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SERUM INHIBIN A AS A MARKER FOR MOLAR PREGNANCY

By

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ABSTRACT

The purpose of this study was to evaluate serum inhibin A as a marker for diagnosis and short term follow up of molar pregnancy , and to compare the results with β-hCG. ***Methods :*** *Serum inhibin A and β-hCG were assayed in a)Ten women with molar pregnancies before and two weeks after evacuation.b)Twenty healthy pregnant women. c) Fifteen healthy non pregnant women.* ***Results :*** *Women with hydatidiform mole had significantly higher serum levels of inhibin A ($P < 0.01$) than healthy pregnant women, 9 fold than the 95% confidence interval of control values, without any considerable overlap with values found in normal pregnant controls.Two weeks after evacuation , the levels of inhibin A declined significantly to the levels of non pregnant controls.Molar β-hCG concentrations were significantly higher than in normal pregnancy ($P < 0.05$), but some values within the 95% confidence interval of normal values. Despite a significant decrease ($P < 0.01$) after evacuation , β-hCG levels were still higher than in non pregnant women.* ***Conclusion:*** *The present data strongly suggest that serum inhibin A measurement may be of better value in diagnosis and short term follow up of molar pregnancy than β-hCG.*

INTRODUCTION

Inhibins (A and B) are heterodimeric glycoprotein hormones assembled from two subunits with a common α subunit. These hormones are mainly produced by the gonads and play a critical role in the control of gamete maturation [24].

Inhibins are produced by the placenta and fetal membranes during pregnancy [18,5]. Indeed, human placenta express inhibin β- and α-subunit transcripts and proteins [11,3]. Inhibins may be important regulators of fetal and placental development as well as being involved in the establishment of pregnancy [18].

This probably explains why serum maternal concentrations vary according to the term of the pregnancy, declining after delivery [23]. Measurement of serum inhibin is useful in cases of various gestational pathologies, including pre-eclampsia, Down's syndrome and molar pregnancies [1,14].

Inhibin has been also postulated to play a role in trophoblastic molar invasion and its presence in molar trophoblast cells has been reported [12,11,17,20].

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High serum levels of inhibin have been reported in cases of hydatidiform mole in comparison to cases of normal pregnancy. However controversy exists regarding the prognostic significance of measuring inhibin in these patients[12,2,17,4].

Aim of the work:

The aim of this work is to investigate whether inhibin A may represent better marker than β human chorionic gonadotrophin (β-hCG) for diagnosis and short term follow up of molar pregnancy

SUBJECTS AND METHODS

This study included 10 female patients from the Obstetrics and Gynecology Department of Benha University Hospital, in addition to 20 healthy pregnant females and 15 non pregnant females as controls.

All patients were newly diagnosed cases of complete molar pregnancy with age range, 18-36 years and gestational age range, 10-17 weeks calculated from the 1st day of last menstrual period. The diagnosis of which was based upon the following criteria:

*The clinical presentation in the form of vaginal bleeding and in some cases, the passage of the characteristic molar vesicles from the uterus.

*High levels of β-hCG relative to the gestational age

*Characteristic "snow storm pattern" of the ultrasonographic picture.

*The histologic appearance of samples obtained at curettage confirmed the diagnosis

The molar pregnancies were evaluated after evacuation over a period of six months. They showed complete remission, as evidenced by regression of β-hCG levels to normal during the first 10 weeks of follow-up except three patients (cases 7, 9, 10) who developed persistent trophoblastic disease (PTD). This was evidenced by plateauing of β-hCG at high levels for 2 successive weeks in two patients and rise of β-hCG level after 2 weeks of evacuation in one patient. These patients subsequently received chemotherapy.

Control subjects were divided into two groups:

Control A: Pregnant Control Group: This group included 20 healthy subjects with age range, 18-36 years and gestational age range, 9-17 weeks, who progressed to deliver healthy single baby. Subjects with multiple pregnancies diabetes, hypertension, fetal anomaly, and maternal or fetal infection were excluded.

Control B: Non- Pregnant Control Group: This group included 15 healthy subjects with age range, 18-35 years in the follicular phase of the menstrual cycle.

All of the studied individuals were

subjected to assessment of their serum inhibin A and β-hCG levels.

Sampling:Five milliliters (mL) of venous blood was collected from each subject and allowed to clot. After centrifugation, serum was divided into 2 aliquots and stored at -70°C until assay.

Only one sample was drawn from each control subject. As regards patients, two samples were collected from each patient the first sample was taken immediately after diagnosis of the case, whereas the second sample were obtained 2 weeks post-evacuation

Methods :

Inhibin A:

Assay was carried out by kit of diagnostic systems laboratories using ELISA teqnique [10].

The method is an enzymatically amplified "two-step" sandwich-type immunoassay. In the assay, standards, controls and unknown serum samples were incubated in microtitration wells which has been coated with anti-inhibin β_A subunit antibody. After incubation and washing, anti-inhibin alpha subunit antibody labeled with the enzyme horseradish peroxidase (HRP) was added. After a second incubation and washing step, the wells were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution was added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm on an automated ELISA plate reader. The absorbance measured was directly proportional to the concentration of inhibin A present. the inhibin A concentrations in the unknowns had been calculated from the standard curve.The inhibin A detection limit was 4 pg/ml , with intra and inter-assay coefficients of variation (CVs) for quality control samples of 4.0 % and 8.0% respectively. Cross reactions for the assay with the various inhibin-related proteins were less than 0.5%.

β-hCG :

Assay was carried out using automated chemiluminesence system by ACS 180 [19].

β-hCG: assay is a two-site sandwich immunoassay using direct, chemiluminometric technology, which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent is a polyclonal goat anti-β-hCG antibody that has been purified and labeled with acridinium ester. The second antibody, in the solid phase, is a purified monoclonal mouse anti-β-hCG antibody, which is covalently coupled to paramagnetic particles. These two antibodies are specific for different epitopes

that are present on both the free β sub-unit and the β subunit of intact hCG . This assay has a sensitivity of 2 mIU/ ml , a within assay CV of 2.1% and an inter assay CV of 3.1%.The assay was highly specific for β-hCG with no cross reactions with Luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and prolactin.

Statistical analysis was by the Wilcoxon rank sum test for non-paired samples and by the Wilcoxon signed rank test for comparison of paired samples before and after evacuation.

RESULTS

Table (1): Serum inhibin A and β–hCG levels in study groups

	Mean	SEM	Lower 95% CI	Upper 95% CI
Inhibin A (pg/ml)				
Pregnant controls	242.3	14.87	211.2	273.4
Non pregnant contols	18	1.3	15.28	20.8
patients' group before evacuation	2076.6	329.4	1331.3	2821.8
patients' group after evacuation	20.3	4.2	10.8	29.7
β–hCG (mIU/ ml)				
Pregnant controls	73258	1843	69400.6	77115.8
Non pregnant contols	1.9	0.33	1.23	2.66
patients' group before evacuation	281517.7	113739	124222.1	638813.3
patients' group after evacuation	2341.09	1022.5	27.96	4654.2

SEM : Standard error of the mean. **C I :** Confidence interval.

Table (2): Statistical comparison of serum inhibin A and β–hCG between patients' group before evacuation versus pregnant controls (control A) and 2 weeks post evacuation versus non pregnant controls (control B) using Wilcoxon's Rank Sum Test:

Parameter	Patients 'group before evacuation versus control A		Patients ' group after evacuation versus control B	
	Z	P	Z	P
Inhibin A(pg/ml)	- 2.8	<0.01*	- 0.051	> 0.05
β–hCG (mIU/ml)	- 2.29	<0.05*	- 2.8	<0.01*

P<0.05* significant difference. P<0.01* highly significant difference.

Table (3): Statistical comparison of serum inhibin A and β–hCG between patients' group before evacuation versus 2weeks post-evacuation using Wilcoxon's Signed Test:

Parameter	Before evacuation versus 2 weeks post-evacuation	
	Z	P
Inhibin A(pg/ml)	- 2.8	<0.01*
β–hCG (mIU/ml)	- 2.7	<0.01*

P<0.01* highly significant difference.

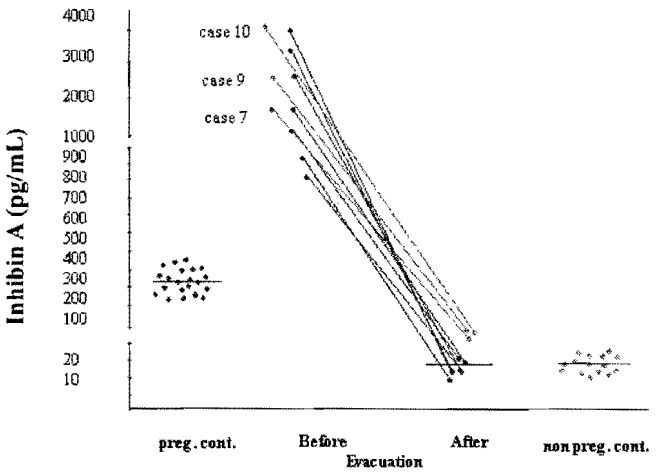

Figure (1): Serum levels of inhibin A in studied groups

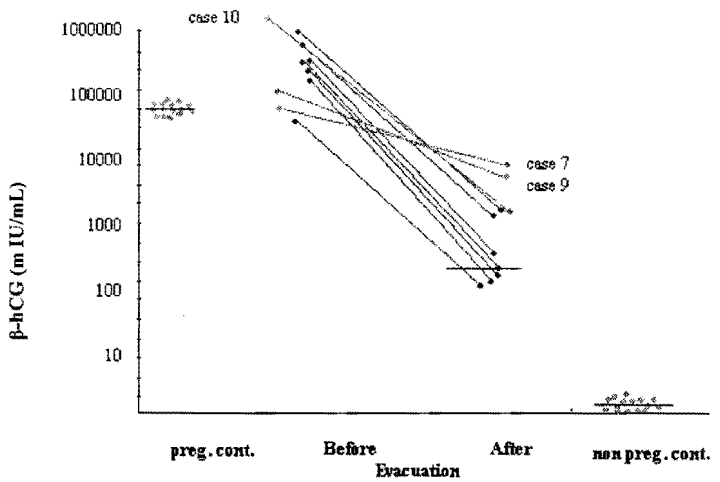

Figure (2): Serum levels of β-hCG in studied groups