Evaluation of Nidogen-٢ Serum Level Assessment as a Screening and Diagnostic Tool for Ovarian Cancer

Thesis
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List of Abbreviations

- **AFP:** Alpha Fetoprotein
- **BRCA:** Breast Cancer Association Gene
- **CA١٢٥:** Cancer or Carbohydrate Antigen ١٢٥
- **CA ١٩-٩:** Cancer or Carbohydrate Antigen ١٩-٩
- **CI**: Confidence Interval
- **ECDU**: Early Cancer Detection Unit
- **FIGO:** International Federation of Obstetrics and Gynaecology
- **hCG:** Human Chorionic Gonadotrophin
- **LASA:** Lipid-Associated Sialic Acid
- **LPA:** Lysophosphatidic Acid
- **Nid-٢:** Nidogen-٢
- **NOS:** Not Otherwise Specified
- **OCPs:** Oral Contraceptive Pills
- **PET:** Positron Emission Tomography
- **PI:** Pulsatility Index
- **PJS:** Peutz-Jeghers Syndrome
- **RI:** Resistance Index
- **SCSTs:** Sex Cord Stromal Tumours
- **SIADH:** Syndrome of Inappropriate Antidiuretic Hormone
- **UGF:** Urinary Gonadotrophin Fragment

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Introduction

Ovarian cancer is relatively common tumour and is the leading cause of death from gynaecological cancer. It is a serious disease particularly in advanced stages with a course that is punctuated by frequent tumour recurrence and negative impact on quality and length of life ***(Vern et al., ٢٠٠٧)***. The majority of patients with ovarian cancer will relapse and ultimately die from their disease. While the prognosis in stage I ovarian cancer is excellent with earlier lower grade stages having a cure rate of greater than ٩٠٪ ***(John et al., ٢٠٠٨)***.

However, despite enormous effort, there is no proof that routine screening with serum markers, sonography, or pelvic examinations decreases mortality. Hundreds of possible markers have been identified, yet no test currently available approaches sufficient levels of accuracy ***(Kuk et al., ٢٠٠٩)***.

Serum biomarker "Carbohydrate antigen ١٢٥" or (CA١٢٥) is limited as regard the utility in screening the disease due to its high false positive rate ***(Tuxen, ١٩٩٥)***. CA-١٢٥ could be elevated in some malignancies as well as some benign gynaecological conditions; it is proven to be elevated in: uterine and fallopian malignancies, colon and gastric malignancies, endometriosis and pregnancy ***(Jacobs et al., ١٩٨٩)***

The need to identify new biomarkers with increased sensitivity and specificity for early diagnosis, prognosis or monitoring of ovarian cancer is crucial for optimal patient management ***(Kulasingam and Diamandis, ٢٠٠٨).***

In ٢٠٠٨, scientists of the ***Canadian Society of Clinical Chemists*** performed extensive proteomic analysis of ovarian cancer ascites identifying over ٤٥٠ proteins but after applying a set of filtration criteria to reduce the number of potential biomarker candidates, they identified ٥٢ proteins for which further clinical validation is warranted, of the ٥٢ candidates, ١٨ of them had reagents available to develop an ELISA to measure the levels of these proteins in biological fluids one of those is nidogen-٢ ***(Kulasingam and Diamandis, ٢٠٠٨)***.

The candidate molecule nidogen-٢ is a basement membrane protein. The major components of the basement membrane include collagen IV**,** laminins, heparin sulfate, proteoglycan (perlecan) and nidogens, the proteins that allow for cell adhesion and the formation of networks to confer the mechanical stability of the basement membrane ***(Ekblom et al., ١٩٩٤; Dziadek, ١٩٩٥)***.

Two nidogens; nidogen-١ and nidogen-٢ have been identified in humans, The two proteins share a ٤٦٪ in primary sequence identity but have similar three-dimensional structure, consisting of three globular domains (G١, G٢, G٣) connected by a flexible link ***(Fox et al., ١٩٩١; Kohfeldt et al., ١٩٩٨)***, The nidogens bind and form a ternary

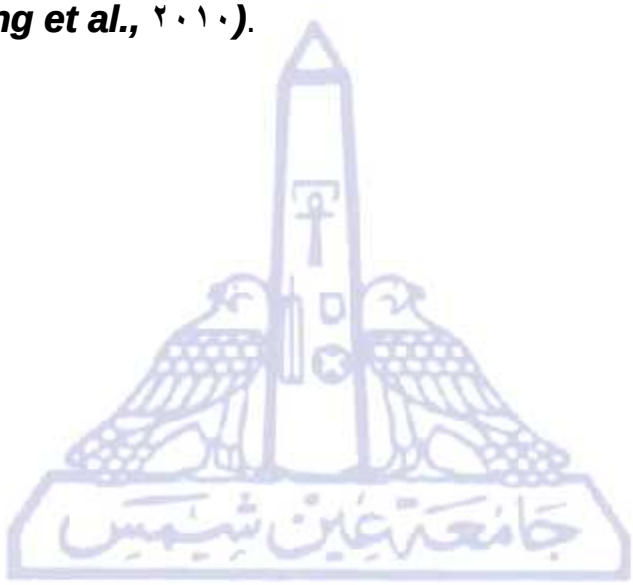
complex with laminin-١ and collagen type IV, connecting the two networks stabilizing and maintaining the structure of the basement membrane ***(Aumailley et al., ١٩٨٩; Timpl & Brown, ١٩٩٦)*.**

Both nidogens are co-expressed in various tissues and it has been proposed that they fulfill similar, if not identical functions and may also play a compensatory role ***(Miosge et al., ٢٠٠١; Miosge et al., ٢٠٠٢)*.**

Physiologically, nidogens have been shown to interact with cell receptor molecules controlling cell polarization, migration and invasion ***(Dedhar et al., ١٩٩٢; Dong et al., ١٩٩٥; Wu et al., ١٩٩٥)***, as well as the fact that interaction between cells and basement membranes in which nidogens are integrated have an important role in regulating various cellular processes including differentiation, proliferation and apoptosis, another function is the interaction with leukocytes, which favor neutrophil chemotaxis during inflammation ***(Yi et al., ١٩٩٨)*.**

In ***٢٠٠٩, Kuk and co-workers*** investigated the levels of nidogen-٢ in serum of ovarian cancer patients and patients with benign gynaecological conditions or normal controls. Elevation of nidogen-٢ was identified in ovarian carcinoma serum samples, mostly associated with the serous histotype. These data support the view that nidogen-٢ is a new serological biomarker of ovarian carcinoma. Its clinical utility needs to be addressed in larger studies ***(Kuk et al., ٢٠٠٩)***.

Zhang and co-workers ٢٠١٠, evaluated the clinical significance of serum nidogen-٢ and carbohydrate antigen ١٢٥ (CA١٢٥) in patients with ovarian serous carcinoma. The serum levels of nidogen-٢ and CA١٢٥ were determined by ELISA and immunoassay in ١٥ cases of normal ovary, ٢٢ cases of ovarian serous cystadenoma, and ٤٠ cases of ovarian serous cystadenocarcinoma. The conclusion stated that Serum nidogen-٢ can be used as a new biomarker for ovarian cancer ***(Zhang et al., ٢٠١٠)***.



Aim of the Work

- Evaluating nidogen-٢ as a new tumour marker of higher sensitivity, specificity and accuracy than CA-١٢٥ and Doppler U\S to improve early diagnosis of ovarian cancer.

Protocol of Thesis

Research question:

Is there a marked difference in accuracy between diagnosis of ovarian cancer using histopathological examination and diagnosis using nidogen-٢ serum level assessment in patients with adnexal masses?

Research Hypothesis:

There is no marked difference in accuracy between diagnosis of ovarian cancer using histopathological examination and diagnosis using nidogen-٢ serum level assessment in patients with adnexal masses.

Objectives:

Primary Objective**:**

- Evaluation of accuracy of Nidogen-٢ serum level assessment in diagnosis of ovarian cancer;

Secondary objectives**:**

- Comparison between U\S Doppler, CA-١٢٥ serum level assessment and nidogen-٢ serum level assessment.

Participants:

The study will include ١٤٤ Women from ***Ain Shams University Hospital of Obstetrics and Gynaecology*** who are preliminary diagnosed of adnexal mass clinically and\or sonographically regardless the patients' complaint, age or parity.

All patients will be subjected to the following after taking a written consent from each patient:

١- Complete history taking.

٢- General physical examination to assess the general condition, metastatic disease if present.

٣- Local pelvic examination; to assess the adnexal mass as regard site, size, shape, surface, tenderness, consistency and mobility if possible.

٤- Abdominal and\or transvaginal ultrasound was performed for assessment of the mass, uterus and ovaries for size and any associated abnormalities.

٥- Colour Doppler imaging of the ovarian tissue to detect neovascularization.

٦- Spectral Doppler analysis using a resistance index and\or pulsatility index.

٧- Quantitative assessment of serum level of CA-١٢٥.

٨- Quantitative assessment of serum level of Nidogen-٢.

٩- Adnexal masses will be resected in all patients and will be sent for full histopathological examination.

Index and reference tests:

Assessment of the serum level of nidogen-٢ in ovarian mass patients is the index test in this study. Serum level will be assessed using an ELISA kits for nidogen-٢. The kits are sandwich enzyme immunoassay for the in vitro quantitative assessment of nidogen-٢ in human serum, plasma and other biological fluids. The manufacturer is Wuhan EIAAB® Science Co., Ltd. imported by Gamma Trade Company. Detection range is ٠-١٠٠ ng/mL; a kit can be used for ٩٦ serum sample and it will be stored at (-٢٠ ºC). Two Kits will be used in this study. All samples will be collected in serum separator tube and allowed to clot for ٣٠ minutes before centrifugation for ١٥ minutes at approximately ١٠٠٠ x g, serum will be removed and stored at -٢٠ ºC until analysis. All samples will be analysed at ***Clinical Pathology Department of Ain Shams University***.

In ٢٠١٠, ***Zhang and co-workers*** studied the serum levels of serum nidogen-٢ in ٤٠ ovarian cancer patients, ١٥ healthy subjects and ٢٢ patients with benign ovarian mass, the study reported that the cut-off value that might be used for ovarian cancer is (٢٨,٣٥ ng\mL).

The reference test will be the histopathological examination which is considered the gold standard

test for diagnosis of ovarian cancer. All tissue specimens were examined in ***Early Cancer Detection Units (ECDU)*** at ***Obstetrics and Gynaecology Hospital, Ain Shams University***.

The results of nidogen-٢ also will be compared to the results of CA-١٢٥ that will be measured by immune-radiometric assay in the ***Clinical Pathology Department of Ain Shams University*** where serum levels above (٣٥ U\mL) were considered abnormal.

The results of nidogen-٢ finally will be compared to transvaginal U\S Doppler diagnosis. Ultrasonic evaluation will be done using trans-abdominal route (using ٣٫٥ MHz curvilinear probe) and\or transvaginal route (using ٦٫٥ MHz intra-cavitary probe).

Outcomes:

١) How many patients with adnexal mass proved to be ovarian cancer by histopathological examination will show elevated serum Nidogen-٢ (above ٢٨٫٣٥ ng\mL) and how many of them will show average levels (below ٢٨٫٣٥ ng\mL).

٢) How many patients with adnexal mass proved to be benign by histopathological examination will show elevated serum Nidogen-٢ (above ٢٨٫٣٥ ng\mL) and how many of them will show average levels (below ٢٨٫٣٥ ng\mL).

(٣) How many patients with adnexal mass proved to be ovarian cancer by histopathological