dr. Hossam Hassan El Sokkary,
E-mail: hossamsokkary@hotmail.com

ABSTRACT

Background: The aim of this study was to compare the level of LRG1 in epithelial ovarian cancer (EOC) cases with benign ovarian masses and to evaluate results in relation to CA125.
Methods: An observational prospective controlled study was done on 70 patients admitted to El Shatby Maternity University Hospital, Oncology department categorized as follows: study group (group I) included 35 patients, with epithelial ovarian malignancy confirmed by histopathological examination and control group (group Il) included 35 patients with benign ovarian tumors confirmed by histopathological examination. Determination of Serum LRGI level by using enzyme-linked immuno sorbent assay with CA125 tumor marker analysis were done for all cases of both groups.
Results: As regard comparison between the two studied groups according to CA125 and LRGI. CA125 in group I ranged from 14.90 to 4600 with a mean value 856.73±1104.03, in group II ranged from 7.45 to 523 with a mean value of 51.97±86.14. LRGI in group I ranged from 62.46 to 653.98 with a mean value of 130.86±119.78, in group Il ranged from 47.73 to 261.78 with a mean value of 77.35±38.75. There was statistically significant difference between the two studied groups regarding CA125 and LRGI ($p \leq 0.05$).
Conclusions: LRG1 can be used as promising tumor marker to diagnose epithelial malignant ovarian cancer with or without CA125 tumor marker as it was significantly higher in epithelial ovarian cancer patients.

Keywords: Epithelial ovarian tumors, Leucine-rich alpha-2-glycoprotein-1

INTRODUCTION

Ovarian cancer is the 8th most common cancer in woman and the 18th most common cancer in general.[1] Most ovarian cancer are diagnosed lately due to it is commonly asymptomatic or unspecific symptomatology and the absence of reliable screening test.[2] The need of a specific and a sensitive marker that can be done periodically to screen ovarian cancer in its early stages is of great importance as this can reduce mortality from this common cancer. Leucine-rich-alpha-2-glycoprotein1 (LRG1), a membrane-associated leucine-rich repeat (LRR) family member, was separated from human serum by Haupt and Baudner in 1977 and overregulated by pro inflammatory cytokines in many types of inflammation as in appendicitis, ulcerative colitis and many other types of inflammation.[3-5] In addition, LRG1 serum level has been shown to be elevated in many types of cancer as in pancreatic cancer, gastric cancer, hepatocellular carcinoma and leukemia.[6-9] But its role in epithelial ovarian cancer need to be evaluated. Many studies showed that LRG1 promotes carcinogenesis through different mechanisms as neovascularization, cell migration, cell adhesion, cell invasion.[10,11] In the present

study, the authors compare the level of LRG1 in epithelial ovarian cancer (EOC) cases with benign ovarian masses and to evaluate results in relation to CA125. The aim of this study is to compare the level of LRG1 in epithelial ovarian cancer (EOC) cases with benign ovarian masses and to evaluate results in relation to CA125.

METHODS

Following approval by our institutional ethics committee, an observational prospective controlled study was done on 70 patients admitted to El Shat by Maternity University Hospital, Oncology department categorized as follows: study group (group I) included 35 patients, with epithelial ovarian malignancy confirmed by histopathological examination and control group (group II) included 35 patients with benign ovarian tumors confirmed by histopathological examination. Exclusion criteria included causes of elevated CA 125 as pregnancy, endometriosis, uterine fibroids, pelvic inflammatory disease, and causes of elevated LRGI including heart failure, hepatic metastasis, associated colorectal malignancy. Informed consent was obtained from all eligible women before starting the study. Biopsy and histopathological examination from ovarian masses were taken from all patients in both groups by surgery as in patients managed by surgical staging, cytoreductive surgery in malignant cases and CT guided biopsy in advanced unresectable malignant cases who were subjected later on to neoadjuvant chemotherapy or by laparotomy in benign cases. Determination of serum LRGI level by using enzyme-linked immuno sorbent assay with CA125 tumor marker analysis were done for all cases of both groups. Collection of result and data for both groups, followed by statistical comparison using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. The used tests were Chi-square test for categorical variables, to compare between different groups, Fisher's Exact, Student t-test for normally distributed quantitative variables, to compare between two studied groups, Mann Whitney test for abnormally distributed quantitative variables, to compare between two studied groups.

RESULTS

Table 1 shows histopathological examination of the 2 studied groups. Cases of group 1 are distributed histopathologicaly as follows: 24 cases were papillary serous adenocarcinoma, three of them where type 1 (low grade) and 21 cases were type 2 (high grade), 7 cases were mucinous adenocarcinoma and 4 were endometrioid adenocarcinoma. As regarding the distribution of cases in group 2, 11 cases were mucinous cystadenoma, 6 papillary serous cystadenoma, 6 serous cystadenofibroma, 4 mature cystic teratoma, 2 benign brenner-mucinous cystadenoma, 1 fibrothecoma, 1 sclerosing stromal tumor, 2 follicular cyst with luteinization, 1 mesothelial mesenteric cyst, 1 hemorrhagic luteal cyst.

Table 1: Distribution of the 2 studied groups according to histopathological result.

Histopathological result	Malignant (n=35)		Benign (n=35)	
	N	%	N	%
Pathology				
Papillary serous cystadenocarcinoma	24	68.6	-	-
Mucinous cystadenocarcinoma	7	20	-	-
Endometroid adenocarcinoma	4	11.4	-	-
Mucinous cystadenoma	-	0	11	31.4
Serous cystadenomafibroma	-	0	6	17.1
Mature cystic teratoma	-	0	4	11.4
Papillary serous cystadenoma	-	0	6	17.2
Benign brenner-mucinous cystadenoma	-	0	2	5.7
Fibrothecoma,	-	0	1	2.9
Sclerosing stromal tumor,	-	-	1	2.9
Follicular cyst with luteinization	-	-	2	5.7
Mesothelial mesenteric cyst	-	-	1	2.9
Hemorrhagic luteal cyst	-	-	1	2.9

Table 2 show comparison between the two studied groups according to CA125 and LRGI levels. CA125 levels in group I ranged from 14.90 to 4600 U/ml with a mean value 856.73±1104.03, in group II ranged from 7.45 to 523 with a mean value of 51.97±86.14. LRGI in group I ranged from 62.46 to 653.98 µg/ml with a mean value of 130.86±119.78, in group II ranged from 47.73 to 261.78 with a mean value of 77.35±38.75. There was statistically significant difference between the two studied groups regarding CA125 and LRGI ($p \leq 0.05$).

Table 2: Comparisons between the two studied groups according to CA125 and LRGI.

	Malignant (n=35)	Benign (n=35)	u	p
CA125				
Min.-Max.	14.90-4600.0	7.45-523.0		
Mean±SD	856.73±1104.03	51.97±86.14	83.50*	<0.001*
Median	352.0	31.0		
LRG1 concentration				
Min.-Max.	62.46 - 653.98	47.73-261.78		
Mean±SD	130.86±119.78	77.35±38.75	211.0*	<0.001*
Median	95.73	66.53		

U, P: U and p values for Mann Whitney test for comparing between the two groups, *: Statistically significant at $p \leq 0.05$

Table 3: Relation between grade with LRGI, RMI and CA125in malignant group (n=35).

	Grade			H	p
	Grade I (n=12)	Grade II (n=10)	Grade III (n=13)		
LRG1					
Min. – Max	62.46 – 653.98	63.47 – 113.50	75.91 – 455.20		
Mean±SD.	151.28±161.60	88.16±15.70	144.87±119.92	2.704	0.259
Median	103.31	84.55	98.03		
CA125					
Min. – Max	60.0 – 3295.0	42.0 – 4600.0	14.90 – 3000.0		
Mean±SD.	783.40±1059.70	765.63±1406.40	994.50±952.78	1.564	0.457
Median	316.50	167.0	626.0		

H, p: H and p values for Kruskal Wallis test

Table 4: Relation between stage with LRGI, RMI and CA125 in malignant group (n=35).

	Stage				H	p
	Stage I (n=15)	Stage II (n=15)	Stage III (n=3)	Stage IV (n=2)		
LRG1						
Min. – Max	62.46 – 163.66	79.11 – 653.98	82.35 – 112.27	73.80 – 78.04		
Mean±SD.	97.21±31.16	179.03±171.6	94.90±15.53	75.92±3.0	6.355	0.096
Median	90.7	100.35	90.07	75.92		
CA125						
Min. – Max	42.0 – 4600.0	149.0 – 3000.0	113.50 – 650.0	14.90 – 1366.0		
Mean±SD.	711.49±1346.98	1125.73±954.05	347.30±274.81	690.45±955.37	6.399	0.096
Median	244.0	875.0	278.40	690.45		

H, p: H and p values for Kruskal Wallis test

Table 5: Agreement (sensitivity, specificity) for LRG1.

	AUC	P	95% C.I		CUT OFF	Sensitivity	Specificity	PPV	NPV
			LL	UL					
LRG1	0.828*	<0.001*	0.730	0.925	78.04	80	74.29	75.7	78.8

AUC: Area under the curve, P value: Probability value, CI: Confidence intervals, PPV: Positive predictive value, NPV: Negative predictive value, *: statistically significant at P≤0.05.

Table 3 shows the relation between tumor grade and mean value of LRG1 and CA125 in malignant group, there was no statistically significant difference between tumor grade and the mean value of LRG1 nor CA125 in malignant group. Table 4 shows the relation between tumor stage and mean value of LRG1 and CA125 in malignant group, there was no statistically significant difference between tumor stage and the mean value of LRG1 nor CA125 in malignant group. Figure 1 show receiver operating characteristic (ROC) curve for LRG1

and Table 5 shows agreement (sensitivity and specificity) for LRG1 According to this agreement table, at cut off level 35 U/ML: The sensitivity was 97.14%, the specificity was 64.29%, the PPV was 68% and the NPV was 95%.

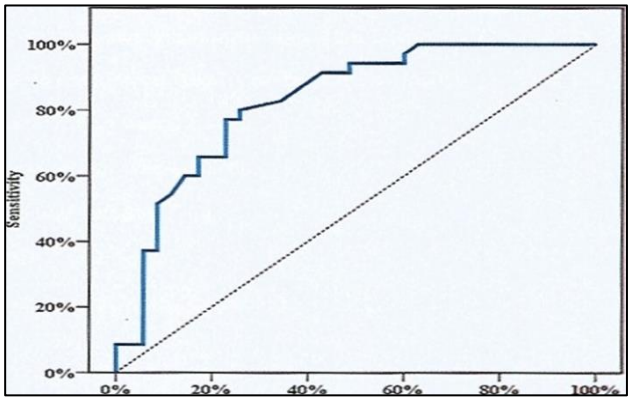

Figure 1: ROC curve for LRG1.

DISCUSSION

Ovarian cancer is the most common Fatal cancer of the familiar productive tract in industrialized countries, it is diagnosed annually in more Than 200,000 women worldwide, with the greatest incidence is united states and Northern Europe, and Lowest incidence in Africa And Asia.[12] It is considered the 5th cause of cancer related mortality in women with most cases are diagnosed in advanced stage.[13] The definitive diagnosis of an ovarian mass is a common problem in the gynecological practice, also the evaluation of pelvic ovarian masses regarding its nature whether benign or malignant masses constitutes one of the most important and challenging tasks facing the gyneoncologist. So, the needs of a soft marker that help the gynecologists to differentiate between Malignant and benign masses are of great importance. LRG1 is a new marker that is overexpressed in many others cancer can help to differentiate between malignant epithelial and benign ovarian masses.[6-9] The present study evaluates its sensitivity and specificity as a marker to differentiate between malignant epithelial ovarian cases and benign ovarian cases and the result was compared to CA125 known serum marker. Leucine-rich-alpha-2-glycoprotein1 (LRG1), a membrane-associated leucine-rich repeat (LRR) family member, was separated from human serum by Haupt and Baudner in 1977.[3] LRG1 is regulated by proinflammatory cytokines.[14] LRG1 has been shown to be expressed in many types of cancer, such as hepatocellular carcinoma, pancreatic cancer, gastric carcinoma, bladder cancer, leukemia but its role in ovarian cancer as a sensitive and specific marker need to be studied.[6-9,15] The present study investigated its serum level with ovarian cancer in a controlled prospective study and correlate its level with CA125 serum marker. The study included 35 patients with epithelial ovarian malignancy compared to 35 patients with epithelial benign ovarian tumors. The present study shows that there are a statistically significant difference regarding both LRG1 and CA125 between malignant epithelial ovarian tumor group and benign ovarian tumor group. In agreement with the present study, Andersen JD et al found that both markers are significantly elevated between both groups.[16] Also, Wu J et al study showed a similar result and recommended to use LRG1 as single marker or in combination with CA125 for the diagnosis of ovarian cancer.[17] The present study shows no correlation between tumor stages and grades in epithelial ovarian cancer group and serum levels of LRG1 and CA125 in contrast with other study as Wu J et al study which found that the levels of CA125 and LRG1 were higher in late stages than early stages and Anderson et al who found that LRG1 levels were significantly higher in late stages than early stages, this finding may be attributed to the few number of late stages cases in this study and the small number of malignant group cases so we recommend to increase number of cases in further study.[17,16] Analysis of ROC of LRG1 curve showed that the sensitivity, specificity, positive predictive value and negative predictive value at cut level 78.04ug/ml were 80%, 74.29%, 75.7% and 78.8% respectively. From the present study we can conclude that LRG1 can be used as tumor marker to diagnose epithelial malignant ovarian cancer with or without CA125.

CONCLUSION

LRG1 can be used as promising tumor marker to diagnose epithelial malignant ovarian cancer with or without CA125 tumor marker as it was significantly higher in epithelial ovarian cancer patients.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer J Clini. 2018;68(6):394-424.
2. Doubeni CA, Doubeni AR, Myers AE. Diagnosis and Management of Ovarian Cancer. Am Fam Physician. 2016;93(11):937-44.
3. Haupt H, Baudner S. Isolation and characterization of an unknown, leucine rich 3.1-S- alpha2-glycoprotein from human serum. Hoppe Seyler Z Physiol Chem. 1977;358(6):639–46.
4. Kentsis A, Ahmed S, Kurek K, Brennan E, Bradwin G, Steen H, et al. Detection and diagnostic value of urine leucine-rich α-2-glycoprotein in children with suspected acute appendicitis. Ann Emerg Med. 2012;60(1):78–83.
5. Serada S, Fujimoto M, Terabe F, Iijima H, Shinzaki S, Matsuzaki S, et al. Serum leucine-rich alpha-2

glycoprotein is a disease activity biomarker in ulcerative colitis. Inflamm Bowel Dis. 2012;18(11):2169–79.
6. Furukawa K, Kawamoto K, Eguchi H, Tanemura M, Tanida T, Tomimaru Y, et al. Clinicopathological Significance of Leucine-Rich α2-Glycoprotein-1 in Sera of Patients With Pancreatic Cancer. Pancreas. 2015;44(1):93–8.
7. Uen YH, Lin KY, Sun DP, Liao CC, Hsieh MS, Huang YK, et al. Comparative proteomics, network analysis and post-translational modification identification reveal differential profiles of plasma con a-bound glycoprotein biomarkers in gastric cancer. J Proteomics. 2013;83:197–213.
8. He X, Wang Y, Zhang W, Li H, Luo R, Zhou Y, et al. Screening differential expression of serum proteins in AFP-negative HBV-related hepatocellular carcinoma using iTRAQ -MALDI-MS/MS. Neoplasma. 2014;61(1):17–26.
9. Wu RC, Yu CS, Liu KC, Huang HY, Ip SW, Lin JP, et al. Citosol (thiamylal sodium) triggers apoptosis and affects gene expressions of murine leukemia raw 264.7 cells. Hum Exp Toxicol. 2012;31:771–9.
10. Zhang J, Zhu L, Fang J, Ge Z, Li X. LRG1 modulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer via HIF-1α activation. J Exp Clin Cancer Res. 2016;35:29.
11. Zhong D, He G, Zhao S, Li J, Lang Y,Ye W, Li Y, Jiang C, Li X. LRG1 modulates invasion and migration of glioma cell lines through TGF-beta signaling pathway. Acta Histochem. 2015;117(6):551–8.
12. Zavesky L, Jancarkova N, Kohoutova M. Ovarian cancer: origin and factors involved in carcinogenesis with potential use in diagnosis, treatment and prognosis of the disease. Neoplasma. 2011;58(6):457-68.
13. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2011;61(2):133-4.
14. Ladd JJ, Busald T, Johnson MM, Zhang Q, Pitteri SJ, Wang H, et al. Increased plasma levels of the apc-interacting protein mapre1, lrg1, and igfbp2 preceding a diagnosis of colorectal cancer in women. 2012;5(4):655-64.
15. Lindén M, Lind SB, Mayrhofer C, Segersten U, Wester K, Lyutvinskiy Y, et al. Proteomic analysis of urinary biomarker candidates for nonmuscle invasive bladder cancer. Proteomics. 2012;12(1):135–44.
16. Andersen JD, Boylan KL, Jemmerson R, et al. Leucine-rich alpha-2-glycoprotein-1 is upregulated in sera and tumors of ovarian cancer patients. J Ovarian Res. 2010;3:21.
17. Wu J, Yin H, Zhu J, Buckanovich RJ, Thorpe JD, Dai J, et al. Validation of LRG1 as a Potential Biomarker for Detection of Epithelial Ovarian Cancer by a Blinded Study. PLoS ONE. 2015;10(3):e0121112.

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