Clinical Utility of Serum Glycodelin as a Novel Marker for Ovarian Cancer

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Abstract: Ovarian cancer is a cancerous growth arising from different parts of the ovary. It has been called "Silent killer" because it frequently causes non-specific symptoms. CA- 125 was the first tumor marker available for detection of ovarian cancer but the major problem is its poor sensitivity and specificity as it is elevated in only 40-50 % of patients with stages I and II ovarian cancer. Glycodelin consists of 180 amino acid and it is a glycoprotein belonging to the lipocalin family. In the present study, ELISA technique is utilized for the selective detection of Glycodelin. The detection of Glycodelin, utilizing ELISA technique, indicated a significantly high glycodelin levels in group one (ovarian cancer) as compared to either control group or benign ovarian disease group. Glycodelin was found to be highly sensitive as well as an early diagnostic marker for ovarian cancer. The utilization of the Glycodelin detection in addition to the current and standard tests available for diagnosis of an early and recurrent ovarian cancer would significantly improve the ability to identify patients who might be missed by the current diagnostic strategies and thus might provide a better therapeutic outcome.
[Ibrahim H. A. Badr, Helmy M. El Sayed, Hala Abdel Al, Mohamed S. Hegab. **Clinical Utility of Serum Glycodelin as a Novel Marker for Ovarian Cancer.** *Life Sci J* 2013;10(3):664-670]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 97

Keywords: Benign ovarian diseases, Glycodelin, Ovarian cancer, CA-125, Progesterone.

1. Introduction

Ovarian cancer is a malignancy that arises from various different cells within the ovaries. It is the fifth leading cause of death from cancer. It has been called "Silent killer" because it frequently causes non-specific symptoms, which contribute to its diagnostic delay, resulting in a late stage diagnosis and subsequent poor prognosis **(Anderiesz and Michael 2003).**

A tumor marker is generally defined as an antigen or protein which is secreted by the tumor itself or in response to its presence. This tumor marker is sometimes found in blood, other body fluids, or tissues. The detection of the tumor marker may suggest the presence of a certain type of cancer **(Sindransky, 2002).** Many tumor markers are found to be linked to ovarian tumor such as: CA-125, CA19-9, carcinomabryonic antigen (CEA), carbohydrate antigen 15-3 (CA15-3), cancer associated serum antigen (CASA), immunoreactive inhibin A, immunosuppressive acid protein (IAP), macrophage colony stimulating factor (MCS-F), and lysophosphatidic acid (LPA) **(Santillan, Garg and Marianna2005).**

CA-125 was the first tumor marker available for detection of ovarian cancer. The major problem associated with this marker, however, is its poor sensitivity and specificity as it is elevated in only 40-50 % of patients with stage I and II ovarian cancer **(Dupont, Tanwar and Thaler 2004).** In the detection of recurrent disease, CA-125 shows an accuracy of about 75%. The lead time of CA-125 elevation to clinically detectable recurrence is about 3 to 4 months **(Sharma and Menon, 2006).**

Glycodelin, a glycoprotein belonging to the lipocalin family, consists of 180 amino acid residues with a molecular weight of 28 kDa and a carbohydrate content of 17.5%. There are three putative *N*-glycosylation sites (Asn-28, Asn-63 and Asn-85). Therefore, four different glycoforms have been described, namely; glycodelin-A (GdA), glycodelin-F (GdF), glycodelin-C (GdC), and glycodelin-S (GdS). Such glycoforms have the same protein core and differ only in their N-glycosylation **(Lee 2009 and Tsviliana 2010).** The glycodelin gene is localized on chromosome 9, band q34. Glycodelin is considered the major lipocalin protein of the reproductive axis. It is mainly synthesized within female reproduction tract, breast or male reproduction tract and bone marrow under the effect of progesterone and relaxin **(Lee, Pang and Yeung 2009)**. In the ovary, expression of glycodelin is seen both in follicular and luteal phase. Glycodelin is localized mainly to theca interna cells through various stages of involuting corpus luteum to corpus albicans **(Seppälä, Koistinen and Koistinen 2001).**

Glycodelin has many immunosuppressive, contraceptive and morphogenic properties (**Alok and Karande, 2009).** Immunosuppressive properties of glycodelin were based on placental protein 14 (PP14)

mediated inhibition of phytohemagglutinin-induced lymphocyte proliferation and synthesis of interleukin (IL)-1 and IL-2. GdA has been found to inhibit NK cell activity. In addition, GdA suppresses IL-2-stimulated proliferative response of large granular lymphocytes (LGLs) **(Lee, Pang and Yeung 2009)**.

Glycoprotein potently and dose-dependently inhibits binding of human sperm to the zona pellucida. **(Seppälä, Koistinen, and Koistinen 2001)**. Because GdA also potently inhibits NK cell activity at similar concentrations to those that inhibit sperm–egg binding, it is possible that the recognition processes between immune cells and gametes have converged (**Seppälä, Koistinen and Koistinen 2001).**

2. Subject and Methods

2.1 Subjects

This study was conducted on female patients complaining of ovarian diseases who were admitted to the Oncology Unit of Ain Shams University Hospitals in the period from 15/8/2010 to 15/3/2011. The study also included twenty age matched apparently healthy females serving as controls. Subjects included in this study were divided into the following groups.

A. Group 1: Ovarian cancer (n =40):

This group included 40 female patients with newly diagnosed ovarian cancer. Their median age was 60 years with an interquartile range (IR) (55.3 – 65). They were complaining of irregular menses, lower abdominal distention, or pain, such as dyspareunia. Diagnosis was confirmed by histopathological examination of ovarian biopsy. Patients of this group were classified according to The TNM system (classification system developed by the American Joint Committee on Cancer [AJCC]) into two subgroups.

Subgroup 1a: Early stages ovarian cancer (n=15).

This subgroup included 15 patients with TNM stages I and II. Their median age was 58 years with (IR) (53 – 65).

Subgroup 1b: Late stages ovarian cancer (n=25).

This subgroup included 25 patients with TNM stages III and IV. Their median age was 60 years with IR (58 – 65).

B. Group 2: Benign ovarian diseases (n=30):

This group included 30 female patients with benign ovarian diseases with a median age of 30 years and IR (24.8 - 43). They were presented with abdominal pain or discomfort, an abdominal mass, bloating, back pain, urinary urgency, constipation, tiredness. The diagnosis was confirmed by histopathological examination of ovarian biopsy.

C. Healthy Control group (n =20):

This group consisted of twenty apparently healthy females with a median age of 54.5 years and IR (34 - 59.8).

2.2 Exclusion criteria

Any female suffering from Human Immunodeficiency Virus, liver diseases, breast cancer, synovial sarcoma, pancreatic cysadenoma, hidradenoma, or parabronchial was excluded from the study.

All subjects included in the study were subjected to the following:

i. Full history taking focusing on family history of ovarian cancer.
ii. Thorough clinical examination.
iii. Radiological examination as pelvi- abdominal ultrasound.
iv. Histopathological examination of ovarian tissue biopsy.
v. Laboratory investigations as:
   a. Serum level of the tumor marker CA-125.
   b. Serum level of glycodelin.

2.3 Analytical methods

2.3.1 Assay of serum CA-125:

CA-125 concentrations were measured using a commercially available electrochemiluminescence immunoassay (ECLIA) kit supplied by Roche Diagnostics (GmbH, D-68298 Mannheim, USA). The analysis was done on Cobas (E411) immunoassay analyzers.

The principle of the assay is based on sandwich immunoassay. Serum was incubated with biotinylated monoclonal CA-125 capture antibody and ruthenylated CA-125 tracer antibody. Streptavidin-coated paramagnetic beads were added forming a sandwich complex. The reaction mixture was then drawn into the measuring cell where the following steps take place: capture of the magnetic beads, addition of buffer containing tripropylamine, suitable voltage application, and measurement of the resulting electrochemiluminescence using a photomultiplier (emission of a photon at 620 nm). Chemiluminescent emission was measured by a photomultiplier. The analyzer automatically calculated CA-125 concentration level of each sample in U/mL. Results were determined via a calibration curve.

2.3.2 Assay of serum Glycodelin:

Glycodelin concentrations were measured using a commercially available enzyme-linked immuno-sorbent assay (ELISA) kit supplied by R&D system R&D system, Inc. (Minneapolis, NE, USA) (Lee et al, 2009). In this technique, a monoclonal antibody specific for Glycodelin pre-coated into the wells of a micro-titer plate was utilized. Standards and samples were pipetted into the wells of the micro-titer plate and any Gycodelin present in the sample was bound by the immobilized antibodies.

After washing away any unbound substances, enzyme-linked polyclonal antibody specific for Glycodelin was added to the wells. Following a wash step to remove any unbound antibody- enzyme reagent, a substrate solution complex oxidizes tetramethylbenzidine (TMB) was added to the wells and the enzymatic color reaction was developed in proportion to the amount of glycodelin bound in the initial step. The reaction was stopped and intensity of the color was measured by a micro-titer plate reader adjusted at a wavelength of 450 nm.

The absorption value of each standard is plotted aganist (y-axis) the corresponding glycodelin concentration (x-axis) on a log log graph paper. The resulting calibration curve is used to determine the concentration of glycodelin in the patients. The absorbance values of the serum samples are correlated with the corresponding glycodelin concentration values by interpolation. Using the absorbance value for each sample, the corresponding concentration of glycodelin in ng/mL was determined from the standard curve. Any diluted sample was further converted by the appropriate dilution factor.

2.4 Statistical Methods:

Statistical analysis was performed on a personal computer using SPSS (statistical software package for social science) (V. 17, Echosoft Corp., USA, 2008) for windows operating system.

3. Results

Table (1) and figure (1) show the descriptive and comparative statistics of the studied parameters in the different studied groups. Group-1 (i.e., ovarian cancer group) exhibited an increasing in concentration of glycodelin when compared to either the control group or the benign ovarian disease group.

Table (3) and figure (2) show the statistical comparison between the different studied groups regarding the studied parameters. CA-125 and glycodelin levels were significantly high in group 1 (ovarian cancer), with $p < 0.01$, as compared to either control group or benign ovarian disease group. Meanwhile, the levels of CA-125 did not show any significance difference between the benign ovarian disease group and the control group ($p > 0.05$) (see data in Table 2). In addition, CA-125 and glycodelin levels were significantly higher in subgroup 1b (late stages ovarian cancer) as compared to subgroup 1a (early stages ovarian cancer) (Figure-2).

Table(4) shows diagnostic performance of serum glycodelin in the ability of discrimination. Glycodelin was able to discriminate between benign group and control group at a cut off 6.2 ng/mL. This cut off had a diagnostic sensitivity 93.3%, specificity 100%, ppv 100%, NPV 90.9% and efficacy 96% (see data in Figure-3). Moreover, glycodelin was able to discriminate between malignant group and benign group at a cut off value of 40 ng/mL. This cut off had a diagnostic sensitivity 97.5%, specificity 90%, PPV 92.9%, NPV 96.4%, and efficacy 94.3% (Figure 4). In addition, Glycodelin was able to discriminate between subgroup 1b and subgroup 1a at a cut off value of 80 ng/mL. This cut off had a diagnostic sensitivity 88%, specificity 66.7%, PPV 81.5, NPV 76.9%, and efficiency 80% (Figure 5).

Table (1): Descriptive statistics of the demographic data and the studied parameters in the different studied groups.

Group / Parameter	Group 1 (n= 40) Median (IR)	Group 2 (n=30) Median (IR)	Group 3 (n=20) Median (IR)
CA-125 (U/mL)	302 (115 – 839)	16 (11 – 28)	12 (6 – 23)
Glycodelin (ng/mL)	88 (77– 98)	22 (14 – 31)	2 (1.5 – 3.8)

IR: Interquartile Range.

Table (2): Descriptive and comparative statistics between different subgroups of ovarian cancer patients using Wilcoxon's Rank Sum test .

Group / Parameter	Subgroup 1a (n= 15) Median (IR)	Subgroup 1b (n=25) Median (IR)	Z	*P*
Age (years)	58 (53 -65)	60 (58 – 65)	0.84	> 0.05
CA-125 (U/mL)	168 (102 – 300)	490 (277 – 761)	3.34	< 0.01
Glycodelin (ng/mL)	76 (56 -96)	95 (84 - 106)	2.39	< 0.05

Group 1a : early stages ovarian cancer. Group 1b : Late stages ovarian cancer.
P > 0.05 : Non - significant difference. *P* < 0.05 : Significant difference. *P* < 0.01 : Highly significant difference.

Table (3): Statistical comparison between different groups regarding the studied parameters using Wilcoxon's Rank Sum Test .

Group / Parameter	Group 1 versus Group 3		Group 1 versus Group 2		Group 2 Versus Group 3	
	Z	*p*	Z	*p*	Z	*p*
Age (years)	3.17	< 0.01	5.97	< 0.01	3.13	< 0.01
CA-125 (U/mL)	5.47	< 0.01	5.95	< 0.01	1.85	> 0.05
Glycodelin (ng/mL)	6.27	< 0.01	6.99	< 0.01	5.48	< 0.01

Table (4): Diagnostic performance of serum Glycodelin in discrimination between different studied groups.

Group / Parameter	Cut off (ng/mL)	Sensitivity (%)	specificity(%)	PPV(%)	NPV(%)	Diagnostic efficiency
Benign from Control	6.2	93. 3	100	100	90. 9	96
Malignant from Benign	40	97. 5	90	92. 9	96. 4	94. 3
Early Stages from Late stages	80	88	66.7 %	81. 5	76. 9	80

P P V : Positive predictive value**.**
N P V : Negative predictive value.

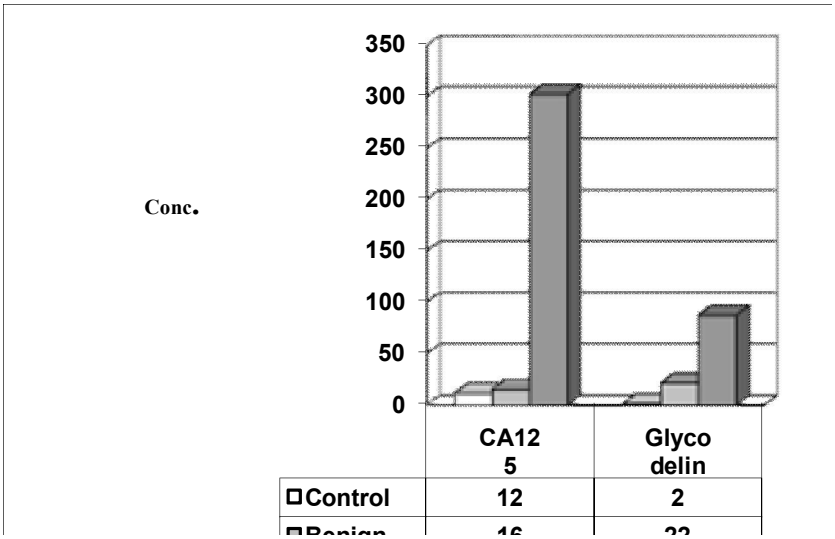

Fig. (1): Comparison between all studied groups as regards CA125 and glycodelin.

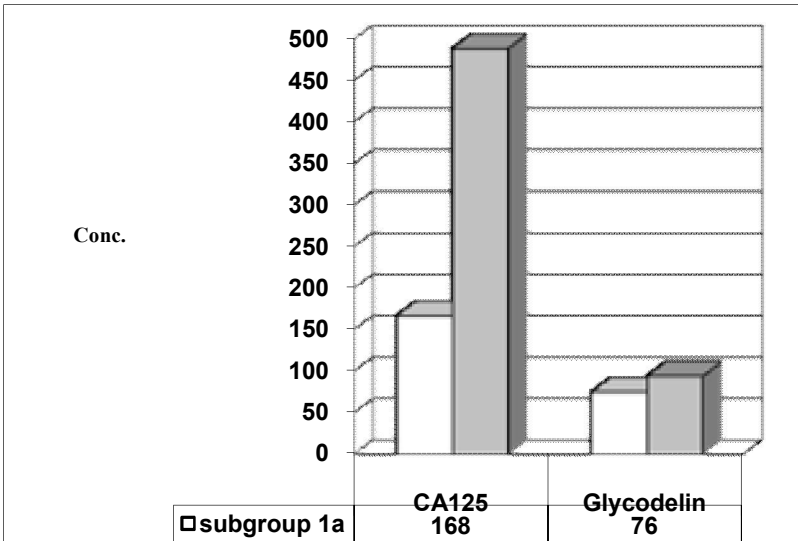

Fig. (2): Comparison between early and late stages of ovarian cancer regarding serum CA125 and glycodelin.

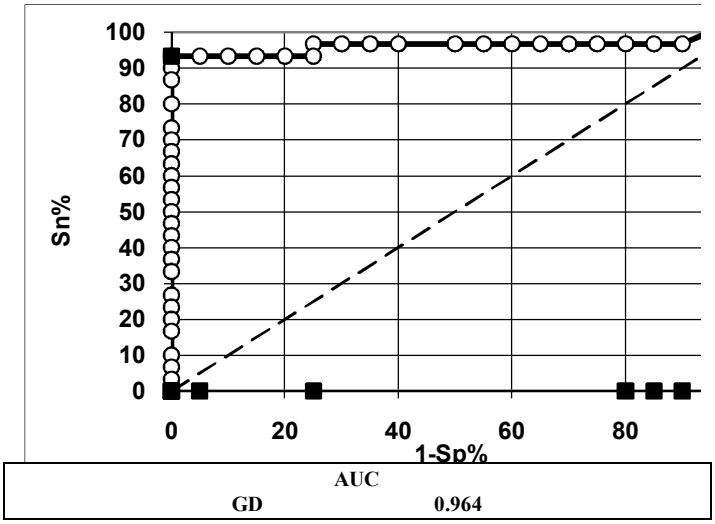

Fig. (3): ROC curve analysis showing the diagnostic performance of Gd for discriminating benign ovarian disease from controls.

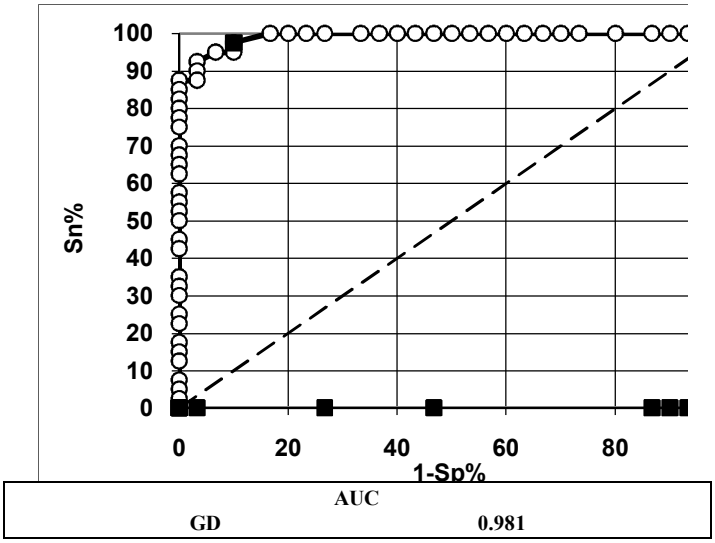

Fig. (4): ROC curve analysis showing the diagnostic performance of GD for discriminating malignant from benign ovarian disease.

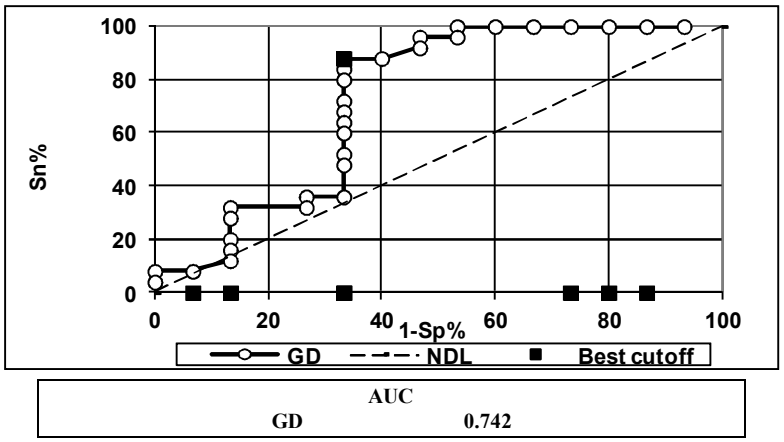

Fig. (5): ROC curve analysis showing the diagnostic performance of GD for discriminating between early and late stages ovarian cancer.

4. Discussion

Ovarian cancer is often known as "the silent killer" as it is difficult to be detected early because the ovaries are deep within the pelvis and initial symptoms are often ambiguous. Usually the cancer goes undiagnosed until after the disease is far advanced and has spread throughout the abdomen or to distant organs **Kiani, Knutsen and Singh (2006)**.

In the present study glycodelin was significantly higher in ovarian cancer patients when compared to either benign or control groups. These results were in accordance with **Bischof, B.riese and Richter (2005)** who demonstrated elevated levels of glycodelin mRNA and protein in the ovarian and endometrial cancer tissue when compared to the normal ovary and endometrium. Increased serum levels of glycodelin have also been found in patients with ovarian cancer as shown table (1).

Our current study is in accordance with **Kosary, Young and Keel (2007)** who demonstrated that older women are at highest risk. More than half of the deaths from ovarian cancer occur in women between 55 and 74 years of age and approximately one quarter of ovarian cancer deaths occur in women between 35 and 54 years of age. This finding could explain the increased risk of ovarian cancer with increased age (Table 2). However, the serum levels of CA-125 did not show any significance difference in patients with benign ovarian disease compared to control group as depicted in Table (3). These results are in accordance with **Tsviliana ,Mayr and Kuhn (2010)** who reported that there is no useful tumor marker for the early diagnosis of ovarian cancer, however quantitative assessment of glycodelin could be used in improving the early diagnosis of ovarian cancer as depicted in Table (3).

Moreover, we found that, the serum level of glycodein was significantly higher in benign ovarian disease group as compared with control group (p<0.01). But a statistically non-significant difference was found between the early and late stages of ovarian cancer (p<0.05) as shown in Table (2). In addition, both CA-125 and glycodelin were significantly higher in the late stages of ovarian cancer (p < 0.01) as compared to the early stages (p < 0.05) as depicted in Table (2).

The present study indicated that glycodelin was able to discriminate between benign group and control group at a cut off 6.2 ng/mL (see data in Table 4). This cut off had a diagnostic sensitivity 93.3%, specificity 100%, PPV 100%, NPV 90.9%, and efficiency 96% with an area under the curve of 0.964 (Table 4, Figure-3). Moreover, Glycodelin was able to discriminate between malignant group and benign group at a cut off 40 ng/mL. This cut off had a diagnostic sensitivity 97.5%, specificity 90%, PPV 92.9%, NPV 96.4%, and efficiency 94.3% with an area under the curve of 0.981 (Table 4, Figure4). In addition, Glycodelin was able to discriminate between subgroup 1b and subgroup 1a group at a cut off 80 ng/mL. This cut off had a diagnostic sensitivity 88%, specificity 66.7%, PPV 81.5, NPV 76.9%, and efficiency 80% and an area under the curve of 0.742 (Table 4, Figure 5).

5. Conclusions

Glycodelin was found to be a sensitive and early diagnostic marker of ovarian cancer. Inclusion of glycodelin to the current standard tests for diagnosis of early detection of recurrent ovarian cancer would improve the ability to identify patients who might be missed by current diagnostic strategies and thus might provide a better therapeutic outcome.

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