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Estimation, Partial Purification of Lipoxygenase and Estimate GGT in the Blood Patients with Prostate Cancer

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Abstract

The goal of study was to evaluate the activity, partial purification of LOX enzyme and the evaluation of GGT activity in serum of prostate cancer patients in Baghdad after having been diagnosed with prostate cancer through CT scans or MRI and a biopsy in the Oncology hospital for the period 19-02-2018 to 28-02-2019. The study included 50 samples of men with prostate cancer. Also, the study comprise 50 men samples (control) As a comparative group. The results showed that serum LOX activity was significantly increased in prostate cancer patients compared to the control. The serum enzyme LOX of prostate cancer was purified by following step: precipitate serum protein (40% ammonium sulfate), dialysed against a buffer solution, The gel filtration technique was use to separation the enzyme from other proteins (Sephadex G.100), we can obtained one peak in this step with purity 6.732 fold and yield27.77 %. The study also included determination the molecular weight of enzyme by electrophoresis technique with polyacrylamide gel, its molecular weight was 70 KD.

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Also, the results showed a significant increase GGT activity in patients ($P \leq 0.000$) compared to control. These blood samples were obtained from the Oncology Hospital - city Medicine - Baghdad.

Keywords: lipoxygenase (LOX), lox enzyme purification, prostate cancer, gamma - glutamyl transferase (GGT).

قياس وتنقية جزئية لانزيم اللايبوكسيجنيز وتقدير انزيم GGT في امصال دم المرضى المصابين بسرطان البروستات

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الخلاصة

الهدف من الدراسة قياس فعالية وتنقية انزيم اللايبوكسيجنيز وقياس فعالية انزيم غاما غلوتامايل ترانسفيريز في مصل دم مرضى سرطان البروستات في بغداد بعد تشخيص اصابتهم بسرطان البروستات من خلال الاشعة المقطعية او الرنين المغناطيسي وفحص خزعة البروستات في مستشفى الاورام للفترة 19-2-2018 الى 28-2-2019. تضمنت الدراسة على 50 عينة لرجال مصابين بسرطان البروستات. وكذلك تضمنت الدراسة 50 عينة من الرجال الاصحاء كمجموعة ضابطة (سيطرة). اظهرت النتائج وجود ارتفاع معنوي في فعالية انزيم LOX في مصل مرضى سرطان البروستات مقارنة مع المجموعة الضابطة . تمت تنقية انزيم LOX من مصل دم مرضى سرطان البروستات بواسطة الخطوات التالية: ترسيب بروتينات مصل الدم (40 % كبريتات الامونيوم) , عملية الديلزة باستخدام المحلول المنظم , استخدمت تقنية كروموتوغرافيا الاستبعاد الحجمي لفصل الانزيم من بقية البروتينات (sephadex G-100) حيث تم الحصول في هذه الخطوة على قمة واحدة، وبعدد مرات تنقية 6.732 مرة و نسبة استرداد 27.77 %. كذلك تضمنت الدراسة حساب الوزن الجزيئي للأنزيم بواسطة تقنية الترحيل الكهربائي وباستخدام هلام متعدد اكرايل امايد وكان وزنه الجزيئي 70KD. ايضا أظهرت النتائج ارتفاع معنوي في فعالية GGT عند المرضى وبمستوى احتمالية ($P \leq 0.000$) مقارنة مع المجموعة الضابطة. تم الحصول على عينات الدم من مستشفى الأورام التعليمي في مدينة الطب / بغداد.

الكلمات المفتاحية: لايبوكسيجينيز(LOX)، تنقية انزيم lox، سرطان البروستات، غاما–غلوتامايل ترانسفيريز (GGT).

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Introduction

Prostate cancer, a type of cancer that grows in the prostate gland, most prostate cancers grow slowly, yet some grow relatively quickly [1]. Cancer cells can spread from the prostate to other parts of the body, especially bone and lymph nodes [2]. Prostate cancer is the most common type of noncutaneous cancer diagnosed in men, with more than 1 million new cases diagnosed in 2012, representing 15% of cancers diagnosed in men[3].In 2018, the American Cancer Society (ACS) estimated that the number of new prostate cancer cases in men diagnosed in the United States was 164,690, accounting for 19% of all new cancer cases [4]. It is a common cancer among males in developed countries [5]. In Iraq, the Iraqi Cancer Board reported in 2016 that prostate cancer affects 4.13 people per 100,000 and it is the fourth among cancers in males of Iraq [6].

Liopxygenases are A family of enzymes that contain non-heme iron, which are classified as oxidoreductase enzymes [7] and are widely dispersed in plants, animals, fungi and some types of bacteria [8-11], where they can Stimulate the reaction of the addition oxygen molecule to unsaturated fatty acids that possess the synthetic formula (cis, cis-1,4-pentadiene) [12]. The acids that have such functions are linoleic, linolenic and arachidonic acid, which are considered as a substrate of Liopxygenases enzymes [13 and14].

More studies have shown that the LOX pathway is linked to certain cancers, especially prostate cancer [15and16]. The arachidonic acid (AA) of phospholipids is released by phospholipase A2, which is an important material and which is later transformed into compounds biologically active by the following enzymes.: lipoxygenase, cyclooxygenase, and cytochrome P450 .lipoxygenase-5 is works on convert arachidonic acid into leukotriene-A4, which are converted by LTA4 hydrolase into leukotriene-B4 or transform to leukotriene-C4 by leukotriene-C4 synthase [17-19].

This Derivatives of the arachidonic acid and lipoxygenase implicated in causing a variety of human diseases, as well as metastasis and promoting tumor progression [20]. Gamma glutamyl transferase is involve in the metabolism of leukotrienes C4, glutathione and his derivatives [21].

The goal of These study is to measure the efficacy and purification of the enzyme lipoxygenase from the serum of prostate cancer patients as well as to measure the effectiveness of gamma glutamyl transferase enzyme.

Materials and Methods

Collection of samples

Prostate cancer blood samples (5ml) were obtained from the Educational Oncology Hospital at Medical City – Baghdad for the period (19-2-2018 -28-2-2019). It consisted of 50 blood samples of the patient and 50 blood samples of healthy men as control group ages of both groups 40-80 years.

Estimation the effectiveness of LOX in serum

The method of estimation LOX activity is based on the stimulating oxygen to reaction with an unsaturated fatty acid that have (cis-cis-1.4-penta diene). It made of a Conjugated double bonds system that expansion absorption at a wavelength of 234 nm. which the absorption density is directly proportional to the enzyme concentration [22]. The method (K. Leo) was followed to determine enzyme activity [23].

Determination of Protein concentration

The Biuret reagent was used to estimate the protein concentration in the serum specimen [24].

Partial purification of lipoxygenase

LOX was purified by following steps:

Precipitate by Ammonium Sulphate salt

Serum proteins were precipitate by adding 1.2 g of ammonium sulphate (0-40%) to 5.2 ml serum for prostate cancer patients, which were gradually added to in the ice bath with magnetic stirring (15 min) until ammonium sulphate was dissolved. The solution was then placed in a centrifuge for 15 minutes at a rate of 17608 g to separate the precipitate from the leachate. The precipitate was dissolved with a minimum amount of buffer solution (buffer phosphate pH 7 (0.001 M)) and measured the enzyme activity and protein concentration.

Dialysis step

The dialysis bag was used to conduct dialysis of the dissolved protein to remove ammonium sulphate residues from protein deposition. The solute protein was added to the bag and immersed in a buffer solution (phosphate buffer (0.001 M) pH 7). This operation was carried out for 24 hours and the solution was changed periodically at 4° C to maintain enzyme activity. Measuring enzyme activity and protein concentration after the end.

Gel exclusion chromatography

Gel exclusion techniques are based on differences in molecular weight. This step is used to purify the lipoxygenase from proteins and related salts. A Sephadex-G.100 filter column was used.

1- The column diameter to this step is (2 cm) and length (50 cm) and contain a filter at its end to prevent the resin granulating to outside. and fill it with a resin solution and pour the resin solution on the wall slowly and evenly to avoid the formation bubble that impediment separation process. the column was washed with a buffer solution (phosphate buffer (0.001 M) pH 7), and the flow rate was set at (1 mL / min).

2- four mL of the product in the dialysis step was slowly and gradually added on the column wall to the resin surface and left for 5 minutes to be soaked into the resin.

3- Start the separation process using a 250 mL solution (buffered phosphate (0.001 M) pH 7). The elution was collected from the gel filtration column with a size 5 mL per part.

4- Evaluation the LOX activity and protein concentration for the elute.

Electrophoresis

Garfine methods was used to electrophoresis in Polyacrylamide gel under non denatured condition [25].

Estimate the activity of GGT in the serum

The Szasz method is used to measure the effectiveness of GGT enzymes [26], and the reaction equation as follows

$$L-GAMMA-Glutamyl-3-carboxy-4-nitroanilid+Glycyl\ glycin \overset{GGT}{\Longrightarrow} L-GAMMA-Glutamyl-\ Glycylglycin+5-Amino-2-nitro-benzoat$$

The activity of the enzyme is directly proportional to the product at a wavelength of 405nm.

Statistical Analysis

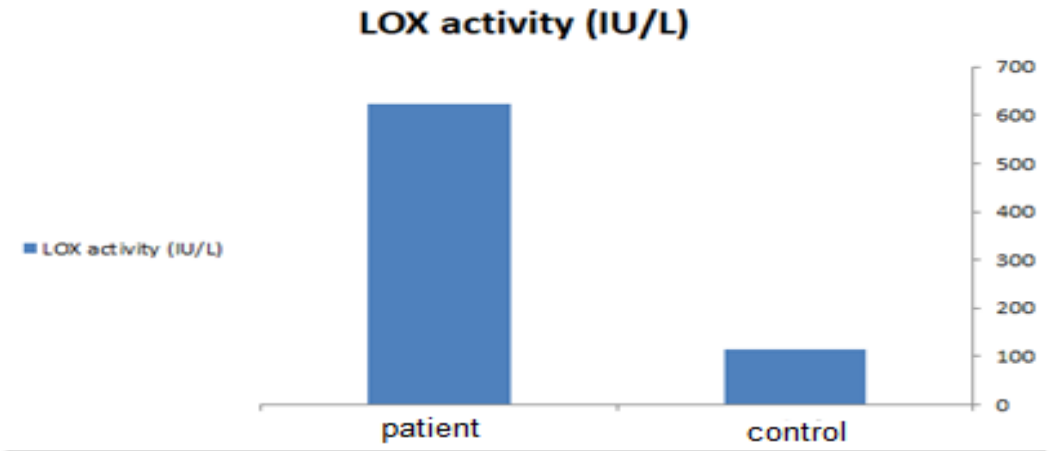
The statistical analysis was done by using SPSS (statistical package for social sciences) version 14 in which mean, standard and error. student t-test and Excel were used for data comparison and a p value of <0.05 was considered to be statistically significant [27].

Results and Discussion

The study included 50 patients with prostate cancer. The study also included 50 samples healthy male as comparison group, patients and healthy aged 40-80 years.

Estimate LOX activity in serum Patients prostate cancer

The results displayed that the LOX activity in the serum of patients with prostate cancer increased. A comparison statistical of LOX activity between the patient's serum and the control group showed that the enzyme activity of the patient was significantly increased with a probability $P \leq 0.000$ compared with control group. As shown in figure 1.



Parameter	Control Mean±S.D N=50	Patients Mean±S.D N=50	T test	P value
LOX (IU/L)	114.6± 49.77	623.25± 256.91	8.69	0.000

Figure 1: The activity of lipoxygenase in control and patients groups

In general, the results indicate an increase LOX activity in serum prostate cancer patients, and previous scientific literature indicates that LOX has increased its activity in human prostate cancer cells lines [28], and this high effectiveness was reported to seems to stimulate angiogenesis in prostate cancer cells of human [29]. In addition, the 5-lipoxygenase product may be acting as a potent growth-promoting factor for human prostate cancer cells [30].

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Partial purification of Lipoxygenase from patients of prostate cancer

LOX has been purified in several steps as shown in table 1. The step one is to precipitate and isolate the enzyme from the serum by using ammonium sulfate at concentration (0-40) %. In the Step two, the dialysis method was performed by using dialysis bag to obtain a degree of purity enzyme and desalting. In the step three, Exclusion chromatography technique is used to purify lipoxygenase from other proteins and salts. We used the column filtration with Sephadex G-100 resin, in this step we can obtain single peak in at yield (27.77) % and (6.732) times purified as shown in figure 2.

Previous literature has shown that LOX was purified from a variety sources, it purified from serum of women with breast cancer [31], purified from serum male patients with asthma [32], and purified from serum male of cardiovascular disease patients [33], also purified from human placenta [34]. Previous scientific literature did not indicate this enzyme is purified from the serum of prostate cancer patients.

Table 1: Partial purification of the lipoxygenase from serum patients of prostate cancer

Step	Elute (ml)	Activity (IU/L)	Total activity (IU)	Protein con. (g/L)	Total protein (g)	Specific activity (IU/g)	Purification (fold)	Yield %
Crude	6	360	2.16	73.641	0.441	4.897	1	100
Ammonium Sulphate (0-40)	5	420	2.1	24.816	0.12408	16.924	3.455	97.22
Dialysis	4	480	1.92	13.244	0.0529	36.294	7.411	88.88
Gel filtration sephadex G100	5	120	0.6	3.641	0.0182	32.967	6.732	27.77

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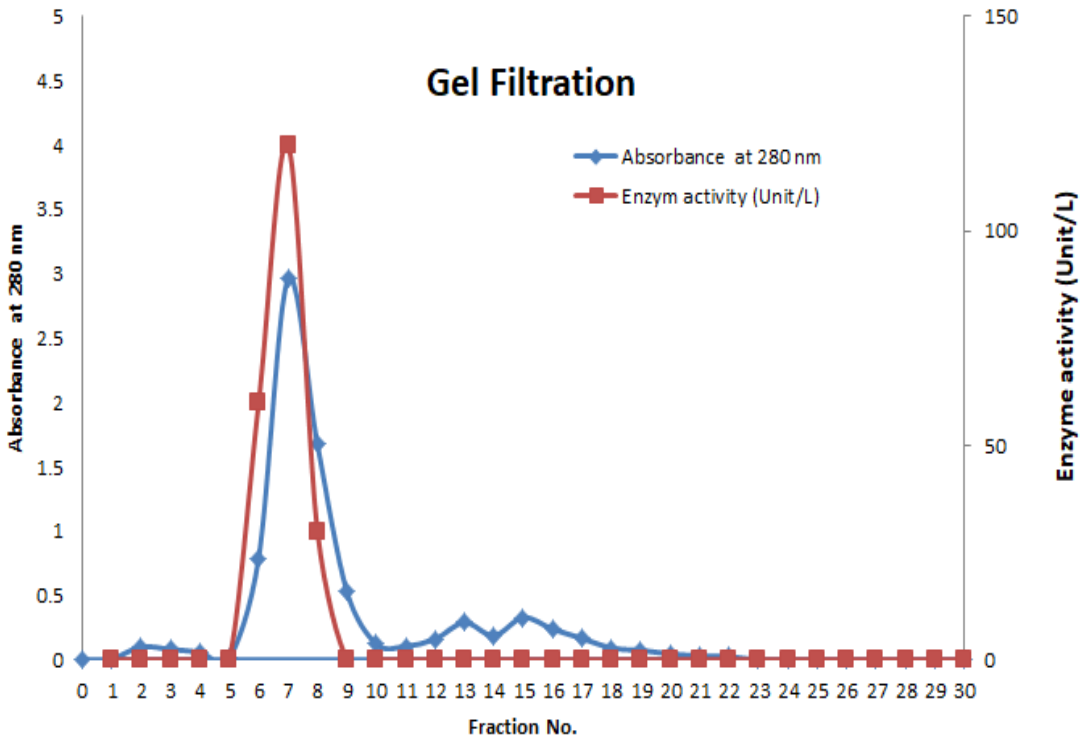


Figure 2: The absorbance at 280 nm and serum LOX Activity for exclusion filtration step in prostate cancer by used column diameter is (2 cm) and length (50 cm) fill it with a Sephadex G – 100 resin solution. It was balanced with a phosphate buffer solution (PH=7(0.001 M)), and the flow rate was set at (1 mL / min). the process started using a 250 mL solution (buffered phosphate (0.001 M) pH 7). The elution was collected with a size 5 mL per part.

Estimation Molecular weight for Enzyme by electrophoresis

The electrophoresis is technique that can be used to determine M. Wt through the linear relationship between protein movement within the gel and molecular weight logarithm. The gel can be calibrated with proteins knows molecular weight to be used later to determine unknown molecular weights.

The standard curve (the relationship of the molecular weight logarithm and the RM relative motion) of the standard proteins was used in polyacrylamide gel under non denatured condition. The molecular weight of the enzyme was (70KD) and is shown in figure 3.

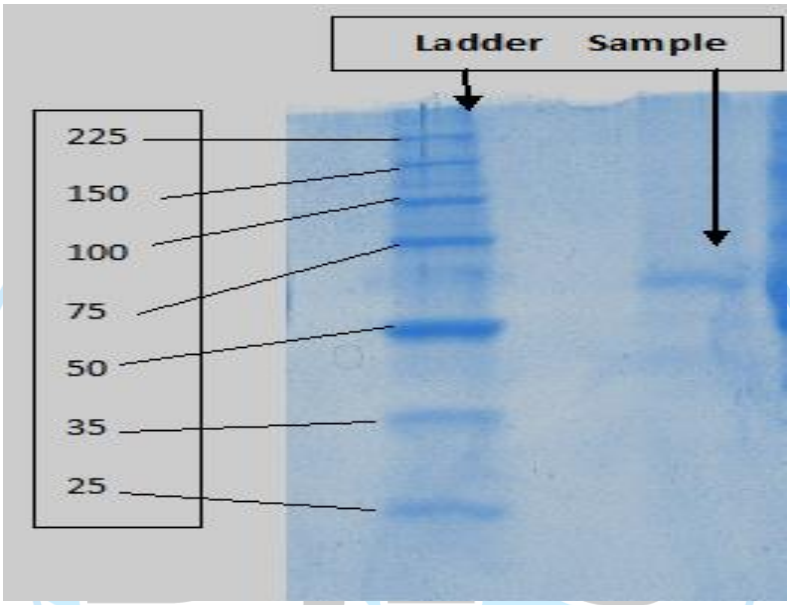


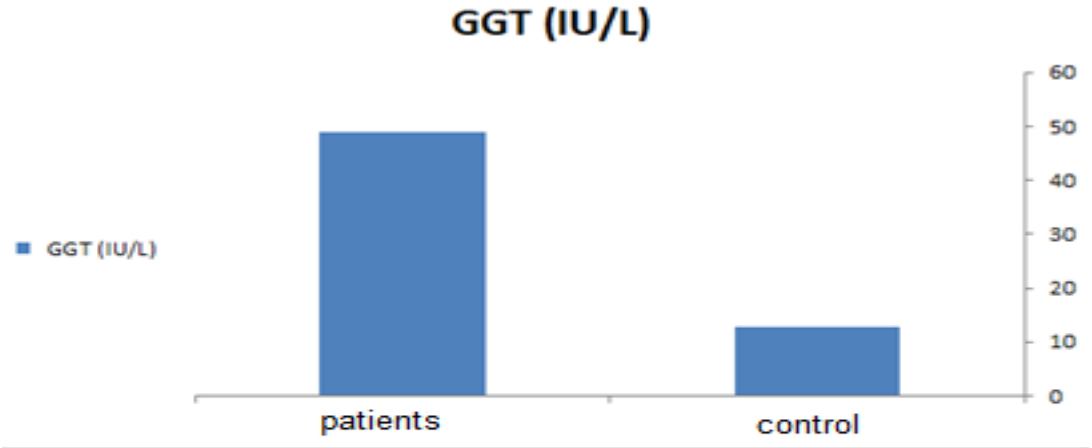
Figure 3: Electrophoresis used Polyacrylamide gel under non denatured condition

The molecular weight of the enzyme varies according to its sources, in humans, the molecular weight of the LOX enzyme was estimated by the - Al-Barqawi researcher in asthmatic patients. It was found that the molecular weight (35KD) of the one synthetic unit, and that the LOX enzyme consists of two synthetic units [32 and 35]. but the purified enzyme from T lymphocytes in humans was molecular weight (79KD) [36].

Estimate the activity of GGT

The statistical analysis results also showed the effectiveness of GGT in prostate cancer patients was higher than that of the control. As shown in figure 4.

Previous literature has shown that patients with prostate cancer have high serum GGT activity [37, 38]. The reason for the high GGT activity may be due to the involvement of GGT in the production of free radicals and the peroxidation of unsaturated fatty acids, which involved in different tumorigenesis [39 and 40].



Parameter	Control Mean±S.D N=50	Patients Mean±S.D N=50	T test	P value
GGT (IU/L)	12.79± 5.68	48.99± 15.43	10.64	0.000

Figure 4: The effectiveness of GGT in serum control and patients' groups

Conclusions

Through the current study, the conclusions were reached:

1-Having a high in the activity of the enzyme lox in patients compared to healthy group. This increase in enzyme activity in patients can be used as a tumor marker to detect the presence of prostate cancer.

2-There was a significant increase in the activity of the enzyme GGT in patients compared to healthy group.

3-The molecular weight of the enzyme was determined where it was 70KD

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