Purification of Gamma Glytamyl Transferase and Study Some Biochemical Variables in Sera Patients with Hepatitis B and C in Kirkuk City

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Abstract

Hepatitis B and C are the most frequent causes of chronic hepatitis diseases in the world. A cross sectional study was carried out in Kirkuk city from 17th of November 2019 to 25th of February 2020 . The number of hepatitis patients under study was 80 hepatitis patients, 44 with hepatitis B (14 acute and 30 chronic) and 36 with hepatitis C (11 acute and 25 chronic) Their ages were between 20-70 years old. These patients admitted to Hepatology and Gastroenterology Center of Azadi Teaching Hospital and Kirkuk General Hospital and The control group matched the patients group included 30 individuals who were apparently healthy who were conducted in this study to determine the level of activity of the enzyme (GGT) as well as to determine the level of a number of biochemical variables (ALT, AST, ALP, TSB, CRP, AFP) in both healthy subjects and patient group with acute and chronic viral hepatitis B and C . The results showed that there was significant increase in the activity of the GGT enzyme in patients with P-value ($p \leq 0.01$) compared to control group. The present study showed that there was significant increase in the levels of (ALT, AST.ALP, TSB) in the blood serums of patients with acute hepatitis B and C compared to chronic and the healthy group. The present study also included the purification and partial isolation of the enzyme (GGT) and find the approximate molecular weight for it, that was purified from the blood serum of patients with hepatitis B and C and control group by using ammonium sulfate, Dialysis and gel filtrationchromatography with sephadex (G-150) to separate the enzyme from other proteins. The degree of purification in type C was (31.2) times, type B (27) times, and with healthy (30.2) times. The highsingle peak were used to determine the approximate molecular weight of the enzyme, and using the gel filtration technique. The approximate molecular weight of the enzyme (GGT) was up to (371,000-346,000-316,000) Dalton for healthy and hepatitis C and B patients, respectively. In acute and chronic viral hepatitis B and C, there was a positive correlation between the efficacy of (GGT) and the efficacy of GPT, CRP and AFP. The study showed that there was significant increase in the activity of the GGT enzyme in patients compared to control group. The results showed in Table 3 that there was significant increase in the activity of the ALT enzyme in patients compared to control group. After performing the process of separating and purifying the GGT enzyme from the serum of patients with viral hepatitis B and C, using 40-60% of ammonium sulfate and after obtaining the required purity separating it from the rest of the components of the blood serum, the activity of the GGT enzyme was measured and it was found to have overall effectiveness in patients with hepatitis Liver C was (0.282) and the number of purification times was (1.63) times and the specific effectiveness was (1843). Hepatitis B was (3.5) times, and the results from the dialysis process of the precipitate resulting from the saline precipitation process showed an increase in the specific activity of the enzyme GGT, so it was (3.4) for healthy people, (3.521) for patients with HCV and (4.3) HBsAg. Then he used gel filtration chromatography using the gel (Sephadex G-150), the results showed that a single protein beam appeared, which is highly effective and has a degree of purification reaching (30.2) And (31.2) and (27) times for GGT enzyme separated by gel filtration technique when the protein solution obtained from the blood serum of healthy subjects and patients with viral hepatitis is passed from the separation column containing gel (G-150).

Keywords: *Gamma GlytamylTransferase; HCV; HBV; Kirkuk; Liver enzymes.*

Introduction

Several enzymes act as catalysts in a specific biochemical reaction that leads to the normal state and the maintenance of regular balance in the body. Any changes in the enzyme level in the body could indicate a specific abnormality in the body. The enzyme Gamma Glutamyl Transferase (GGT) is a hepatic and biliary enzyme with a molecular weight of (68000) Dalton that is synthesized by hepatocytes as well as by the epithelial cells of the bile ducts [1], and it is a two-molecular glycoprotein, the large secondary unit and the small secondary unit are associated with a non-covalent [2]. The GGT enzyme (EC2.3.2.2) is one of the main antioxidants in many of the body's defense mechanisms [3]. It plays a key role in the metabolism and metabolism of glutathione [4]. it is classified within the enzymes that transport the peptide (Transpeptidase), as it stimulates the process of transferring the Gamma Glutamyl group of peptides and compounds that contain this group to some receptors that form the main substance Substrate or some amino acids or peptides or even water A simple decomposition process takes place.

Gamma-glutamyl-X + acceptor Gamma-glutamyl-acceptor + X

It also has an important role in the liver, and it has other functions in the body as a transporter molecule, helping to transport other molecules around the body, such as glutathione, to a receptor that may be an amino acid, peptide, or water [5]. The GGT enzyme is present in the cell membranes of many tissues [6] and is located on the outer surface of the plasma in almost all cells, but it is mainly involved in the epithelial tissues with their secretory and absorptive functions [7], and includes the kidneys, bile duct, pancreas, gallbladder, spleen, heart., Brain, and seminal vesicles[8]. In adult liver, the enzyme (GGT) is found in the bile duct from hepatocytes and in gallbladder cells and is responsible for secreting bile into the bile duct [9]. Glutathione is the basis for the physiological enzyme (GGT) in mammals and The relationship between glutathione and GGT is presumed to be a means of supplying the liver with cysteine and Glycin.

Materials and Method

Thirty samples were collected from the blood of healthy people of varying ages from (20-50) years, These samples were collected by blood donors. (80) samples were collected from the blood of patients infected with viral hepatitis (B, C) after they were diagnosed by specialized doctors in the lobbies of the digestive system and the public health laboratory in Kirkuk. The hepatitis patients under study was 44 with hepatitis B (14 acute and 30 chronic) and 36 with hepatitis C (11 acute and 25 chronic) Their ages were between 20-70 years old, The samples collection period is from 11/17/2019 to 2/25/2020.

Estimation of GGT Activity in Serum: The basic principle of this method is known as Szasz, A kinetic chromatometric method for determining GGT activity [10]. The interaction can be explained as follows: This process liberates the compound (5-amino-2-nitrobenzoate, which can be measured at 405 nm). The increase in absorption of this compound at this wavelength is directly related to the increase in GGT activity. Where 1000 µmol of puffer is mixed with 100 µmol of serum and incubated at a temperature of 37 for a minute, then (250 µmol) of the base material is added to the mixture and incubated for a minute, then the absorbance is recorded at (405 nm) within 3 minutes.

Separation and purification of Gamma-Glutamyl Transferase (GGT) f: Ammonium sulfate is the first stage in the purification process during which the proteins present in the serum are deposited, depending on the degree of saturation of the serum with ammonium sulfate [13]. Where (1.88 gm) of ammonium sulfate was added slowly with constant stirring to (4.7 ml) of serum over a period of 60 minutes at low temperatures in order to obtain a saturation rate of (40%). The filtrate was collected and placed in a centrifuge cooled at a temperature of (4° C) and for a period of (20 minutes) the filtrate was taken and an ammonium sulfate (2.4 gm) was added to obtain a saturation rate of (60%), then It was centrifuged at (14600 xg) for (20 minutes) to obtain the precipitate, the precipitate dissolved in (mL) of (0.125M) Tris-HCl with a pH of (8.3). Then it is placed in a centrifuge at a speed (14600 xg) for a period of (20 minutes) at (4° C), after which the total protein concentration is measured.

The dissolved protein is placed in the hemodialysis bag membrane after measuring GGT activity and protein concentration, and the pouch is immersed in buffer solution (HCl-Tris 0.125 M).) From a pH of 8.3. The solution is changed from time to time for an entire night. This step is performed at (4° C) to maintain GGT activity.

Gel filtration is also used as a method for estimating the approximate molecular weight of protein substances[14]. The Laemmli method was used to prepare electrolyte separation gel with modifications using 10% acrylamide gel and Coomassie bright blue [15].

Results

The study showed that there was significant increase in the activity of the GGT enzyme in patients compared to control group.

Table 1: GGT enzyme level in acute and chronic hepatitis C and B patients and the control group.

Studied groups	N	GGT (U/L)	P. value
		Mean±SD	
Patients with acute hepatitis C	10	202.4±52.0	<0.01
Patients with chronic hepatitis C	25	76.68±10.53	
Patients with acute hepatitis B	14	192.07±26.04	
Patients with chronic hepatitis B	30	63.47±15.64	
Control group	30	40.43±15.29	

The results showed in Table 3 that there was significant increase in the activity of the ALT enzyme in patients compared to control group, Table 2.

Table 2: GPT enzyme level in acute and chronic hepatitis C and B patients and control group.

Studied groups	N	ALT (U/L)	P. value
		Mean±SD	
Patients with acute hepatitis C	10	671.3±164.1	P<0.01
Patients with chronic hepatitis C	25	34.04±8.18	
Patients with acute hepatitis B	14	606.0±151.6	
Patients with chronic hepatitis B	30	27.00±7.05	
Control group	30	14.96±6.91	

Proteins were precipitated from their protein solution by removing the solvent to make them insoluble. After performing the process of separating and purifying the GGT enzyme from the serum of patients with viral hepatitis B and C, using 40-60% of ammonium sulfate and after obtaining the required purity separating it from the rest of the components of the blood serum, the activity of the GGT enzyme was measured and it was found to have overall effectiveness in patients with hepatitis Liver C was (0.282) and the number of purification times was (1.63) times and the specific effectiveness was (1.843) as shown in the table (3-6). In hepatitis B patients, the overall effectiveness of GGT enzyme was found (0.27), the number of purification times (1.63), and the specific effectiveness (2.077), as shown in Table (3-7). The enzyme was concentrated and a degree of purity was obtained and the salts were eliminated during the separation process by membrane sorting by Tris-HCl with an acidic function of PH (8.3). Hepatitis B was (3.5) times, and the results from the dialysis process of the precipitate resulting from the saline precipitation process showed an increase in the specific activity of the enzyme GGT, so it was (3.4) for healthy people, (3.521) for patients with HCV and (4.3) HBsAg. Then he used gel filtration chromatography using the gel (Sephadex G-150), the results showed that a single protein beam appeared as shown in Figures (3-3), (3-4) and (3-5), which is highly effective and has a degree of purification reaching (30.2) And (31.2) and (27) times for GGT enzyme separated by gel filtration technique when the protein solution obtained from the blood serum of healthy subjects and patients with viral hepatitis is passed from the separation column containing gel (G-150).

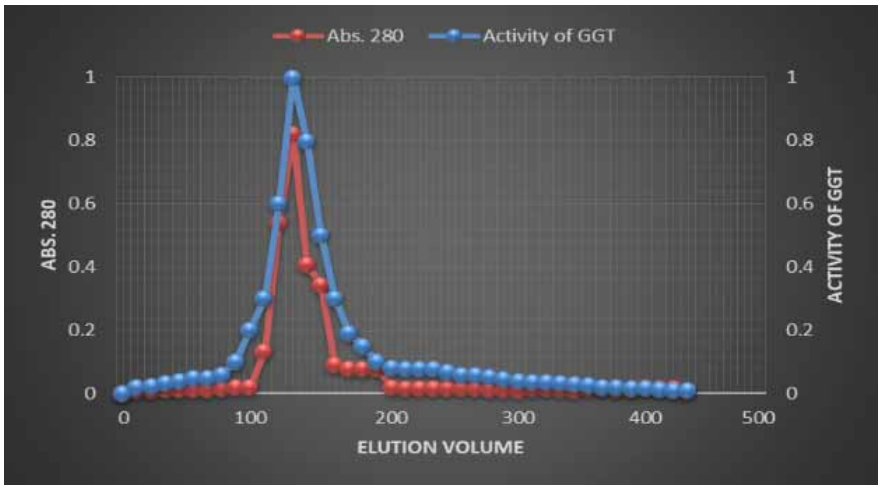

Figure 1: The profile shows the demonstration of the highly effective GGT protein packet from the gel filtrate column of the healthy blood serum.

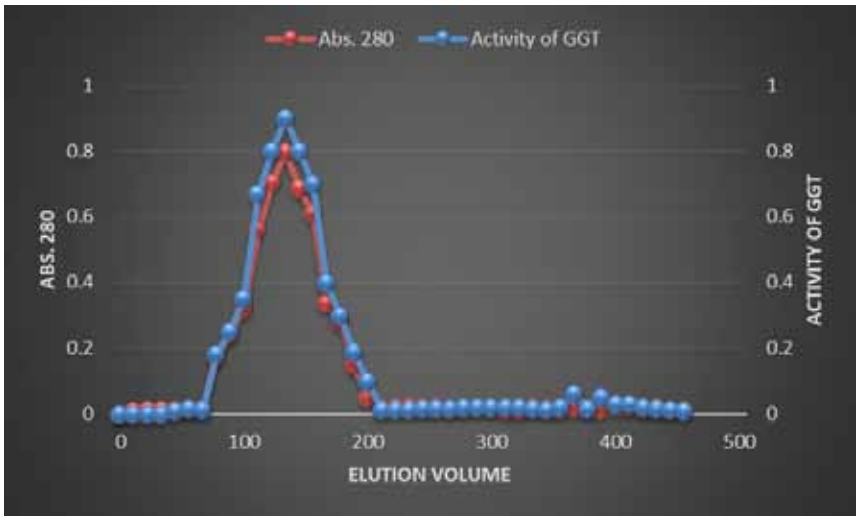

Figure 2: The profile Rogan shows the high-potency GGT protein packet from the gel-filtration column of the serum of patients with viral hepatitis C.

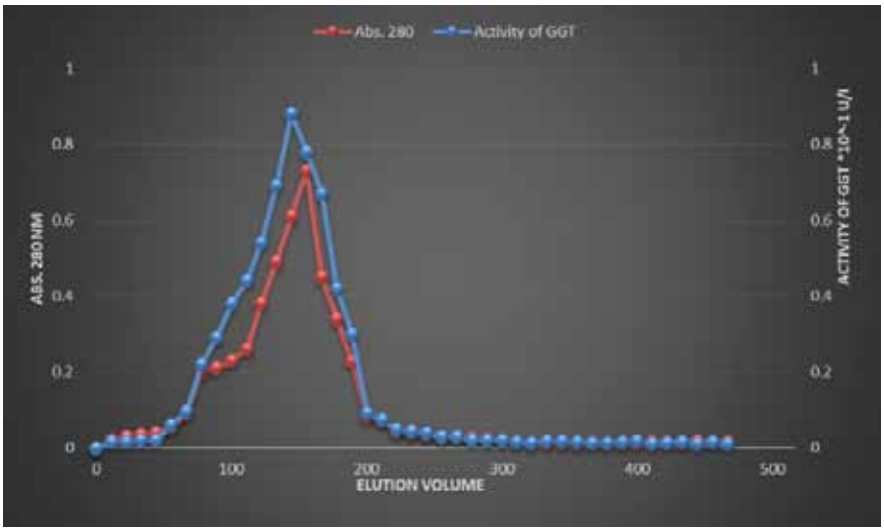

Figure 3: The profile shows the Rogan of the high-activity GGT protein packet from the gel filtrate column of the serum of patients with hepatitis B.

Table 5: Steps of GGT enzyme purification from blood serum of healthy subjects

Purification Step	Volume (L)	Protein Conc (g/L)	Total Protein (g)	Enzyme Activity (U/L)	Total Activity U	Specific Activity (U/ mg)	Fold of Purification	Yield 100 %
Serum	0.005	61.2	0.306	51	0.25	0.817	1	100
Precipitatioby Ammonium sulphate 60%	0.0048	24.1	0.115	40	0.192	1.67	2	76.8
Dialysis	0.0032	12.2	0.039	42	0.134	3.4	4.16	53.6
Gel Filtration Sephadex G-150	0.0027	1.9	0.005	45	0.121	24.2	30.2	48

Table 6: Steps for GGT enzyme purification from blood serum for people with viral hepatitis C.

Purification Step	Volume (L)	Protein Conc (g/L)	Total Protein (g)	Enzyme Activity (U/L)	Total Activity U	Specific Activity (U/ mg)	Fold of Purification	Yield 100 %
Serum	0.0051	68.4	0.348	77	0.392	1.126	1	100
Precipitationby Ammonium sulphate 60%	0.0047	32.6	0.153	60	0.282	1.843	1.63	71.9
Dialysis	0.0038	18.2	0.069	64	0.243	3.521	3.1	61.9
Gel FiltrationSephadex G-150	0.0032	2.1	0.006	66	0.211	35.166	31.2	53.8

The approximate molecular weight of the protein bundle produced when the serum resulting from the dialysis step was passed into the separation column that contains the gel of the type sephadex G-150 and the molecular weight was The enzyme had limits (371000) Dalton For healthy subjects, also for patients with HCV the approximate molecular weight of the enzyme was about (346000) Dalton approximately, as well as for patients with HBsAg, the approximate molecular weight of the enzyme was approximately (316000) Dalton .These values were taken from the Figures 4,5,6.

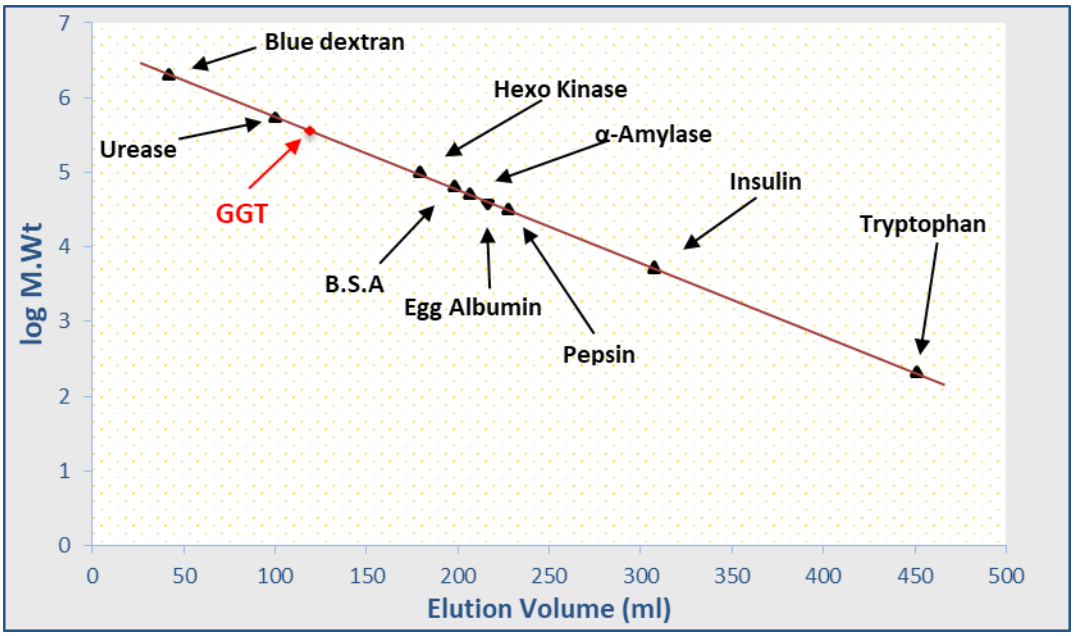

Figure 4: Standard curve for estimating the approximate molecular weight of the GGT enzyme using a sephadex G-150 gel-containing separator column for healthy subjects.

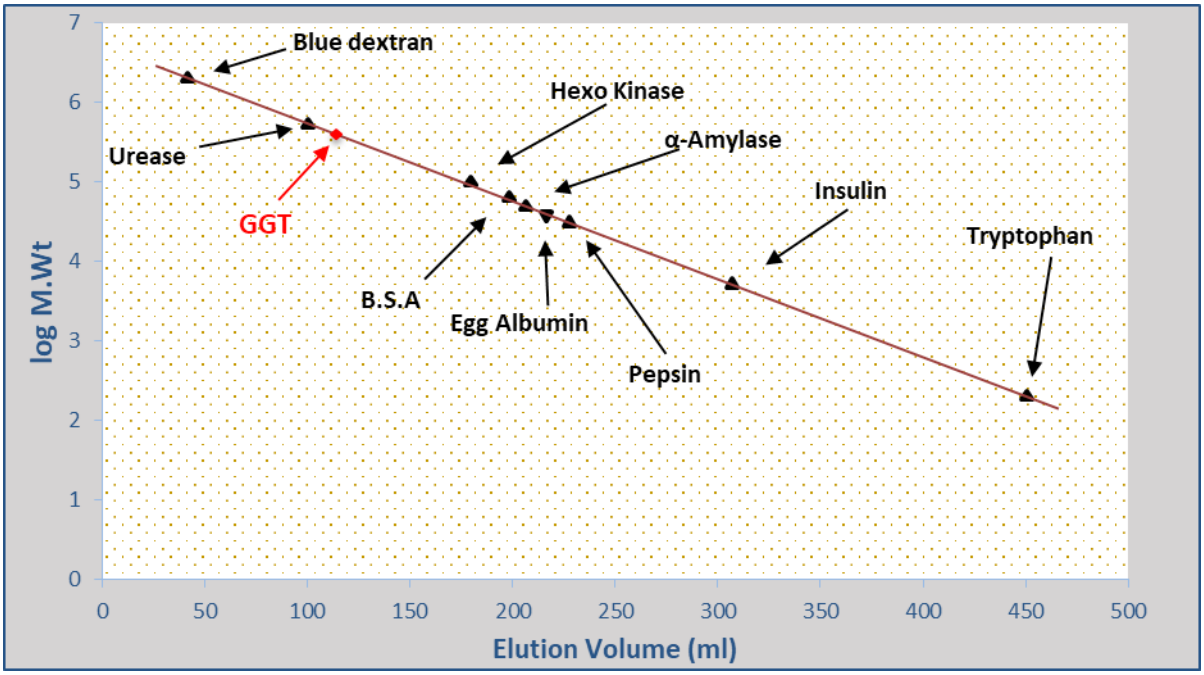

Figure 5: Standard curve for estimating the approximate molecular weight of GGT enzyme using a gel-containing separation column sephadex G-150 for viral hepatitis C.

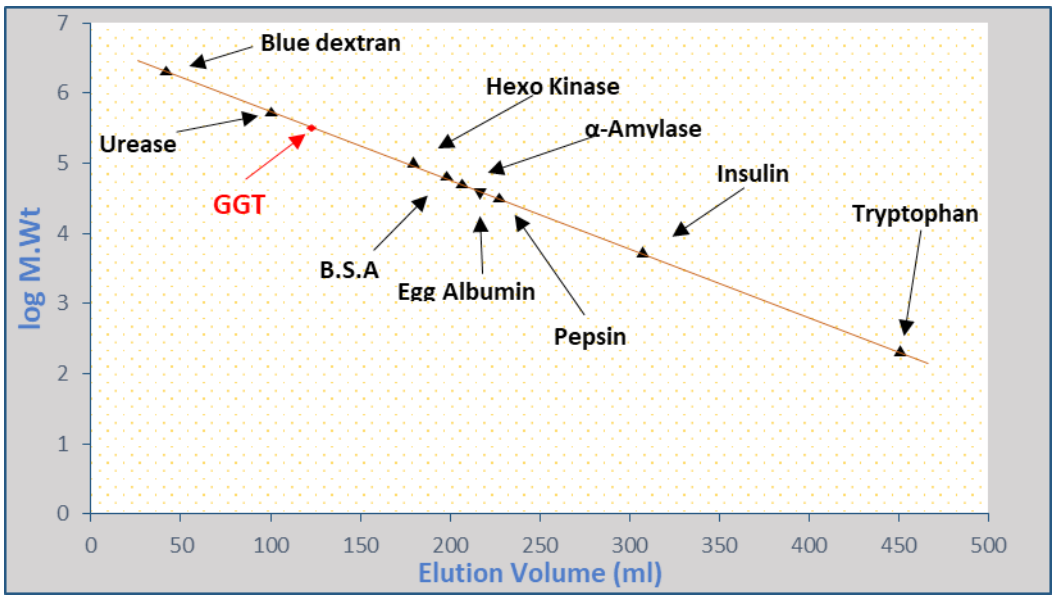

Figure 6: Standard curve for estimating the approximate molecular weight of GGT enzyme using a gel-containing separation column sephadex G-150 for HBsAg patients.

The molecular weight of the GGT enzyme was determined by the method of electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). This method is widely used and is used in the process of separating large biomolecules such as enzymes, proteins and nucleic acids, where the enzyme is treated with sodium dodecyl sulfate, which works to break down The protein gives chains of different sizes surrounded by particles of negatively charged sodium dodecyl sulfate, which removes the original charge of the protein, so these chains migrate to the positive electrode as the movement of the protein in the gel depends on the charge it carries mainly and then depends on the size and shape of the protein, Figure 7 shows the shape of the GGT enzyme bundle when migrated with standard solutions of known molecular weight [16,17]. and in comparison between

the GGT enzyme package and the standard compound packages, it was found that the molecular weight of the purified GGT enzyme from the sera of HCV patients and HBsAg is (68 K.D), and this is consistent with the findings of previous research[18-21].

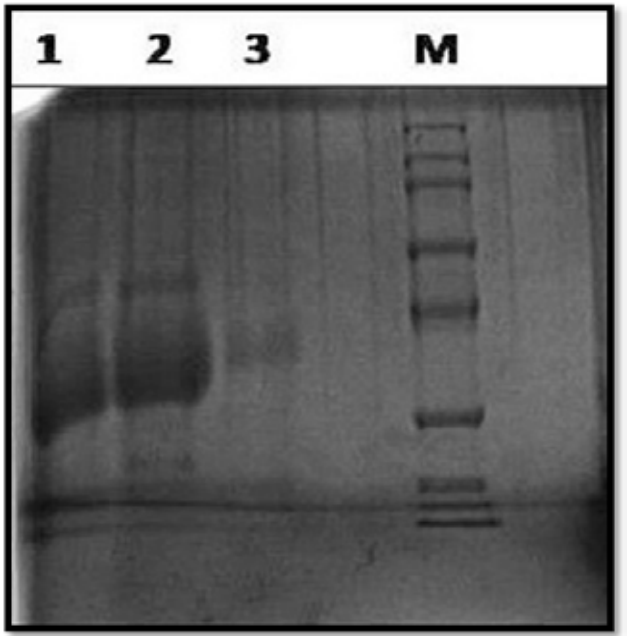

Figure 3-9: Electrophoresis of purified GGT enzyme to find approximate molecular weight compared to standard solutions. (1 : Healthy, 2 : HCV, 3 : HBV, M : protein marker)

Discussion

The increase in the GGT rate in hepatitis B and C patients compared to the control group indicates a difference, and this gives evidence of the importance of GGT activity as a reliable marker for long-term (HCV, HBV) infection. It is considered as an independent indicator for both viral response and clinical outcome among patients with advanced liver disease due to hepatitis C and B. These general results in this research are consistent with previous work and are supported by many studies. Increased GGT activity is observed in all forms of liver disease especially in blockage of bills within or after the liver [22]. In addition to increasing the level of the enzyme in cases of high triglycerides and liver disease. The factor of excessive alcohol consumption [23]. Previous studies [24-27] showed an increase in the level of GGT activity in all patients with viral hepatitis B and C and most importantly, all of them showed a chronic increase in GGT, so it remained elevated repeatedly for a long time after the ALT level had returned to normal, as it is a closely related marker for predicting severity and GGT has been shown to have greater predictive significance than ALT or AST. Therefore, the association of GGT to any clinical point of treatment or liver disease in patients with viral hepatitis and liver cancer over relatively longer periods. And among (Rui-danZheng et al) and (Rui Huang) [28,29], the levels of ALT, AST, and GGT were significantly higher in patients with hepatitis B virus type, the GGT levels reached about 8 times higher than the limit. Normal highest and a significant positive association was found between serum GGT levels and serum ALT levels in chronic hepatitis B patients. Previous studies [30-36] showed that the level of ALT, AST, and ALP in acute infection was higher than that in patients with chronic hepatitis . Previous literature indicated that GGT was purified from Helicobacter pylori from the gastric mucosa[37], Bacillus subtilis SK11.004 [38], human kidney [39] and human liver[40], and with However, there is no evidence to suggest iso-GGT enzyme purification in the serum of patients with hepatitis B and C. The molecular weight of 68 KDa found in this study is consistent with previous research[41-44]. All enzymes operate at an ideal pH that results in a change in the hydrogen ion concentration[45]. Any change in H + reduces the activity of the enzymes[46]. The pH can affect the activity of the enzyme due to the difference in its nature and chemical structure in addition to the presence of different ionic groups carried by the enzyme[47]. as the enzyme has a complex three-dimensional configuration that plays a role in the creation and functioning of the enzyme's active site[48].

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kirkuk University and all experiments were carried out in accordance with approved guidelines.

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