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The diagnostic/prognostic significance of serum Golgi phosphoprotein 3 in ovarian cancer

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ABSTRACT

Ovarian cancer (OC) is a serious healthcare problem and early detection is a fundamental stone in improving patient survival. Golgi phosphoprotein 3 (GOLPH3) is found to be increased in various cancers including OC. This study aimed to investigate GOLPH3 levels in OC patients and explore its clinical significance. A total of 68 females were enrolled in the study: 43 OC patients and 25 healthy volunteers as the control group, Peripheral blood samples were collected and serum GOLPH3, cancer antigen (CA) 125, and CA19-9 were measured using ELISA. The diagnostic value of GOLPH3, CA125, and CA19-9 was investigated by the receiver operating characteristic (ROC) curve. Serum GOLPH3 levels showed about a 3-fold increase in OC patients compared to healthy control subjects ($P< 0.001$). Advanced stages OC patients had higher GOLPH3 levels than early stages ($P= 0.006$). Also, higher GOLPH3 levels were detected in serous histological OC compared to non-serous subtypes ($P= 0.011$), linking GOLPH3 to OC aggressiveness and poor prognosis. Moreover, serum GOLPH3 showed positive associations with tumor grade and lymph node metastasis ($P< 0.01$) but not distant metastasis. Interestingly, there is a strong positive correlation between GOLPH3 and neutrophils-to-lymphocytes ratio ($r= 0.732$ at $P< 0.001$), indicating its possible link to inflammation, a key hallmark of cancer. In addition, serum GOLPH3 levels showed AUC and sensitivity superior to CA125 and CA19-9. In conclusion, this study highlights the role of GOLPH3 in OC as both a promising diagnostic biomarker for OC detection and a key contributor to disease prognosis.

Keywords: *Golgi phosphoprotein 3; Ovarian Cancer; CA125; CA19-9; neutrophils-to-lymphocytes ratio.*

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1. Introduction

Ovarian cancer (OC) is the primary cause of fatality among females diagnosed with gynecological cancers, as well as the fifth most common cause of death among women in general **[1, 2]**. According to the GLOBOCAN 2022 report, there were 3,070 new cases of OC in Egypt, placing it as the 12th most common cancer overall in the country. The 5-year prevalence rate for OC in Egypt was 16.5%, and the number of fatalities reached 1,944, resulting in a mortality rate of 2.1% **[1]**.

Early detection of ovarian cancer strongly correlates with the 5-year survival rate which is about 93% for patients diagnosed in stage I, but reaches below 15% in stage IV **[3, 4]**. Early detection is a challenge for gynecologists as only 15% are diagnosed at stage I and 75% of cases at late **[3, 5]**. The deficiency of reliable early detection markers and the propensity for tumor

metastasis significantly contribute to the high mortality rates associated with OC **[3, 6]**.

Cancer antigen (CA)125 is considered the primary tumor marker widely used for OC diagnosis. However, it has some features that limit its clinical use. It was found that about 20% of epithelial OC cases fail to show significantly high CA125 levels **[5, 7]**. Also, elevated CA125 levels were detected in benign diseases and some inflammatory diseases such as rheumatoid arthritis and lupus **[8]**. Moreover, pregnancy, menstruation, and cyclic hormonal therapy may be associated with elevated CA125 levels, and that limits its specificity [9,10]. In addition, it has low sensitivity in early OC stages **[8, 11]** Therefore, there is a pressing need to combine it with other biomarkers to improve its specificity and sensitivity to reduce OC mortality associated with late diagnosis.

Golgi phosphoprotein 3 (GOLPH3), a recently identified 34-kDa phosphorylated matrix protein, is a crucial player in the Golgi secretory pathway and DNA damage signaling **[12]**. Levels of GOLPH3 were found to be low in normal and benign tissues in contrast to high levels in malignant OC **[13, 14]**, suggesting its possible diagnostic value in OC. In addition, recent studies have linked GOLPH3 to cancer progression, including adverse prognosis in various cancer types, including OC **[13, 15, 16]**. However, the specific biological role and underlying mechanisms of GOLPH3 in OC as well as its correlation with different clinicopathological features in OC remain incompletely understood.

The inflammatory microenvironment significantly influences the pathogenesis of cancers including OC and monitoring inflammatory responses is essential for OC prognosis **[17–19]**. Being the largest group of inflammatory cells, numerous subtypes of white blood cells such as neutrophils and lymphocytes are utilized as fundamental inflammatory biomarkers **[20, 21]**. Also, several inflammatory indices like platelets-to-lymphocytes ratio, neutrophile-to-lymphocytes ratio (NLR) and others are widely used inflammatory markers with better clinical significance than single ones in OC **[18, 22]**. A study by *Templeton et al* revealed that high NLR levels independently predict overall survival and disease-free survival in many solid tumors **[23]**. Although GOLPH3 was found to be linked to inflammation in various cancers through activation of nuclear factor (NF)-kappaB, and mTOR signaling pathways **[24, 25]** its contribution to inflammatory response as well as its link to NLR in OC has not addressed yet.

We aimed to comprehensively investigate the involvement of Golgi-associated proteins; GOLPH3 in OC pathogenesis. The primary objectives include evaluating the serum levels of GOLPH3 in OC patients compared to controls and investigating its correlation with clinical pathological parameters such as stage, grade, and lymph node involvement. Additionally, we aimed to assess the diagnostic utility of serum GOLPH3 levels in discriminating between OC patients and controls. Also, we assessed the association between GOLPH3 and NLR as a marker of inflammation in OC patients.

2. Subjects and Methods

2.1. Sample size calculation

Using an online sample size calculator https://clincalc.com/stats/samplesize.aspx and based on a previous study **[14]**, the number of cases in two independent study groups with a ratio 1:1 required to achieve a power of 90% and a type I error alpha < 0.5 is only 26 cases in study groups.

2.2. Study design

This study is a case-controlled retrospective mono-center study.

2.3. Ethical approval and informed consent

This study was approved by the ethical committees of the Faculty of Pharmacy, Ain Shams University (REC no.241; 8 May 2019) and El-Demerdash Hospitals, Cairo, Egypt. We adhered to the regulations and recommendations of the Declaration of Helsinki (WMA, 2013). Every subject signed an informed consent before participating in the study and after informing them of the study objectives.

2.4. Clinical and pathological features of OC patients

The **inclusion criteria** for patients were adult females with pathologically confirmed OC. The **exclusion criteria** include subjects having blood diseases, chronic liver diseases, receiving chemotherapy or radiotherapy, chronic renal diseases, or subjects having tumors other than OC.

Tumor clinical evaluation of OC patients was done by El-Demerdash Hospitals, Faculty of Medicine, Ain Shams University, Egypt using tumor-node-metastasis (TNM) staging and FIGO staging system. The data of histopathological subtypes, tumor grade, tumor stage, tumor size, and lymph node metastasis (LNM) were obtained from patient pathological reports.

From patients' files, the following parameters were obtained, CA125, CA19-9, neutrophils, and lymphocytes absolute numbers. The latter two parameters were used to calculate NLR, an inflammatory. The index of NLR is calculated by dividing the number of neutrophils (cells/mm^3) by the number of lymphocytes (cells/mm^3). In addition, patient demographic data as age, weight, and height as well as detailed family and medical history were obtained from their files.

2.5. Study subjects

A total of 68 females enrolled in the study: 43 OC patients and 25 healthy volunteers as the control group. The patients group has a median age of 51.05 (47.00-59.00) years while the control group has a median age of 50.60 (39.50-57.00) years. The OC patients undergoing oophorectomy were recruited from the Gynecology and Obstetrics Surgery Department at El-Dermerdash Hospitals, Ain Shams University.

2.6. Blood sampling

Peripheral blood samples were obtained from OC subjects just before undergoing oophorectomy surgery and after overnight fasting from the healthy control subjects. Blood samples were collected on plain vacutainer tubes, following standard international biosecurity safety procedures. Collected blood samples were allowed to clot by standing for 15 min at 25 °C. After 15 min., clotted blood was centrifuged for 15 min. at 4000 rpm and supernatant was collected. Sera were aliquoted and stored at -80 °C till required.

2.7. Quantification of serum GOLPH3

Serum levels of GOLPH3 were determined using an enzyme-linked immunosorbent assay (ELISA) kit provided by BT-LAB with a sensitivity of 0.11ng/mL, detection range of 0.2-60.0 ng/mL, and inter-assay precision of CV< 10% (kit catalog no. BT-E2724Hu). Briefly, 40 µL sample and 10 µL human GOLPH3 antibody were added to the appropriate well of the pre-coated plate. Then, 50 µL of peroxidase was added to each well but not to blank wells. After 60 min incubation at 37 °C, the wells were washed 5 times with 300 µL wash buffer. After that, 50 µL of provided substrate A solution and 50 µL of substrate B solution were added. After 10 min incubation at 37 °C, 50 µL stop solution was added and the absorbance was measured at 450 nm.

2.8. Statistical Analysis

The data was assessed for normality using the Kolmogorov–Smirnov test with SPSS 23.0 statistical software for social sciences (IBM, Armonk, NY). Numerical parameters were non-parametric and expressed as median (interquartile range). Nonparametric unpaired Student's t-test (Mann-Whitney U) was used to compare between two independent groups. While categorical data were expressed as numbers (percentages) and compared using the Chi-square test. Correlations between continuous parameters were performed using Spearman's ranked coefficient r and point-biserial correlation if one of the parameters is dichotomous.

GraphPad Prism Version 9 (GraphPad Software, San Diego, USA) and Microsoft Excel 2019 were utilized for the graphical representation of the data. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the optimal cutoff points and calculate sensitivities and specificities, along with the area under the curve (AUC). The ROC curve analysis was carried out using MedCalc Statistical Software version 19.2.6 (MedCalc Software by Ostend, Belgium). *P*-values were two-tailed and considered statistically significant if it is ≤ 0.05.

3. Results

3.1. Study populations characteristics

The clinical data of the studied groups are outlined in **Table 1**. Results revealed no significant difference between control healthy volunteers and OC patients regarding age, BMI, or menopause status. On the contrary, there was a significant difference between the studied groups regarding family history at $P= 0.003$.

3.2. Serum GOLPH3, traditional protein OC markers (CA125 and CA19-9) and NLR

As shown in **Table 1**, OC patients showed significantly elevated levels of serum GOLPH3 compared to the control group by about 2.4-fold (9.30 ng/mL versus 3.93 ng/mL, at $P< 0.001$) (**Fig 1A**). Both CA125 and CA19-9 were significantly increased in the patient group than the control group with a median of 117.60 and 22.99 U/mL versus 10.54 and 9.31 U/mL, respectively at $P< 0.001$. In addition, OC patients had significantly higher levels of NLR (3.45) than the control group (1.43) at $P< 0.001$; indicating the prevalence of systemic inflammation in the OC population.

Table 1. Study participants' demographic and clinical data

	Groups		
Groups Characteristics	**Control (n=25)**	**Malignant (n=43)**	**Statistics**
Age (years)	50.60 (39.50-57.00)	51.05 (47.00-59.00)	U= 419.00, P^2= 0.131
<50 (n, %)	(12, 48.0%)	(16, 37.2 %)	χ^2 = 0.760, P^1= 0.448
≥50 (n, %)	(13, 52.0%)	(27, 62.8%%)	
BMI (Kg/m^2)	25.65 (24.70-26.40)	25.78 (24.54-28.26)	U= 442.00, P^2= 0.227
<25 (n, %)	(12, 48.0%)	(19, 44.2%)	χ^2 = 0.093, P^1 = 0.761
≥25 (n, %)	(13, 52.0%)	(24, 55.8%)	
Menopause			χ^2 = 0.655, P^1 =0.458
Yes (n, %)	(13, 52.0%)	(25, 58.1%)	

No (n, %)	(12, 48.0%)	(18, 41.9%)	
Family history			
Yes (n, %)	(0, 0.0%)	(12, 27.9%)	χ^2 = 8.472, P^1 =0.003**
No (n, %)	(25, 100.0%)	(31, 72.1%)	
NLR	1.43 (1.33-1.65)	3.45 (2.52-5.64)	U= 32.00, P^2< 0.001**
CA 125 (U/mL)	10.53 (7.37-17.46)	117.60 (27.29 – 262.30)	U= 114.00, P^2< 0.001**
CA 19-9 (U/mL)	9.21 (3.01-13.85)	22.99 (14.34-102.07)	U= 223.50, P^2< 0.001**
Serum GOLPH3 (ng/mL)	3.93 (3.19-4.27)	9.30 (6.74- 26.66)	U= 33.00, P^2< 0.001**
Histological status			
Serous (n, %)	-	(24, 55,8%)	*NA*
Non-serous (n, %)	-	(19, 44.2%)	
FIGO stage			
Early stage I, II (n, %)	-	(17, 39.5%)	*NA*
Late stage III, IV (n, %)	-	(26, 60.5%)	
Tumor grade			
G-I/II (n, %)	-	(18, 41.9%)	*NA*
G-III (n, %)	-	(25, 58.1%)	
Ovarian involvement			
Unilateral (n, %)	-	(21, 48.8%)	*NA*
Bilateral (n, %)	-	(22, 51.2%)	
Tumor size (cm)			
< 10 (n, %)	-	(13, 30.2%)	*NA*
≥ 10 (n, %)	-	(30, 69.8%)	
LNM			
Absent (n, %)	-	(20, 46.5%)	*NA*
Present (n, %)	-	(23, 53.5%)	
Distant Metastasis			
Absent (n, %)	-	(36, 83.7%)	*NA*
Present (n, %)	-	(7, 16.3%)	

Categorical data are expressed as numbers (percentages) and numerical data are expressed as median (25^{th}-75^{th} percentiles)
P^1; indicates a comparison between studied groups using the Chi-square test (X^2),
P^2; indicates the comparison between studied groups using the Mann-Whitney (U) test,
NA; not-applicable
[BMI, Body mass index; CA, Carcer antigen; GOLPH3, Golgi phosphoprotein 3; LNM, Lymph node metastasis; NLR, Neutrophil-to-lymphocytes ratio]
*; statistically significant at P< 0.05 level
**; statistically significant at P< 0.01 level

3.3. Serum GOLPH3 levels in OC stratified subgroups

The OC group was stratified into various subgroups with regard to age, BMI, menopause status, family history, histopathological OC subtypes, and other pathological features, as depicted in **Table 2**. With respect to age (< or ≥ 50 years) and menopause state, the levels of serum GOLPH3 did not differ significantly. Results showed significantly increased serum GOLPH3 levels in patients whose BMI ≥ 25 Kg/m^2 compared to those with BMI< 25 Kg/m^2. Also, higher GOLPH3 levels were detected in patients with a positive family history than those without a family history at P= 0.004.

According to the histopathological subtype of OC, serous OC patients showed significantly higher levels of GOLPH3 than non-serous OC patients (19.21 versus 7.45 ng/mL at *P*= 0.011) (**Fig. 1B**). In addition, patients with early FIGO stages (I and II) had decreased GOLPH3 levels compared to advanced stages (III and IV) patients (6.61 versus 22.00, at *P*< 0.001) (**Fig. 1C**). Regarding tumor grades, there is statistically significant increase in serum GOLPH3 levels in grade III OC patients in comparison with grade I and II patients (21.51 versus 7.53 at *P*= 0.004), as illustrated in **Fig 1D**.

Table 2. Serum levels of GOLPH3 in ovarian cancer patients stratified subgroups

Characteristics	n (%)	Median (percentiles)	*P* value
Age (years)			
<50	16 (37.2 %)	8.67 (7.06-26.97)	0.940
≥50	27 (62.8%)	9.74 (6.81-24.50)	
BMI (Kg/m^2)			
<25 (n, %)	19 (44.2%)	7.61 (6.53-9.61)	0.007**
≥25 (n, %)	24 (55.8%)	19.21 (8.72-28.43)	
Menopause status			
Pre-menopause	18 (41.9%)	8.67 (6.86-26.66)	0.370
Post-menopause	25 (58.1%)	9.74 (7.27-25.50)	
Family History			
No	31 (72.1%)	8.45 (6.32-16.92)	0.004**
Yes	12 (27.9%)	26.81 (9.72-30.01)	
Histology status			
Serous	24 (55,8%)	19.21 (8.35-28.43)	0.011*
Non-serous	19 (44.2%)	7.45 (6.32-11.88)	
FIGO Stage			
I/II	17 (39.5%)	6.61 (5.22-8.55)	< 0.001**
III/VI	26 (60.5%)	22.00 (9.42-28.73)	
Tumor grade			
G I/II	18 (41.9%)	7.53 (6.05-13. 50)	0.004**
G III	25, (58.1%)	21.51 (8.45-28.96)	
Tumor size (cm)			
< 10	13 (30.2%)	6.30 (5.77-9.64)	0.006**
≥ 10	30 (69.8%)	16.07 (8.14-27.40)	
LNM			
Absent	20 (46.5%)	8.67 (5.50-9.46)	0.003**
present	23 (53.5%)	21.51 (9.54-29.20)	
Distant metastasis			
No	36 (83.7%)	9.55 (6.60-25.99)	0.062
Yes	7 (16.3%)	22.51 (13.54-28.50)	
NLR			
< 3 (n, %)	17 (39.5%)	6.53 (5.21-8.45)	< 0.001**
≥ 3 (n, %)	26 (60.5%)	22.00 (9.42-28.73)	

Categorical data are expressed as numbers (percentages) and numerical data are expressed as median (25^{th}-75^{th} percentiles) [BMI, Body Mass Index; GOLPH3, Golgi phosphoprotein 3; LNM, Lymph node metastasis; NLR, Neutrophil-to-lymphocytes ratio]. *; statistically significant at *P*< 0.05 level. **; statistically significant at P< 0.01 level

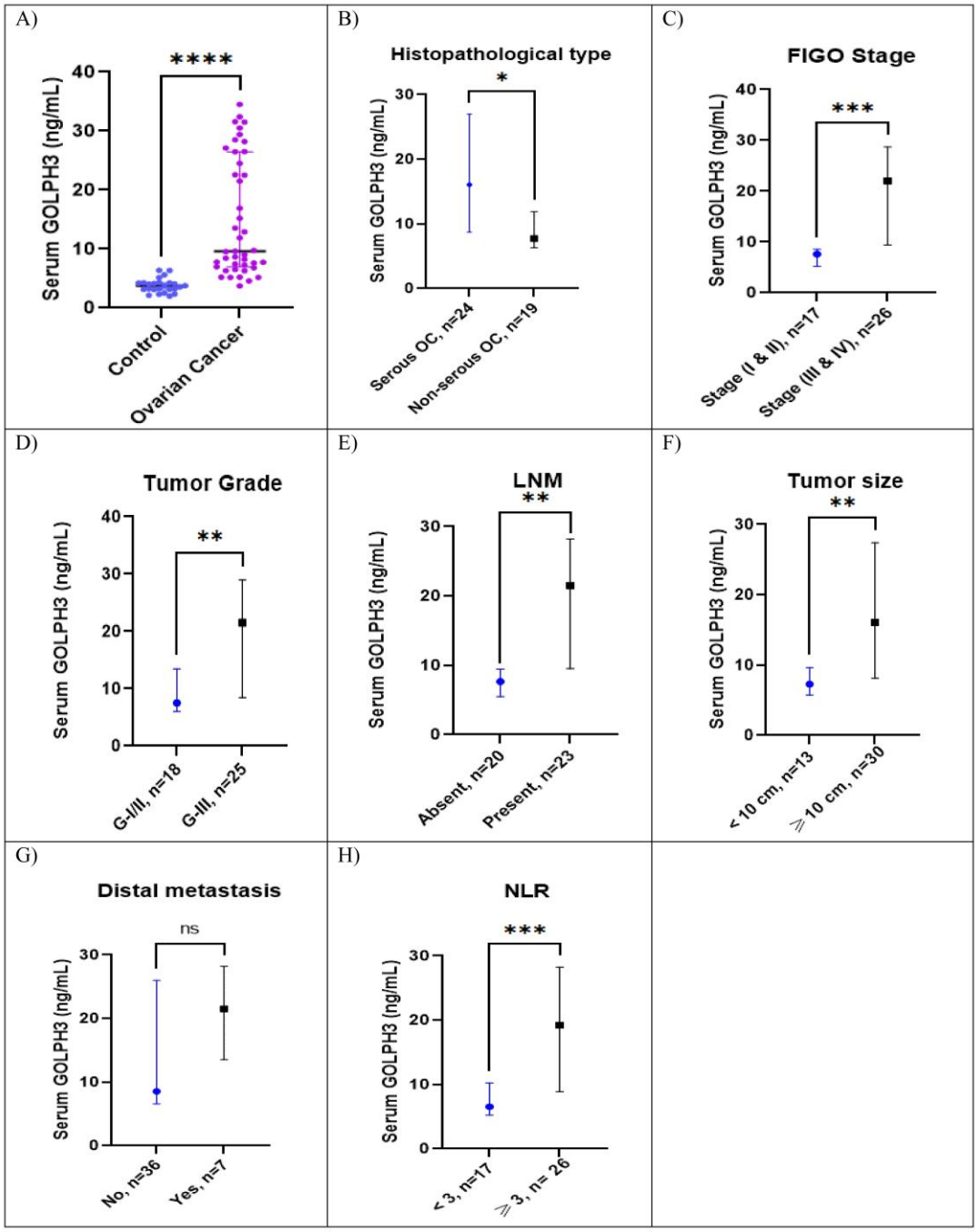

Fig. 1. Levels of serum GOLPH3 (ng/mL) in (A) studied groups, (B-H) different ovarian cancer stratified groups. Ovarian cancer patients are stratified with respect to histological type (B), FIGO stage (C), tumor grade (D), lymph node metastasis (LNM) (E), tumor size (F), distant metastasis (G) and neutrophil-to-lymphocytes ratio (NLR) (H). Data are expressed as median (25^{th}-75^{th} percentiles) and analyzed by Mann-Whitney (U) test using SPSS and graphically plotted using GraphPad Prism 9.0.0 *, **, ***, **** statistically significant at $P < 0.05$, 0.01, 0.001, 0.0001, respectively.

Subgrouping of OC patients based on LNM, patients with confirmed LNM (n= 23) showed elevated levels of serum GOLPH3 than patients without LNM (n= 20) at P= 0.003 (**Fig 1E**). Also, patients whose tumor size was 10 ≥ cm had higher GOLPH3 levels than those with tumors <

10 cm (16.07 versus 6.31ng/mL at *P*= 0.006 (**Fig 1F**). On the contrary, there is no significant difference in GOLPH3 levels between patients with or without distant metastasis (**Fig 1G**).

In addition, OC patients whose NLR ≥ 3 (n= 26) had increased levels of GOLPH3 than those with NLR < 3 (n= 17) at *P*< 0.001, as shown in **Fig 1H**. This highlights the possible link between GOLPH3 and systemic inflammation, a crucial hallmark of cancer.

3.4. Correlation analysis between serum GOLPH3 and clinical data

As summarized in **Table 3**, serum GOLPH3 showed significant positive correlations with BMI and positive family history (*r*= 0.480 and 0.439, respectively). Also, GOLPH3 levels are positively correlated significantly with serous histological OC status, advanced FIGO stages, and higher tumor grades (*r*= 0.394, 0.560, and 0.422 at P= 0.009, 0.001, and 0.003, respectively). In addition, a positive correlation between GOLPH3 and LNM (*r*= 0.462 at *P*= 0.002) was reported. Correlation analysis revealed a significant strong correlation between serum GOLPH3 levels and NLR (*r*= 0.732 at *P*< 0.001). Conversely, there was no discernible correlation between GOLPH3 and either age, menopausal status, tumor size, or distant metastasis.

Table 3. Correlation between serum GOLPH3 levels and clinicopathological parameters in OC patients (n= 43)

	Correlation coefficient (*r*)	***P*- value**
Age (years)	- 0.109	*0.486*
BMI (Kg/m^2)	0.480**	*<0.001*
Menopause Status	0.053	*0.735*
Family history	0.439*	*0.003*
Histological status	0.394*	*0.009*
FIGO Stage	0.560**	*<0.001*
Tumor grade	0.422*	*0.003*
Tumor size	0.318	*0.052*
LNM	0.462*	*0.002*
Distant metastasis	0.290	*0.090*
NLR	0.732**	*<0.001*

Correlation is significant at P< 0.01 level (*) or < 0.001 (**) (2-tailed). [BMI, Body Mass Index; GOLPH3, Golgi phosphoprotein 3; LNM, Lymph node metastasis; NLR, Neutrophil-to-lymphocytes ratio]

3.5. Diagnostic significance of serum GOLPH3 and traditional protein tumor markers (CA125 and CA19-9)

As illustrated in **Fig 2** and **Table 4**, serum GOLPH3 levels showed excellent diagnostic utility to discriminate between OC patients and

healthy controls at a cut-off value of 4.31 ng/mL (AUC= 0.969, sensitivity= 97.7%, specificity= 84.0%, and confidence interval (CI): 0.896 - 0.996). This diagnostic value of serum GOLPH3 is superior to what was observed for both CA125 and CA19-9, whose AUC= 0.894 and 0.790, respectively, while their sensitivities= 81.4 and 76.7%, respectively.

Results revealed a significant improvement of AUC after combining serum GOLPH3 with either CA125 or CA19-9 (AUC= 0.987 and 0.981, sensitivity= 97.7% and 95.0% while specificity= 96.0% and 100.0%, respectively). Such improvement did not appear upon combining serum CA125 and CA19-9. On the other hand, combining the 3 markers achieved much improvement in diagnostic value for differentiating between the OC group and control group (AUC= 0.993, sensitivity= 97.7%, and specificity= 100%, and confidence interval (CI): 0.935 – 1.000). Therefore, adding serum GOLPH3 to traditional tumor markers might be a promising panel to diagnose OC.

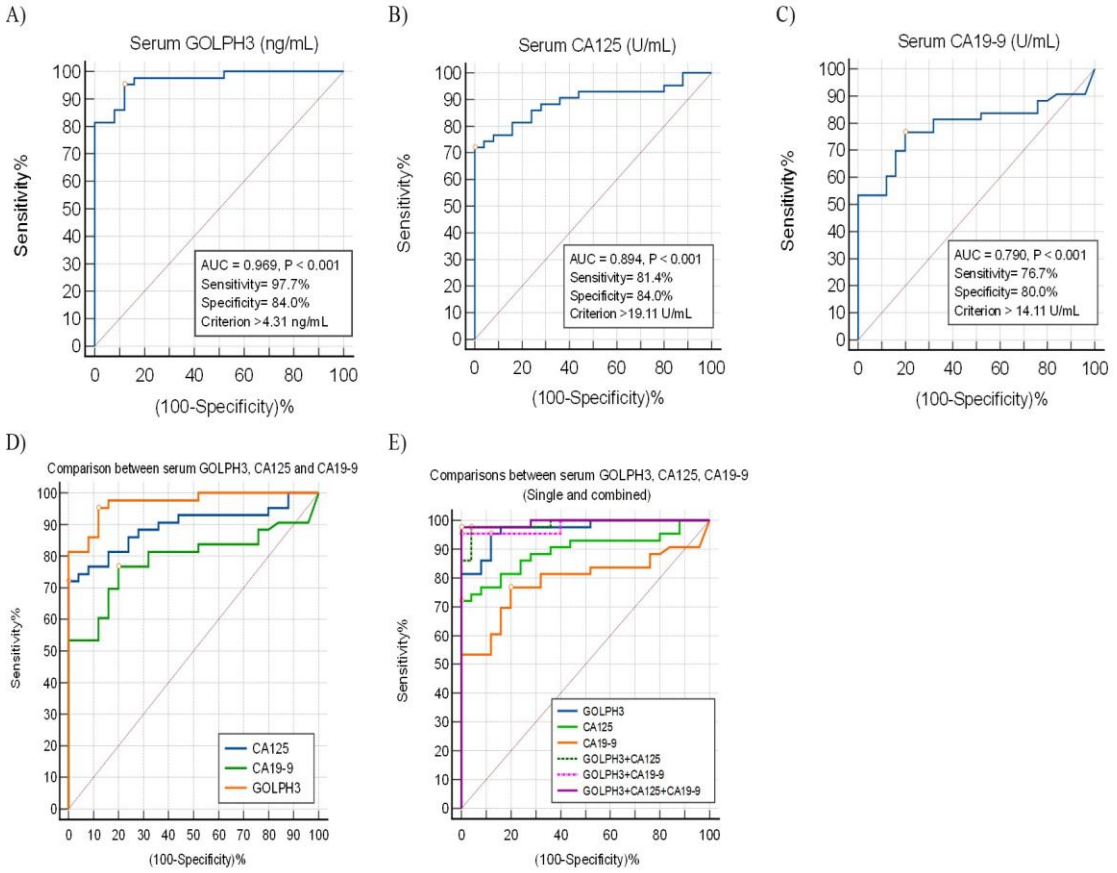

Fig. 2. ROC curve analysis for the diagnostic value of serum GOLPH3 and serum traditional tumor markers (CA125 and CA19-9) to discriminate between ovarian cancer patients (n= 43) and the control group (n= 25). (A) serum GOLPH3, (B) serum CA125, (C) serum CA19-9, (D) comparison between the 3 parameters in single forms, and (E) comparison between the 3 parameters in single and combined forms. Analyzed and plotted using MedCalc Software version 19.2.6

Table 4. Receiver-operating characteristic (ROC) curve analysis, sensitivities, and specificities for serum GOLPH3, CA125, and CA19-9 in ovarian cancer patients (n= 43) versus control group (n= 25)

Markers	Cut-off value	AUC	S.E.M	*P*-value	95% CI	Sensitivity %	Specificity %
GOLPH3 (ng/mL)	> 4.31	0.969	0.017	< 0.0001	0.896 - 0.996	97.7	84.0
CA125 (U/mL)	> 19.11	0.894	0.039	< 0.0001	0.795 - 0.956	81.4	84.0
CA19-9 (U/mL)	> 14.11	0.790	0.055	< 0.0001	0.674 - 0.880	76.7	80.0
GOLPH3+CA125	--	0.987	0.010	< 0.0001	0.924 -1.000	97.7	96.0
GOLPH3+CA19-9	--	0.981	0.014	< 0.0001	0.914 - 0.999	95.0	100.0
CA125+CA19-9	--	0.924	0.032	< 0.0001	0.833 -0.974	86.1	92.0
GOLPH3+CA125+CA19-9	--	0.993	0.007	< 0.0001	0.935 - 1.000	97.7	100.0

Data were obtained from the ROC curve analysis using MedCalc software. [AUC, Area under the curve; CI, Confidence interval; S.E.M, Standard error of means]

4. Discussion

Early detection of ovarian cancer is essential for successful management of this disease and strongly correlates with the 5-year survival rate which is 90% for patients diagnosed at stage I, but below 45% in late stages. Early detection is a challenge for gynecologists as only 15% are diagnosed at stage I and 75% of cases at late stages **[3, 4, 26]**. Extensive evidence highlights the significant role of intracellular vesicle trafficking deregulation in various aspects of cancer phenotypes **[27, 28]**. Among the proteins associated with this process, GOLPH3 stands out as the first example of an oncoprotein resident in the Golgi apparatus **[12, 29]**. Also, GOLPH3 has been identified as a potential oncogenic player in various cancers including OC **[13, 15, 16]**. However, its specific role and association with underlying mechanisms and clinicopathological features in OC are not yet fully understood. More research is required to illustrate the GOLPH3 biological functions in OC, which could potentially lead to the development of target therapies and improved outcomes for patients with this devastating disease.

The current study demonstrated significantly elevated serum levels of GOLPH3 in OC patients compared to the control group. This comes following the previous study by *Fan et al.*, **[14]** who reported increased serum GOLPH3 levels in Chinese OC women. However, our study is the first study to investigate GOLPH3 levels in the Egyptian population. Also, serum levels of GOLPH3 in OC patients showed a significant increase based on histopathological subtypes manifested as significant elevation in serous OC patients versus other non-serous subtypes. This finding was also reinforced by a positive correlation between elevated GOLPH3 levels and serous subtypes. These findings might point to the possible link between the upregulation of GOLPH3 and aggressiveness of OC as serous OC is associated with increased metastasis risk and elevated death rates than other subtypes **[30]**.

Based on clinicopathological features of OC patients, serum GOLPH3 was elevated in patients with advanced FIGO stages compared to patients with early stages. Furthermore, higher serum GOLPH3 levels were detected in OC patients with poor tumor differentiation than those who had well or moderated-differentiated tumors. In addition, OC patients with LNM had elevated GOLPH3 levels than those without LNM. Correlation analyses also revealed that serum GOLPH3 levels were positively correlated with advanced FIGO stages, poor tumor differentiation, and LNM but not distant metastasis; indicating a crucial role of GOLPH3 in OC progression and suggesting its possible use as a prognostic marker for OC.

The reported correlation between GOLPH3 and LNM could be attributed to the role of GOLPH3 in regulating cell-cell interactions via

protein trafficking and protein glycosylation and subsequent induction of cancer cell migration and invasion **[12, 28]**. A study by *Sun et al*., **[13]** reported that downregulation of GOLPH3 decreased the migration and invasion in several OC cell lines. In addition, GOLPH3 is linked to tumor invasion by upregulation of matrix metalloproteinases and Wnt/β-catenin signaling pathway in different types of cancer **[31, 32]**.

We also reported a significant correlation between serum GOLPH3 and family history of OC. In addition, patients with OC family history showed elevated levels of serum GOLPH3 compared to OC patients without such a history. Family history plays a significant role in increasing the risk of developing OC, particularly in those with close relatives. Some mutations in genes like BRCA1 and BRCA2 increase the likelihood of OC and other cancers like breast cancer **[33, 34]**. Such a relation between serum GOLPH3 and OC family history needs further investigations on a larger population as serum GOLPH3 might be used as a screening tool in female relatives of OC patients.

This study also revealed an interesting finding, the strong positive correlation between GOLPH3 and NLR, an index of systemic inflammation. Accumulating evidence has shown that NLR is an independent predictor of poor prognosis in many solid tumors **[23]**. To our knowledge, this is the first study to report such a correlation between GOLPH3 and NLR in OC, highlighting the possible impact of this oncoprotein in the pathogenesis of OC. The contribution of GOLPH3 to inflammation could be explained by its role in upregulating several pathways such as NF-kappaB, mTOR, and Wnt/β-catenin signaling pathways **[24, 25, 32]**. Therefore, the relationship between GOLPH3 and inflammation in OC warrants further investigation.

Traditional protein tumor markers including CA125 and CA19-9 are widely used to diagnose OC, however, their diagnostic performance faces some drawbacks such as low early diagnostic rates and elevated levels in non-malignant diseases. Therefore, finding another diagnostic tumor marker is an urgent need to improve the sensitivities and specificities of current biomarkers. ROC curve analysis showed that serum levels of GOLPH3 had the highest AUC, sensitivity, and specificity compared to both serum CA125 and CA19-9 levels in discriminating between OC and healthy controls. In addition, combining GOLPH3 with either CA125 or CA19-9 caused significant improvement in their diagnostic value compared to combining them. These observations suggested the potential use of serum GOLPH3 in combination with other traditional OC tumor makers as a non-invasive diagnostic tool for early detection of OC, an assumption that needs more investigation.

Conclusions

In conclusion, this study revealed an elevation in serum GOLPH3 in OC patients. Serum levels of GOLPH3 were correlated with poor prognosis of OC manifested as advanced FIGO stages, poorly differentiated tumor grades, and lymph node metastasis. To our knowledge, this is the first study to report the link between GOLPH3 and NLR in OC. In addition, combining GOLPH3 with traditional protein tumor markers (CA125 and CA19-9) might provide a surrogate diagnostic panel for OC detection. Finally, the study portrays the diagnostic/prognostic role of GOLPH3 in OC and warrants further mechanistic studies.

Recommendations

Based on our study findings, we recommend conducting longitudinal studies to assess the

dynamic changes in biomarker levels during the course of OC, including pre- and post-treatment measurements. This could provide insights into the prognostic value of these biomarkers. Also, validating the study results in a multicenter setting with a larger and more diverse cohort to ensure the generalizability and robustness of the findings.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of the Faculty of Pharmacy, Ain Shams University (No. 241; 8/5/2019).

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in the main manuscript.

Competing interests

The authors declare that they have no competing interests.

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