# Binding Studies of LH in Benign and Malignant Uterine Tumors Homogenate with $^{125}$I-Anti LH Antibody.

*Hiba Itemad Nahab**

Date of acceptance 5/10/2005

## Summary

The sera levels of luteinizing hormone were investigated prior to surgery in 10 postmenopausal women with benign and 10 postmenopausal women with malignant uterine tumors. The sera concentrations of LH were compared with those of 10 healthy postmenopausal age matched controls. Student's T-test analysis revealed that there is no significant decrease of serum LH levels in benign tumor and malignant uterine tumor.

## Introduction

Luteinizing hormone is a glycoprotein hormone of an approximate molecular weight of 28 kDa, it is secreted by specialized cells of the anterior pituitary gland. LH is composed of two distinct, non-covalently linked subunits designated α and β [1,2].

Luteinizing hormone place a key rules in the control of reproductive function and act a specific in the cell membrane of target cells in the gonads [3].

LH receptor together with FSH, TSH receptor are members of the supper family of G protein coupled receptors [4,5,6]. The release of LH is affected both positively and negatively by estrogen and progesterone, whether estrogen and progesterone stimulate or inhibit LH release depend upon the level of exposure and duration of the steroid receptors for estrogen and progesterone are found various parts of thebrain,including the hypothalamus[7,8].

## Experimental Chemicals

All laboratory chemicals and reagents were of annular grade. Triss buffer, were obtained from Fluka Company, Switzer land. Hydrochloric acid, were obtained from BDH limited pool, U.K. kit of radio active ($^{125}$I- anti LH antibody) was manufactured by ***Immunotech, Abeckman coulter company***. The radioactivity of ($^{125}$I-anti LH antibody) was approximately 370 KBq.

## Instruments

The instruments used in this work were, LKB gamma counter type 1270-Rack gamma II, centrifuge type Hitachi, Pye-Unicam pH meter.

## Patients

Two groups of postmenopausal patients where include in this study, group I consisted of 10 postmenopausal patients with benign uterine, (Age = 51- 60 years), group II consisted of 10 postmenopausal patients with malignant uterine tumor,

* Chemistry Department , College of Science , University of Baghdad

(Age = 54- 62 years) and this limitation of samples due to difficult to collect those samples and at the same time they enough to complete the study.

All patients were admitted for treatment to **(Medical City, Baghdad Teaching Hospital), Al-Yarmuk Teaching Hospital, Al Habibia Hospital,** under the supervision of specialists, **Dr. Nada Salih Ameen, Dr. Akram Al-Shareef, Dr. Wafaa Al- Omary.**

They were histologically proven from the supervision of specialists **Dr. Raja Al-Hadethy, Dr. Eman Alash.** The patients were newly diagnosed and not underwent of any type of therapy. Patients did not suffer from any disease that may interfere with our study were excluded.

## Collections of Specimens

The tumor tissues were surgically removed from uterine patients by hysterectomy. The specimens were cut off and immediately rinsed with ice-cold isotonic saline solution. They were collected individually in plastic receptacles and stored at –20 °C until homogenization.

## Preparations and Uterine Tissue Homogenate .

The frozen tissue was thawed, sliced finely with a scalped in petridish standing on ice bath. The slices were further minced with scissors, then homogenized at 4 °C in (Triss) buffer solution (0.01M) with ratio of 1:5 (weight:volume), using manual homogenizer. The homogenate was filtered through several layers of nylon gauze, and then centrifuged at 1500xg for 15 min in a cooling centrifuge at 4 °C. The supernatants were used through out our study.

## Solutions

The Triss buffer solution (0.01M, pH = 7.6) was prepared by dissolving (0.303 gm) of Triss buffer in 200ml distilled water. The required pH was adjusted by adding HCl solution (0.2M), and then the volume was completed to 250ml by distilled water.

## Methods

### Determination of LH levels in Sera of Benign and Malignant Uterine Tumors Patients and Controls.

Serum levels of human LH were determined by an Immunoradiometric assay (IRMA). The method is based on the use of antibody-coated tubes, employing to mouse monoclonal antibodies directed against different epitopes on the LH molecule. The assay protocol is described in Table (1).

**Table (1): IRMA assay protocol of serum LH (IU/L).**

| | LH standard (IU/L) | | | | | | Control | Unknown samples | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | —— | 1 | 2 |
| Coated tube no. | 1,2 | 3,4 | 5,6 | 7,8 | 9,10 | 11,12 | 13,14 | 15,16 | 17, 18 |
| Standard (µl) | 50 | 50 | 50 | 50 | 50 | 50 | - | - | - |
| Control serum or sample (µl) | | | | | | | 50 | 50 | 50 |
| Tracer (µl) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |

- Prepare a series of tubes in duplicate.
- Add sequentially, 50 µL of standard or sample and 50 µL of tracer, shake gently.
- Prepare separately two tubes, containing 50 µL of tracer only for the determination.
- Incubate the tubes for 90 min with gently shaking (350 rpm) at room temperature (18-25 °C).
- Aspirate or decant the contents of the tubes, except of tubes T.
- Wash with 2mL of wash solution aspirate. Repeat this step. No trace of the dye should remain.

- Determine the radioactivity of all tubes: count for 1 min, or better 2 min in gamma counter with window adjusted for $^{125}I$.

## Reagents

The reagents provided in the human LH IRMA kit from DiaSorin-Italy were used:

1- **Tracer**: one vial contains 5.5ml IgG to LH (mouse monoclonal) labeled with $^{125}I$, Radioactivity is 370kBq(10μci).

2- **LH standards**: the vials contain increasing amounts of LH. The standards concentrations are the following: 0 to 180 IU/L.

3- **Coated tubes**: the inner surface of each tube is coated with IgG to LH (mouse monoclonal) directed against an LH epitope that is different from the one against which the IgG used for the tracer is raised.

4- **Wash solution**: the bottle contains 50ml saline solution and detergents.

5- **Control serum**: the vial contains LH, human serum and preservatives.

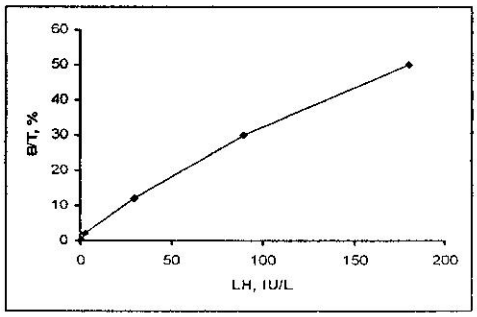


Figure (1): Standard curve of LH in human serum samples

## *Calculations*

1- The mean net count for each group of tubes was counted in a gamma counter for 1min.

2- The B/T ratio was computed for each standard and unknown sample as follows:

$$B/T\% = \frac{\text{Standard or samples mean counts}}{\text{Total activity mean counts}} * 100$$

3- A standard curve was drawn by plotting the percent value for each standard against the corresponding LH standard concentration (in log- log coordinates), Figure (1).

4- LH concentrations of the unknown were calculated from the standard curve by using the mean of their duplicate counts.

**Preliminary Test of the Binding of LH in Benign and Malignant Uterine Tumors Homogenate with $^{125}I$-Anti LH Antibody.**

1- Fifty-five microliters which contain (50μg protein) of postmenopausal benign tumor homogenate were incubated with 25μl (93.67μg/ml protein) of $^{125}I$ – anti LH antibody (mouse monoclonal IgG) in duplicate tubes and the volume was completed to 400μl with triss buffer pH(7.6) at room temperature (25 °C) for 90 min.

2- Two additional tubes containing 25μl of $^{125}I$ – anti LH antibody only (for total activity computation) were set a side until counting.

3- After incubation, the tubes were centrifuged for 15 min in order to separate the complex formed.

4- Supernatant was discarded by decanting the assay tubes. Then, the tubes were inverted on a filter paper for 10min.

5- Rims of the tubes were swabbed with cotton piece.

6- Amount of bound radioactivity (C.P.M) was counted in a gamma counter for one min.

7- Steps 1 to 6 above were repeated for other patient groups postmenopausal malignant tumor homogenate.

## *Calculation*

The counted radioactivity in each tube (expressed in C.P.M) represents the bound fraction (B), ($^{125}$I- anti LH antibody/LH) complex.

The counted radioactivity in the tubes containing $^{125}$I – anti LH antibody only represents the total activity (T).

The B/T ratio for each tube was counted as follows :

$$(B/T)\% = \frac{\text{Sample mean counts (B)}}{\text{Total activity mean count (T)}} \times 100$$

## *Results and Discussion*

### Determination of LH Levels in Sera of Benign and Malignant Uterine Tumors Patients and Controls

Serum LH levels were measured with an Immunoradiometric assay (IRMA) in two groups of postmenopausal patients with uterine tumor, matched with one group of control subject. Group I contained (10) postmenopausal patients with benign uterine tumor, group II contained (10) postmenopausal patients with malignant uterine tumor. Table (1) shows the results obtained form this study. The level of serum LH in postmenopausal patient with benign uterine tumor was found to be (30.84 IU/L), whereas that of postmenopausal patients with malignant uterine tumor was founded to be (27.5 IU/L). But in controls, the level was found (33.26 IU/L). Student's T-test analysis revealed that there is no significant decrease of serum LH levels in benign and malignant uterine tumor, this result shown in table (1).

**Table (1): Serum LH Levels (IU/L) in patients with benign and malignant uterine tumors.**

| Group | No. of cases | Age (year) | Serum LH (IU/L) |
|---|---|---|---|
| Postmenopausal of benign uterine tumor | 10 | 51-60 | 30.84 ± 5.1 |
| Postmenopausal of uterine cancer. | 10 | 54-62 | 27.5 ± 2.97 |
| Control | 10 | 50-60 | 33.26 ± 8.105 |

### Preliminary Test of the binding of LH in Benign and Malignant Uterine Tumors Homogenate with $^{125}$I-Anti LH Antibody.

Benign and malignant uterine tumor homogenate were used as the source of LH in this study. The homogenate was incubated with $^{125}$I-anti LH antibody (mouse monoclonal IgG) for one hour. The $^{125}$I-anti LH antibody/LH complex formed was separated form the unbound particular by centrifugation at 1500xg for 15 min. This centrifugal speed was sufficient to precipitate the complex. After centrifugation the tubes were decanted in order to get rid of the unbound antibody or antigen present in the supernatant fraction. While the $^{125}$I-anti LH antibody/LH complex remained as a pellet in the bottom of the tube. The preliminary conditions used in this experiment resulted in 25 % binding in the postmenopausal patients with benign uterine tumor, 27 % binding in the postmenopausal patients with malignant uterine tumor.

The B/T % represents the binding percent of $^{125}$I-anti LH antibody with LH in benign and malignant uterine tumors, and then the concentration of LH Accordingly, in postmenopausal patients with malignant uterine tumors, the binding percent (27 %) shows that LH concentration in tissue is higher than those of postmenopausal patients with benign uterine tumor. In the

postmenopausal patients with benign tumors, the binding percent (25 %).

## *References:*


1- Roberk, K. Murray, Daryl K. Granner, Peter A. Mayes and Victor W. Rodwell. 2000, "***Harper's Biochemistry***", 25th ed. Appleton and Lange. pp.500-550.

2- Cornell, J.S. and Pierce J.G. , 1973 "***J. Biol. Chem.***";148:4327.

3- Carl, A. Burtis, Edward R. Ashwood, 1999. "***Tietz Textbook of Clinical Chemistry***", 3rd ed., W.B. Saunders company. p. 1466.

4- GW, Montomery, ML Tate, HM Henry, JM Penty and RM Rohan. 1995; "***J. of Molecular Endocrinol***" 15:259.

5- Combarnous. 1992; "***Endocrine Review***" 13:670.

6- Segaloff, DL and Ascoly M, 1992;"***Oxford Reviews of Reproductive Biology***" 14:141.

7- Kmobil, E., Plant TN and Wildt TL, 1980; "***Science***" 207:1371.

8- Piva, F, Motta M, and Matini L, 1979,"***Endocrinology***" Gruneanel Startton, New York. spp. 12-33.


# دراسات في ارتباط الهرمون الليوتيني باورام الرحم الحميدة و الخبيثة مع الجسم المضاد ($^{125}$I-Anti LH)


هبة اعتماد نهاب*

*جامعة بغـــداد – كلية العلـــوم – قسم الكيمـــــياء



## الخلاصة

تم قياس مصل الهرمون الليوتيني قبل الجراحة لعشرة نساء بعد سن اليأس مصابات بمرض ورم الرحم الحميد و عشرة نساء بعد سن اليأس مصابات بمرض ورم الرحم الخبيث .تم مقارنة مستوى الهرمـــون مـــع عشرة نساء أصحاء بعد سن اليأس ، تم اجـــراء الاختبار(Student's T-test) و بين انه لا يوجـــد تنـــاقص جوهري في مستوى مصل الهرمون الليوتيني في الأورام الحميدة و الخبيثة.