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### RESEARCH ARTICLE

## A NEW METHOD FOR DETERMINATION THE ACTIVITY OF 25-HYDROXYCHOLESTEROL 7-ALPHA-HYDROXYLASE (CYP7B1) IN TISSUES OF WOMEN WITH BREAST TUMORS USING ELISA TECHNIQUE WITH SOME MODIFICATION.

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##### Key words:-

CYP7B1: 25-hydroxycholesterol 7 $\alpha$ -hydroxylase, CYP27: cytochrome P450 sterol 27-hydroxylase.

#### Abstract

**Background:** CYP7B1 expression has been decreased in ER-positive tumors compared with normal breast tissues. Tumors get 27-HC from the blood and also convert cholesterol into 27-HC within the own cells. In addition, there are greater 27HC levels in tumor samples compared with controls. Furthermore, survival of cancer patients is markedly poor for patients with low versus high tumor CYP7B1 expression.

**Objective:** Attempt to use a simple and new method for determination the activity of CYP7B1 in tissues of women with benign and malignant breast tumors using ELISA technique with some modification.

**Methods:** This case control study was conducted on sixty patients with breast diseases were divided into three group, group I contained twenty patients with benign breast diseases, group II consisted of twenty premenopausal patients with breast cancer. Group III comprised twenty postmenopausal patients with breast cancer with the mean age and standard deviation (25.25 $\pm$  7.87, 38.65 $\pm$  6.28, 58.5 $\pm$  7.02 years). Oxysterol 7 $\alpha$ -hydroxylase (CYP7B1) were measured in tissues by instrument ELISA technique with some modification.

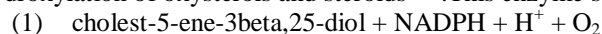
**Result:** The activity of CYP7B1 in tissue homogenates of women with breast tumors are increased when the time increased to limited time (1,5,10,15 min) then decreased when the time increasing to 30 minute. This activity was increased in benign compared with pre and postmenopausal woman with breast cancer

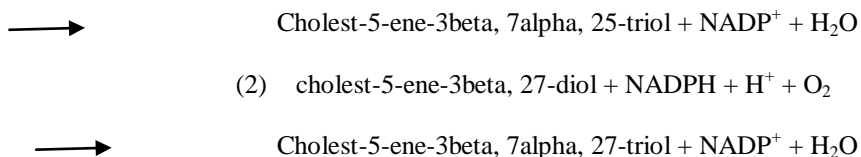
**Conclusions:** A simple and new method for determination of the activity of 25-hydroxycholesterol 7-alpha-hydroxylase (CYP7B1) in tissue homogenates of women with breast tumors was developed using ELISA technique.

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#### Introduction:-

25-hydroxycholesterol 7 $\alpha$ -hydroxylase (EC 1.14.13.100) Is an enzyme with systematic name cholest-5-ene-3beta,25diol, NADPH:oxygen oxidoreductase (7alpha hydroxylating)<sup>(1,2,3,4)</sup>. 25-hydroxycholesterol 7 $\alpha$ -hydroxylase is a heme-thiolate protein (P-450). Cytochrome P450 enzymes contain a single heme group and give a Soret peak at 450 nm, is reduced iron forms and bound to CO (carbon monoxide), (cytochrome P450, family 7, subfamily B, polypeptide 1) CYP7B1 is a microsomal enzyme that utilizes NADPH-cytochrome P450 reductase as an electron donor and stimulates 7 $\alpha$ -hydroxylation of oxysterols and steroids<sup>(5)</sup>. This enzyme stimulates the following reaction





Oxysterol 7 $\alpha$ -hydroxylase, is found in a lot of tissues including brain, lung, liver, prostate and kidney is linked with several physiological functions depending on tissue localization, including;

- 1- Bile acid biosynthesis <sup>(6)</sup>.
- 2- Pathway of steroid hormones (including neurosteroids) <sup>(7)</sup>
- 3- Regulation of immunoglobulin production <sup>(8)</sup>.
- 4- Metabolism of estrogen and androgen receptor ligands <sup>(9,10,11)</sup>.

In breast cancer, Cyp27a1 expression is similar in normal breast and tumors, in contrast, cyp7b1 expression has been decreased in ER-positive tumors compared with normal breast tissue. In addition, there are greater 27HC levels in tumor samples compared with controls. Furthermore, survival of cancer patients is markedly poorer for patients with low versus high tumor cyp7b1 expression. In mouse models, 27HC promoted the tumor growth and metastasis by independent mechanisms <sup>(12)</sup>

### Methods:-

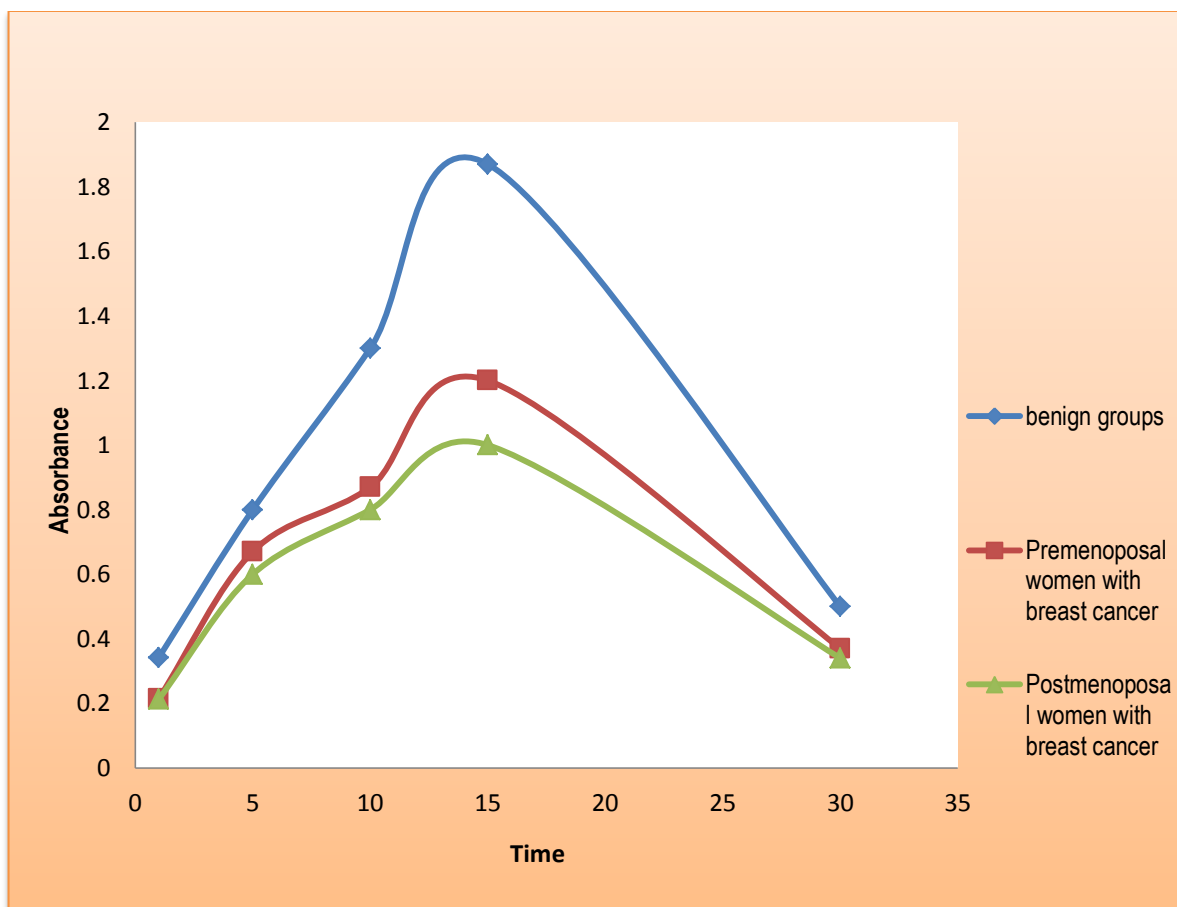
The study was executed during the term from February 2017 to May 2017 this study included sixty patients of woman with breast tumor. Group I contained twenty patients with benign breast tumor. Group II consisted of twenty premenopausal patients with breast cancer. Group III comprised twenty postmenopausal patients with breast cancer. All samples were collected from Al-Imameen Al-Kademen Medical City, Medical City of Baghdad Teaching Hospital, AL-Kademyah Privet Hospital and Al-Numan Hospital. They were histologically proven, newly diagnosed and not underwent any type of therapy. Patients suffered from any disease that may interfere with this study were excluded. Collection of specimens. The tumor tissues were surgically removed from breast tumor patients by either mastectomy or lumpectomy. The specimens were cut off and immediately immersed in ice-cold isotonic saline solution. They were collected individually in plastic receptacle and stored at -20 °C until homogenization. The CYP7B1 was measured by monoclonal antibody Enzyme Linked Immuno Sorbent Assay (ELISA) technique with some modification.

### Results:-

The activity of CYP7B1 in tissue homogenates of women with breast tumors are increases when the time increased to limited time (1,5,10,15 min) then decrease when the time increasing to 30 minute. This activity was increased in benign compared with pre and postmenopausal woman with breast cancer as shown in table (1) and Figure (1).

**Table (1):-**The activity of oxysterol 7 $\alpha$ -hydroxylase CYP7B1 in women with breast tumors.

Time in minute	Group I Benign		Group II Pre		Group III Post	
	Absorbance	Conc. Of CYP7B1 (U/ml)	Absorbance	Conc. Of CYP7B1 (U/ml)	Absorbance	Conc. Of CYP7B1 (U/ml)
1	0.342	0.133	0.215	-1.67	0.213	-1.79
5	0.8	6.97	0.67	5.0	0.6	4.0
10	1.3	14.47	0.87	8.0	0.78	6.7
15	1.87	22.9	1.2	12.9	1.0	9.95
30	0.5	2.49	0.37	0.55	0.34	0.1



**Figure 1:-** The activity of oxysterol 7 $\alpha$ -hydroxylase CYP7B1 in women with breast tumors.

### Discussion:-

The development of enzyme-linked immunosorbent assay ELISA through insert external antibody which study the characterization of antibody- antigen interactions in tumor tissues at constant PH 7.4 and temperature 37C° in different time (1,5,10,15, and 30) in minute result activity of enzyme was increased until 30 minute the incubation was used from the study by Margrit Schwarz that in analyzed by thin-layer chromatography on Silica Gel LK5D 150-Å plates (Whatman) in a solvent system containing toluene/ethyl acetate <sup>(13)</sup>.

The effect of incubation time on the relative activity of the enzyme was investigated as shown in Figure (1). The activity of the CYP7B1 enzyme initially increases rapidly and then essentially reaches at 15minute, after 15 minute leading to slightly decreased enzyme activity. According to this observation, the reaction time was optimized at 15minute. This Behavior show on the other enzymes belonged to a family of oxidoreductase in the presence of NADPH as co factor, the present curve and result compatible with <sup>(14,15,16)</sup>.

The enzyme linked immunosorbent assay (ELISA) is an intense technique for distinguishing and evaluating a particular protein in a mind boggling blend. Initially depicted by Engvall and Perlmanthe <sup>(17)</sup> method enables analysis of protein samples immobilized in microplate wells using specific antibodies. The technique has revolutionized immunology and is commonly used in medical research laboratories. ELISA also has commercial applications, including the detection of disease markers and allergens in the diagnostic and food industries. The ELISA strategy was made conceivable on account of logical advances in various related fields. Innovation empowering the creation of antigen-particular monoclonal antibodies by Kohler and Milstein <sup>(18)</sup>.

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**Author contribution:-**

Dr. Hassan H. AL-Saeed suggests the study; Prof. Dr. Anees K. Nile select the suitable patients and both of them co-writes the manuscript for study and Miss Zahraa K. Mohammed collected the tissue samples, conducted the necessary analysis of the study, writes the paper and analyzed the results statistically.

**Conflict of interest:-**

There was no conflict of interest

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