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Lipoxygenase and Colon Cancer

Muslim Abas Abed AlAdlee

Abstract

Colon cancer is an abnormal growth of cells that occurs in the large intestine. Sometimes growth remains restricted for a relatively long time before it becomes a malignant tumor and then spreads through the intestinal wall to the lymph nodes and other parts of the body. The study aims to estimate the effectiveness and partial purification of lipoxygenase (LOX) enzyme and measure gamma-glutamyl transferase (GGT) activity in serum patients of colon cancer in Baghdad. The study included (80) case male patients with colon cancer with (50) samples of apparently healthy males (control) as comparison group. The result displayed a noteworthy increase in lipoxygenase effectiveness (805.0 ± 517.23 IU/L) in serum of patients with colon cancer (T3 stage) compared with control (114.6 ± 49.77 IU/L). The enzyme was purified by the precipitation of the serum protein using (40% $(\text{NH}_4)_2\text{SO}_4$) then removing the remaining salts by dialysis. The column of gel (sephadex G.100) was used to separate the enzyme from another protein, in this step a single peak was obtained. The effective part of lipoxygenase at yield (71.42%) and folds (11.033). The ion exchange chromatography (DEAE-CeA50) was used to isolate LOX isoenzyme, two bands (LOX1 and LOX2) were acquired with different degree of purity (16.372) and (12.16) folds respectively. The result displayed a noteworthy increase in the (GGT) activity in patients (58.69 ± 16.94 IU/L) (probability $P \leq 0.000$) compared with control (12.79 ± 5.68 IU/L). The increase in activity of LOX can be used as a tumor marker to detect the colon cancer disease.

Keywords: Colon cancer, Lipoxygenase (LOX), Gamma-glutamyl transferase (GGT)

1. Introduction and literature review

1.1 Cancer

Is abnormal and uncontrolled cell growth that may spread to the surrounding tissues, and subsequently spread to other parts of the body [1]. It is also known as a group of tumors that result from abnormal cell division and growth and have clear behavior and close characteristics [2]. However, the behavior of cancer may differ depending on the organ in which it originated, some of them grow rapidly, and some of them have slow growth and may be destroyed very quickly and in an unregulated manner. The risk of cancerous tumors is the possibility of continuous proliferation in tumor cells in a specific area, which increases pressure Within the tissue and leads to paralysis of the blood flow process, which ultimately leads to tissue damage [3], and the rupture of the tissues surrounding the tumor weakens the natural barriers and the rupture results due to the increase in pressure in the tissues as a result of the influence of enzymes or toxic substances produced, stored

and secreted by the invading tumor cells [4]. Tumors secrete digestive enzymes that cause, at a high rate, to damage tissues and facilitate tumor invasion, and these enzymes are known as hydrolytic enzymes. These enzymes possess the ability to separate tumor cells from one another, making it easier for tumor cells to move freely and then penetrate into the normal tissues of the host [5]. That changing the genetic makeup of cells that may occur due to internal or external factors leads to an abnormal division, which leads to the formation of tumors, and tumors are of two types, benign tumors, these tumors are slow-growing, defined by a membrane, they remain in one place and do not extend to nearby tissues or healthy organs [6]. As for malignant tumors, these tumors are rapidly growing. They move to the surrounding healthy tissues through the blood and lymphatic system from their original position (the primary tumor) to other organs of the body to form secondary tumors [7]. Tumors usually arise due to changes or mutations in the DNA deoxyribonucleic acid, and the change is in two types of genes, the first type is oncogenes that stimulate cell division, and that increasing the activity of these genes helps cancer cells to grow abnormally and work on protect cells from apoptosis, while the second type is the tumor suppressor genes or apoptosis genes that work to stop cell division and help the immune system protect tissue [8]. In the case of a tumor, these genes stop, because they oppose its formation by correcting errors in DNA transcription.

It should be noted that cancer occurs in all cases due to mutation, but not all mutations cause cancer. Cancer results from the activation of abnormal activation of cellular genes that regulate cell growth and divisions. Determining the stage of the tumor expresses the extent of the tumor's progress and exacerbation and is necessary before starting the treatment, and thus we conclude that cancer is a disorder that results from the failure of cells to die, rather than the process of cell proliferation, as the proliferation is not matched by a sufficient number of cells that die, which leads to their accumulation [9].

1.1.1 Colon cancer

It is the abnormal growth of cells that occurs in the large intestine, sometimes the growth remains restricted for a relatively long period before it turns into a malignant tumor and then spreads through the intestinal wall to the lymph nodes and other parts of the body [10].

The site of colon cancer extends from the cecum to the sigmoid colon (about 15 cm above the anus), and rectal cancer extends from the sigmoid colon to the anus [11]. Usually colon cancer begins in the form of a non-cancerous growth called a polyp, which then develops on the inner lining of the colon and grows slowly for a period of [10–20] years [12].

The metabolism of fats in the human body, especially the arachidonic acid metabolism pathway, plays a major role in chronic inflammation and colon cancer [13], as phospholipase A2 (PLA2) enzymes stimulate the formation of free fatty acids such as arachidonic acid from phospholipids associated with the cell membrane, which have been shown to participate in the formation of Cancer in laboratory mouse models [14].

Lipoxygenase is found in the human body, has an important role in stimulate inflammatory reactions. Immoderate amounts of reactive oxygen free radical can cause inflammation that activate the release of cytokines and the posterior activation of LOX. Inflammation is associate with many diseases, such as cancer, cardiovascular, and stroke and neurodegenerative diseases. LOX is contribute in the synthesis of leukotrienes and prostaglandins. They are associated with disease development [15]. The most important enzymes in the pathway of arachidonic acid

metabolism [16] are lipoxygenase and cyclooxygenase, which were found in high concentrations in many tumors, including lung cancer [17], prostate cancer [18], brain cancer [19], rectal cancer [20], Skin cancer [21], and breast cancer [22]. where the GGT enzyme enters in the metabolism pathway of Leukotrienes C4 [23].

Spread of colon cancer are common in northwest Europe, North America, while at least in the continent of Africa, Asia and some parts of North America [24]. It is classified fourth among the common types of cancer, and is the second among cancers that cause death in the United States for both sexes, and the incidence and death rates for males are much higher than for females [25].

1.1.2 Staging of colon cancer

Tumor (T):-

The TNM classification was used to show stages of colon cancer:

T1: the tumor grows only in the inner layer of the the intestinal wall.

T2: the tumor has grown through the intestinal wall and penetrates the muscle layer, but does not include the lymph nodes.

T3: the tumor grows through the intestinal wall and reaches the lymph nodes.

T4: becomes a metastatic tumor, usually spreading to the liver, lungs, and distant lymph nodes [26].

The **Figure 1** shows colon cancer stages.

Node (N):

describes whether the cancer has spread to the lymph nodes.

N0 means there are no lymph nodes containing cancer cells.

N1 is split into 3 stages – N1a, N1b and N1c:

- **N1a** means there are cancer cells in 1 nearby lymph node
- **N1b** means there are cancer cells in 2 or 3 nearby lymph nodes
- **N1c** means the nearby lymph nodes do not contain cancer, but there are cancer cells in the tissue near the tumor

Figure 1.
Shows colon cancer stages.

N2 is split into 2 stages – N2a and N2b:

- N2a means there are cancer cells in 4 to 6 nearby lymph nodes
- N2b means there are cancer cells in more than 7 nearby lymph nodes

Metastasis (M):

M0 means the cancer has not spread to other organs.

M1 means the cancer has spread to other parts of the body such as the lung or liver. It is split into 3 stages, M1a, M1b and M1c:

- M1a means the cancer has spread to 1 distant site or organ, for example the liver, but it has not spread to the tissue lining your tummy (peritoneum).
- M1b means the cancer has spread to 2 or more distant sites or organs, but it has not spread to the tissue lining your peritoneum.
- M1c means the cancer may have spread to distant organs and it has spread to your peritoneum.

1.1.3 Causes of colon cancer

The infection occurs by more than 75–95% in people, where the genetic infection in this percentage is very little or no, The following risk factors include: aging, race, gender, high intake of fat, sugar, alcohol, red meat, and processed meat, as well as obesity, smoking and lack of physical activity [27].

1. Pathological Causes:

People who suffer from inflammatory bowel disease (Ulcerative colitis) and Crohn's disease are more likely to develop colon cancer [28, 29], and the risk of cancer increases with the increase in the duration of the disease, and is worse when the severity increases Inflammation, people with large intestine inflammation make up less than 2% of colon cancer annually. 2% of people with Crohn's disease will develop colon and rectal cancer over a period of 10 years, 8% after 20 years, 18% after 30 years [29].

2. Genetics Causes:

People who have a family history of colon cancer related relatives (such as a father or brother) have the risk of colon cancer two to three times more, and that this group constitutes 20% of all cancer cases. There are a number of genetic syndromes that are also associated with a high rate of colon cancer. The most common syndrome is Lynch syndrome, which accounts for about 3% of colon cancer cases [26]. Other syndromes that are strongly associated with colon cancer is Gardner syndrome. And familial adenomatous polyposis (FAP) [30], people with these syndromes develop colon cancer in 1% of the total cases [31].

The most cases of death due to colon cancer are associated with metastatic disease, where he was identified and isolated gene (metastasis associated in colon cancer 1 (MACC1)) that shows the possibility of contributing metastatic disease [32].

As well as non-genetic factors such as abnormal methylation of DNA tumor suppressor promoters also play a role in the development of colon cancer [33].

2. Materials and methods

2.1 Collection of samples

Blood samples of colon cancer patients (40–80 years) were obtained from the Teaching Oncology Hospital at the City of Medicine and the National Center for Oncology - Baghdad for the period (18-2-2018 to 28-2-2019). The samples were 80 samples of blood.

A total of 50 blood samples were collected from apparently healthy individuals as a control group (40–80 years). The samples were collected by drawing blood from the vein (5 mL) using a syringe and placing the blood in a gel tube. The tubes were placed in the centrifuge at 1252 g for 10 minutes to obtain serum. The serum was kept by eppendorf tube in deep-freeze at -20°C until testing.

2.2 Measuring the LOX activity in blood serum

The method of measuring the activity of the LOX enzyme (liu,1998) [34] is based on stimulating the oxygen reaction with the unsaturated fatty acids containing (cis, cis –1.4-pentadiene). It consists of a sequential system of double bonds that increase absorption at a wavelength of 234 nm where the absorption intensity is directly proportional to the concentration of the enzyme [35]. The unit of enzyme is defined as the amount of enzyme that changes in absorbance by 0.001 / sec at wavelength (234 nm) under ideal conditions.

2.3 Estimation protein concentration

The biuret method was used to estimate the concentration of the protein in the samples [36].

2.4 Separation and purification of LOX from serum patients of colon cancer

LOX is purified using the following steps:

2.4.1 Precipitation by ammonium sulphate

The serum proteins were deposited by adding (0.9) gm of ammonium sulphate (0–40%) to 4 ml of serum for patients with colon cancer, which was gradually added in ice bath with magnetic stirrer (15 minutes) until all the ammonium sulphate has been dissolved. Then the solution was placed in the centrifuge for 15 minutes and at a speed of 17608 g to separate the precipitation from the leachate, the precipitate was dissolved with the least amount of the buffer solution (Buffer phosphate pH 7(0.001 M)). Then, the enzyme activity and protein concentration were measured.

2.4.2 Dialysis

The process of dialysis for the dissolved protein was done to remove the ammonium sulphate residues that was used to precipitate the proteins, using a dialysis bag. The dissolved protein was added into the bag and immersed in the buffer solution (Buffer phosphate (0.001 M) pH 7). This process was carried out for 24 hours, with the solution being changed periodically. This step of purification was

done at 4° C to maintain the activity of the enzyme. The activity and protein concentration of the enzyme were measured after the end of the process.

2.4.3 Gel filtration

The gel filtration technique is based on the difference in molecular weights. This step was used to purify the LOX enzyme from proteins and associated salts. The filter column of the Sephadex G.100 was used.

- A column separating diameter (2 cm) and length (70 cm) with a filter at the end of which prevents the granulation of the resin outside was used, the process of casting the column was performed by using resin solution and pouring the resin solution on the walls slowly and homogeneously so as not to form air bubbles that impede the separation process, the column was then washed with a quantity of buffer solution (Buffer phosphate(0.001 M) pH 7), and the flow velocity was set at (1 mL / min).
- Four mL of product in dialysis step were added slowly and gradually over the resin surface and on the column walls and left for 5 minutes to soak into the resin.
- The gel filtration process was initiated using 250 mL of the buffer solution (Buffer phosphate(0.001 M) pH 7). The extracts were extracted from the gel filtration column at a size of 5 mL per part.
- The activity and the protein concentration of the lox enzyme were evaluated.

2.4.4 Ion exchange chromatography

This technique was used to purify the isoenzyme of the LOX.

- DEAE-Cellulose (A50) was prepared by dissolving 20 gm of DAEA-Cellulose A50 in 250 mL of Buffer phosphate pH [7], leaving the solution suspended for 24 hours and at 4° C. The solution was switched several times from time to time to remove the soft minutes from the suspended solution until the pH reaches 7.
- NaCl solution (1 M): was prepared by dissolving 5.85gm of sodium chloride in 100 mL of (Buffer phosphate (0.001 M) pH 7) solution. Other solution were obtained with graduated concentrations of NaCl (0.1,0.25,0.5,0.75 M).
- A glass column diameter (3 cm) and length (30 cm) contains a filter at the end which prevents the resin granules from leaking out of it was used, the process of casting the column was performed by using resin solution with pouring the resin solution on the walls slowly and homogeneously so as not to form air bubbles that impede the process of ion exchange, then The column was washed with 250 mL of the buffer solution (Buffer phosphate (0.001 M) pH 7) and the flow time and velocity were set at 1 mL / min.
- Three ml of protein from the gel filtration step were added slowly on the column walls and left to soak into the column. The separation process was initiated using (500 mL) of the buffer solution containing NaCl (25,50,75,100 mM) progressive concentrations and the elute parts (3 mL) were collected for each part. Then the activity of the LOX and the protein concentration was evaluated.

2.4.5 Measuring GGT activity in blood serum

The Szasz method [37] was used to measure the effectiveness of the GGT enzyme, and the reaction equation is shown in Eq. (1):

Equation (1) the reaction of measured the effectiveness of the GGT.

The activity of the enzyme is directly proportional to the formation of 5-amino-2-nitro-benzoate at a wavelength of 405 nm.

2.5 Statical analysis

Statistical analysis was carried out using SPSS (version 16). Graphs were drawn using the Excel (2010), where ANOVA, arithmetic mean and standard deviation were used. The minimum probability factor ($p \leq 0.05\%$) was statistically significant.

3. Results and discussion

The study included (80) males with colon cancer. The study also included [38] samples of healthy (control) males, as comparison groups, and the range the age for patients and healthy between (40–80) years.

3.1 Measurement of LOX activity in blood serum

The activity of LOX was estimate in patients with (T3 stage) of colon cancer.

The results of the study included the statistical values of colon cancer patients and the biochemical variables measured in serum patients and control group.

The results showed that there was an increase in the activity of LOX in the blood serum of patients with colon cancer. A statistical comparison between the effectiveness of LOX in patients' and control showed a significant excess in enzyme effectiveness in patients with probability ($P \leq 0.000$) compared with control, as shown in **Figure 2**.

Overall, the results indicated an increase in the activity of LOX in the serum of colon cancer patients, previous scientific literature did not indicate that the enzyme's activity was measured from the serum of colon cancer patients, but indicated an increase in the activity of the enzyme in human colon cancer cell lines [30, 39, 40], this high effectiveness was reported to be highly correlated with reproduction of cancer cells, angiogenesis, and, resistance to apoptosis [41, 42].

Also the increase in enzyme activity is due to the increase in the digestion of unsaturated fatty acids and the release of Eicosanoid compounds that promote the growth of cancerous tumors [43].

Separation and Purification of LOX from Serum Patients of Colon Cancer:

LOX was separated and purified in several steps as shown in the **Table 1**.

The first step was precipitating and separating the enzyme from blood serum by using ammonium sulphate salt at a concentration (0–40)%. In the second step, the dialysis was performed to obtain a degree of purity and desalting. In the third step size-exclusion chromatography technique was used to purify the Lox from the proteins and other salts associated with the enzyme. The filtration column of the

Figure 2.
The effectiveness of LOX in sera of control and patient.

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	420	2.52	78.3	0.4698	5.363	1	100
Ammonium Sulphate (0–40)	5	480	2.4	24	0.12	20	3.729	95.23
Dialysis	4	540	2.16	13.6	0.0544	39.705	7.403	85.71
Gel filtration sephadex G100	5	360	1.8	6.093	0.0365	59.17	11.033	71.42
Ion exchange DEAE-C A50 Isoenzyme-II	3	180	0.54	2.05	0.00615	87.804	16.372	28.57
Isoenzyme-I	3	120	0.36	1.84	0.00552	65.217	12.16	21.42

Table 1.
Separation and purification of the lox enzyme from serum patients of colon cancer yield.

sephadex G-100 resin was used in this step, a single peak was obtained at yield (71.42) % and (11.033) times of purification as shown in **Figure 3**. In the final ion exchange chromatography technique step was used to separate the LOX isoenzyme that based on the difference in charge. DEAE-Cellulose A50 resin was used, two isoenzyme were obtained with varying degrees of purity at a yield (28.57)%, (21.42)%, respectively and times of purification (16.372), (12.16) as shown in **Figure 4**. It has been noted in previous scientific literature that LOX was purified from various sources purified from the serum of male patients with cardiovascular disease [44],purified from serum in men with asthma [45] and it was also purified

Figure 3.
Activity and absorbance at 280 nm for the fraction of gel filtration step of Sephadex G-100 resin.

Figure 4.
Activity and absorbance at 280 nm for the fraction of ion exchange step by using DEAE cellulose A-50 resin.

from the serum of women with breast cancer [46]. Previous scientific literature has also indicated that the enzyme was purified from the colon cancer cell line [47] but did not indicate that the enzyme was purified from blood serum of colon cancer patients. Also the scientific literature indicated that the enzyme was purified from various other sources, including soybeans, where the number of times of purification (7.7 times) at yield (41%) [48]. The enzyme was also purified from Human Placental at yield (21.84%) [49].

3.2 Measurement of GGT activity in blood serum

The results of the statistical analysis also showed a higher activity of GGT in colon cancer patients compared to control as shown in **Figure 5**.

Figure 5.
The activity of GGT in sera of patients and control groups.

Previous scientific literature has indicated a high GGT activity in serum colon cancer patients [38, 50]. The reason for the high activity of GGT is due to that the GGT is involved in generating free radicals and peroxidation of unsaturated fatty acids, which are involved in various tumorigenesis [51, 52].

4. Conclusion

1. There is an increase in the activity of LOX enzyme in patients compared to the healthy group. This increase in enzyme activity in patients can be used as a tumor marker to detect the presence of colon cancer with other tumor markers.
2. There was a significant increase in the activity of the enzyme GGT in patients with colon cancer compared to the healthy group.
3. Two isoenzymes of LOX were obtained using ion exchange chromatography.

Authors’ declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in Tikrit University.

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Author details

Muslim Abas Abed AlAdlee
General Directorate of Diyala Education, Ministry of Education, Republic of Iraq

*Address all correspondence to: mu_mh_2006@yahoo.com

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