MMSL
Military Medical Science Letters
Since 1925

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2023, 92(1), 2-13
ISSN 0372-7025 (Print)
ISSN 2571-113X (Online)
DOI: 10.31482/mmsl.2022.013

ORIGINAL ARTICLE

BIOCHEMICAL STUDY OF SERUM β-HYDROXYBUTYRATE DEHYDROGENASE IN β-THALASSEMIA

Hiba N. Sami [1], Mahmoud A. M. Fakhri [2] ✉

[1] Department of Chemistry, College of Science, University of Mosul, Iraq
[2] Department of Biophysics, College of Science, University of Mosul, Iraq

Received 20th January 2022.
Accepted 10th March 2022.
Published 3rd March 2023.

Summary

Introduction: Thalassemia is a healthcare challenging disease all over the world. It imparts a great burden on patients' families and healthcare institutions. Scientists focus on new aspects to overcome these challenges and increase patient tolerance of disease complications. This study aims to quantify β-Hydroxybutyrate Dehydrogenase BHBDH activity in thalassemia patients compared to the control group and their correlation with the patient's demographic characteristics.

Methods: To do so, serum was collected from patients and the control group and analyzed biochemically for targeted laboratory tests. We determined β-Hydroxybutyrate Dehydrogenase from normal human serum using biochemical molecular techniques.

Results: The results showed that BHBDH activity is significantly higher in the patients compared to the control group regardless of age, sex, or marital status. The results confirmed that enzyme activity and the purification folds were (0.0214 U/ml) and (51.7) respectively for the partially purified enzyme. Furthermore, the proportional molecular weight of the incompletely isolated β-Hydroxybutyrate Dehydrogenase was (125.8±0.5 kDa) using gel filtration chromatography. The comparative molecular weight of the subunit of partially isolated β-Hydroxybutyrate Dehydrogenase was (32.1±0.5 kDa) using SDS–PAGE.

Conclusion: we demonstrate that BHBDH enzymatic activity is higher than control and this could be a prognostic or diagnostic tool in thalassemia patients.

Key words: β-Thalassemia; β-Hydroxybutyrate Dehydrogenase; Myeloperoxidase; isolation; purification

Introduction

Thalassemia or Mediterranean anaemia (1), is an inherited disease that affects the ability to produce haemoglobin in affected children leading to acute anemia (2) and causes the appearance of symptoms of anaemia such as stress and fatigue as a result of a lack of haemoglobin eventually resulting in disturbing the oxygen transport (3). Thalassemia is a degenerative genetic disease that leads to a break in red blood cells, and it is a common disease worldwide in general and at the level of the Mediterranean region in particular (4). Thalassemia occurs as a result of the reduction of one of the alpha and beta-globin chains and depending on the type of the reduced chain, thalassemia is classified into alpha-thalassemia if the alpha chain is reduced and beta-thalassemia if the chain is reduced

✉ University of Mosul, College of Science, Department of Biophysics, Iraq
drmahmoudahmed@uomosul.edu.iq
☎ +9647722977221

to beta (5). As a result of recurrent blood transfusions, thalassemia patients acquire iron excess (6). As a result, extra iron builds up in the liver, heart, kidneys, and pancreas, among other organs (7). Iron chelator, which can bind iron and excrete it out of the body through urine and faeces, is the only way to get rid of excess iron (8). For every 1ml of red blood cells, the body can tolerate 1mg of excess iron, but excess iron can exceed ferritin's storage capacity and eliminate toxins, causing excess iron to accumulate in the blood and tissues. Due to free iron interactions, it produces hazardous chemicals such as hydroxyl radicals (9). Excess iron accumulation in the heart is still the leading cause of heart disease (10).

Beta-hydroxybutyrate dehydrogenase (BHBDH) (EC 1.1.1.30, BRENDA; enzyme information system) is a redox enzyme belonging to the dehydrogenase class. The enzyme BHBDH is present on the inner surface of the mitochondrial membrane (11). BHBDH catalyzes the oxidation of β-hydroxybutyrate to acetoacetate (ACAC) using NAD^+ as a catalyst as shown in the following reaction (12):

$$\text{D-3-Hydroxybutyrate} + NAD^+ \rightleftharpoons \text{Acetoacetate} + NADH + H^+$$

BHBDH enzyme in the liver converts acetoacetate into beta-hydroxybutyrate, a reducing force ready for export outside the liver, such as the brain, heart, kidneys, and muscles. The enzyme works oppositely during ketosis, to prevent the increase of acetoacetate, a strong acidic form of ketone bodies that are overproduced during fasting, diabetes mellitus, and an excessive fat diet and cause ketosis. The BHBDH enzyme is found in the liver, heart, muscles, kidneys, brain, and blood serum (13). The greatest activity of the enzyme is observed in the liver. BHBDH is the only product of ketogenesis and is a reverse non-regulated enzyme with high selectivity for β-hydroxybutyrate (14). BHBDH enzyme is the first to break down ketone compounds and the last enzyme to form ketone bodies. In addition, the BHBDH reaction isn't required for the production and breakdown of ketone bodies, Physiologically, because the liver can release acetoacetate into the bloodstream during an acidic period, BHBDH is critical for the appropriate flow of ketone bodies and energy metabolism (14).

Arylesterase enzyme belongs to the esterase family (15). This enzyme binds to HDL, a type of antioxidant. It's present in the location of lesions to prevent the oxidation of LDL, the enzyme works to protect the lipids in lipoproteins and atherosclerotic lesions from oxidation (16). The liver is the primary source of the arylesterase enzyme, which is ultimately expelled in the blood plasma. Furthermore, because arylesterase is an antioxidant enzyme that attaches to HDL to prevent LDL oxidation, it protects the arteries by lowering lipoprotein oxidation in the circulation (17). Patients with atherosclerosis, myocardial infarction, and heart attack had a low level of arylesterase, indicating a high level of oxidative stress (18), In thalassemia patients, the enzyme declines with a drop in haemoglobin in the blood associated with an increase in the risk of atherosclerosis (19).

Myeloperoxidase (MPO) enzyme is a type of peroxidase (20) that contains a heme group that contains the ferric ion and is connected to the protein part of the enzyme to give its name. This is the enzyme's active form (21). It's abundant in immune cells including lymphocytes, leukocytes (22), and neutrophils (23). It's also the cause of the green tint in pus-infested areas (24). MPO enzyme requires the presence of heme as a cofactor to create hypochlorous acid (HOCl) from hydrogen peroxide (H2O2) and chloride ion Cl^-. In the presence of hydrogen peroxide as a cofactor, tyrosine is also oxidized to tyrosyl radical (25). MPO enzyme is involved in tissue damage and cell death, and its increased levels are often associated with diseases such as heart disease, nervous system disorders, vascular disorders, and respiratory disorders (26).

The present study aims to determine the activity of BHBHBDH in Beta-thalassemia patients alongside the determination of its biochemical activity based on measuring biochemical test of protein, lipid profile, enzyme activity, electrolytes, metals.

Materials and methods

Study subjects

The study was conducted in Thalassemia Center (Ibn Al-Atheer Teaching Hospital, Mosul). Blood was withdrawn from 112 thalassemia patients and 46 healthy subjects were enrolled in the study. as well as 46 healthy control subjects (aged 1.5 to 47), serum was separated and stored for further analysis.

Measurement of parameters

To measure β-hydroxybutyrate dehydrogenase, sodium salt of β-hydroxybutyrate (160 mM) (the substrate) and nicotinamide adenine dinucleotide solution 30 mM were used based on the assay reported by Tal, *et. al*. (27), the reaction solution was maintained at 100 mM Tris-HCl buffer pH 7.8, at 37 °C.

Myeloperoxidase was determined using Kumar, *et. al*. method (28), based on using a Citrate buffer 0.1 mM pH 5.5, O-dianisidin 20.568 mM as a substrate, and 1.5 % hydrogen peroxide solution in Dimethyl sulfoxide solution.

Arylesterase was tested using Tomas, *et. al*. method (29) in Tris-HCl buffer 9 mM pH 8 with Calcium chloride in distilled water and phenylacetate (5 mM) as substrate in a Tris-HCl buffer 9 mM pH 8 with Calcium chloride in distilled water.

Iron, magnesium, calcium, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and total iron-binding capacity, total protein, albumin levels, total cholesterol, triglyceride, and high-density lipoprotein were measured according to manufacturer instruction, the specification and suppliers of used kits are mentioned below Table 1.

The Friedewald equation (30) was used to compute low-density lipoprotein cholesterol: LDL-cholesterol = TC – (TG/5) – HDL-cholesterol. Fischbach equation VLDL Concentration (mg/dl) =Triglyceride/5 was used to compute very-low-density lipoprotein cholesterol. Globulin was estimated using the Richterich, Scimone, and Rothstein equation: Globulin=Total protein – Albumin (31, 32). Atomic absorption spectrophotometer was used to measure zinc and copper (33).

Purification of β-Hydroxybutyrate Dehydrogenase

The purification of B-hydroxybutyrate dehydrogenase was done on a single serum sample collected from a healthy 35-year-old male donor in the blood bank. The activity of beta-hydroxybutyrate dehydrogenase was estimated, the serum was kept in the freezer for use in the purification steps that followed.

Protein was separated by precipitation with ammonium sulfate, which was gradually added to (19 ml) of serum in a solid-state and saturated (0 - 75 %). while stirring the mixture with an electric motor at a temperature of (4 °C) for 60 minutes, then the solution was placed in the refrigerator for 24 hours to completely precipitate. Separating the precipitate from the filtrate was accomplished using a coolant centrifuge at a speed of 18000 rpm for 30 minutes, then taking the precipitate and dissolving it in distilled water, and finally estimating the amount of protein and enzyme activity in solution before proceeding with the following steps.

Dialysis was performed on a previously prepared protein solution in a cellophane tube, which was then immersed in a volumetric container containing (2.5 L) of (0.1 M) ammonium bicarbonate (NH_4HCO_3) solution (34). Dialysis was performed at (4 °C) with continuous stirring with a magnetic stirrer, and the process was continued for 24 hours, taking into account the change of the Dialysis solution. After the Dialysis process was completed, the final volume of the resultant solution was calculated, and the amount of protein and enzyme activity in the solution was estimated. The dialysis solution was maintained in the freezer (-20 °C) and then dried using a Lyophilizer, the protein solution was concentrated, and the volume of the solution, protein concentration, and enzyme activity were all determined.

The compounds were then separated using chromatographic technique employing a separation column with dimensions of 60 x 2.5 cm that contained a gel (Sephadex G-100) that separated compounds with molecular weights up to (150 kD). Protein elution was continuously scanned at 340 nm, and BHBDH activity was measured in each fraction, with known molecular weight materials, the molecular weights of the materials in the molecular range (207 – 2,000,000 D), and to determine the approximate molecular weight of the separated protein compounds obtained by the gel filtration technique, and to detect their molecular weight of BHBDH.

The resulting protein from the gel filtration process was dried using a lyophilization apparatus to acquire this package in the form of a dry powder, which was then stored in the freezer (-20 °C) until it was employed in the following tests.

Finding the molecular weight of the protein package with the highest BHBDH enzyme activity utilizing an electrophoresis approach on a polyacrylamide gel in the presence of SDS. The current utilized was 50 mA.

Table1. Kits are used for measuring the studied parameters.

Kit Specification	Catalogue No.	Suppliers
ALBUMIN BCG Method	80002	Biolabo (France)
ALT GPT (IFCC)	80027	
AST GOT (IFCC)	80025	
CALCIUM CPC method	80004	
CHOLESTEROL CHOD PAP	80106	
HDL-CHOLESTEROL (PTA)	86516	
IRON Direct Method (Ferene)	92108	
Total Iron Binding Capacity (TIBC)	92308	
MAGNESIUM Calmagite	87212	
TOTAL PROTEIN Biuret Method	80016	
TRIGLYCERIDES GPO Method	80019	
ALKALINE PHOSPHATASE (DEA)	92214	

Results and Discussion

The results showed the enzyme activity of BHBDH in serum of healthy adults aged (1.5 - 47) years of both sexes (control group) was (125.3 ± 4.87 U\L), and the enzyme activity of BHBDH in serum of people with Thalassemia ages ranging from (1.8 to 50 years) of both sexes, was (328.9 ± 8.21 U\L).

Comparison of All Patients Group with Control Group

Beta-Hydroxybutyrate Dehydrogenase Activity

According to age groups, sex, social status, and body mass index, all thalassemia patients were compared to the control group as shown in Table 2.

Table 2. Shows the effectiveness of the BHBDH enzyme in the blood serum of all thalassemia patients and the control group.

Influencing factors	Group	The activity of BHBDH (U/L)		Level of probability
		Control group (Mean ± S.E) U/L	Cases of thalassemia (Mean ± S.E) U/L	
Age group	1.5 - 10	132.3 ± 10.71	340.7 ± 17.21	≤ 0.001
	11 – 20	111.9 ± 7.37	326.6 ± 10.59	≤ 0.001
	> 20	133.4 ± 7.52	320.6 ± 17.71	≤ 0.001
Sex	Male	129.0 ± 7.15	329.2 ± 11.73	≤ 0.001
	Female	119.4 ± 5.57	328.8 ± 11.58	≤ 0.001
Social status	Single	124.2 ± 5.79	329.9 ± 8.46	≤ 0.001
	Marriage	127.4 ± 9.23	318.0 ± 34.12	≤ 0.001
Body Mass Index	18.5 - 24.5	125.1 ± 5.85	318.8 ± 12.86	≤ 0.001
	24.5 - 29.5	125.6 ± 8.98	317.9 ± 39.87	≤ 0.001

Table 2 shows the effect of age on the enzyme level in the Thalassemia case group, which was separated into three age groups like the age groups in the control group, as shown in Table 2. When comparing the activity of BHBDH enzyme among thalassemia patients with the activity of the control group within the same age group, a very high significant increase was seen at the probability level ($P \leq 0.001$) among thalassemia patients.

When comparing the males of the patient group to the males of the control group, as well as when comparing the females of the patient group to the females of the control group, Table 2 demonstrates a very high significant rise. There is also a significant increase in the level of probability ($P \leq 0.001$) among the single patients compared to the control group, and a very high significant increase between the married patients and the married control group, as well as the BMI, was divided into two groups in a manner consistent with the control group's BMI. When compared to the control group, the activity of the enzyme increases in thalassemia patients.

Clinical Variables of Patients Group Compared to Control Group

Some clinical variables were measured in all thalassemia patients and compared to the control group. Table 3 shows a very high significant decrease in the level of haemoglobin in the blood in thalassemia patients at the level of probability ($P \leq 0.001$) compared to the control group, which is consistent with what Khawaja, *et al*. (35) stated, which is attributed to a defect in all beta-globin chains that affects haemoglobin production in humans. Anaemia is the effect of this abnormality. We also see a substantial reduction in blood PCV in patients with thalassemia compared to the control group at a probability level ($P \leq 0.001$), which is consistent with previous findings Kadhem Abeid (36). The reason for this is that PCV is primarily determined by the amount, shape, and size of red blood cells.

Table 3. Comparison of all clinical characteristics measured in the serum of the control and thalassemia case groups.

Clinical variables	Control group Mean ± S.E	Cases of thalassemia Mean ± S.E	P-value
Hb (g/dl)	13.15 ± 0.19	8.57 ± 0.09	≤ 0.001
PCV (%)	39.47 ± 0.57	23.80 ± 0.32	≤ 0.001
Arylesterase (U/L)	117.7 ± 2.65	70.04 ± 1.23	≤ 0.001
MPO (U/L)	27.52 ± 1.19	48.55 ± 1.38	≤ 0.001
Total protein (g/dL)	7.55 ± 0.17	6.75 ± 0.14	≤ 0.05
Albumin (g/dL)	4.94± 0.09	4.33 ± 0.07	≤ 0.001
Globulin (g/dL)	2.61 ± 0.16	2.41 ± 0.14	≥ 0.05
Iron (µg/Dl)	135.0 ± 12.66	244.3 ± 8.83	≤ 0.001
UIBC	324.7 ± 7.78	155.5 ± 11.97	≤ 0.001
TIBC	456.7 ± 9.65	400.9 ± 14.9	≤ 0.05
Ca^{+2} (mg/dL)	9.77 ± 0.27	8.74 ± 0.19	≤ 0.05
Mg^{+2} (mg/dL)	2.11 ± 0.03	1.98 ± 0.009	≤ 0.001
HDL (mg/dL)	38.18 ± 2.85	29.12 ± 1.78	≤ 0.01
Total Cholesterole (mg/dL)	164.1 ± 4.22	100.5 ± 3.18	≤ 0.001
Triglyceride (mg/dL)	118.1 ± 8.12	121.9 ± 7.09	≥ 0.05
LDL (mg/dL)	102.3 ± 4.36	47.05 ± 2.68	≤ 0.001
V LDL (mg/dL)	23.62 ± 1.62	24.4 ± 1.42	≥ 0.05
GOT (IU/L)	11.54 ± 0.71	41.51 ± 2.98	≤ 0.001
GPT (IU/L)	17.15 ± 1.19	64.28 ± 2.76	≤ 0.001
Zn^{+2} (µg/dl)	94.69 ± 2.07	157.9 ± 3.32	≤ 0.001
Cu^{+2} (µg/dl)	113.5 ± 3.19	214.5 ± 9.84	≤ 0.001
ALP (IU/L)	16.55 ± 1.40	16.23 ± 0.69	≥ 0.05

The table also shows a very high significant decrease in the activity of the arylesterase enzyme in patients with thalassemia at a probability level ($P \leq 0.001$) compared to the control group, and this is consistent with what was stated by Asif, *et al*. (37) because the low level is associated with a deficiency of Oxygenation as a result of haemoglobin deficiency and antioxidants as a result of frequent blood transfusions that affect oxidative stress and antioxidants.

We also notice that, when compared to the control group, patients with thalassemia had a very high significant increase in MPO enzyme activity at the level of probability ($P \leq 0.001$), which is consistent with previous findings by Tripathi, *et al*. (38) as a result of repeated blood transfusions, red blood cells decompose, increasing white blood cells and, as a result, an increase in the MPO enzyme for its presence in white blood cells.

It was also discovered that individuals with thalassemia had a very high significant drop in total protein and albumin levels at a probability level ($P \leq 0.001$) when compared to the control group, which is similar to Abd & Zainal (39). The decrease in total protein is related to the liver's decreased ability to synthesize protein. Low amounts of albumin in the blood can be caused by hepatic dysfunction or malnutrition. There was also a significant decrease in total iron-binding capacity (TIBC) at the probability level ($P \leq 0.05$), which is consistent with the findings of Ghone, *et al*. (40). The oxidation of red blood cells and cellular organelles occurs as a result of an excess of iron.

In Thalassemia patients, there was a very high significant rise in iron levels at the probability level ($P \leq 0.001$) when compared to the control group, which is consistent with El-Karaem, *et al*. (41) and is due to frequent blood transfusions and increased iron absorption in the gastrointestinal tract in Thalassemia patients. While there was a significant decrease in the level of unsaturated iron-binding capacity (UIBC) in the thalassemia patients group compared to the control group at the probability level ($P \leq 0.001$), which is consistent with Smesam, *et al*. (42) and the reason is due to the high level of iron in patients.

We also found a very high significant decrease in the level of LDL and total cholesterol at the probability level ($P \leq 0.001$), as well as a very high significant decrease in the level of HDL at the probability level ($P \leq 0.01$) in patients with thalassemia compared to the control group, which is consistent with Ghorban, *et al*. (43) and Bordbar, *et al*. (44) It's because liver damage is a big factor in lipid patterns and the effect of regular blood transfusions.

At the probability level ($P \leq 0.01$) in patients with thalassemia compared to the control group, a substantial drop in calcium level was noted, which is consistent with El-Karaem, *et al*. (41). The cause is calcium removal by citrate anticoagulants in the blood unit or eating a low-calcium lunch, or a low level of albumin in Thalassemia patients, where albumin carries calcium, and patients also suffer from vitamin D deficiency due to lack of sunlight and limited physical activity, or hypothyroidism and genital insufficiency.

In addition, patients with thalassemia had a very high significant decrease in magnesium levels at the probability level ($P \leq 0.001$) when compared to the control group, which is consistent with Karim, *et al*. (45) The cause is low thyroid hormones caused by high iron and a poor diet. However, in patients with thalassemia, a very high significant rise in zinc levels was seen at the probability level ($P \leq 0.001$) when compared to the control group, which is consistent with Mehdizadeh, *et al*. (46) The reason for this could be the patients' regularity with blood transfusions, as regular transfusion compensates for Zinc deficiency. In individuals with thalassemia, a significant rise in copper level was seen at the probability level ($P \leq 0.001$) when compared to the control group, which is consistent with previous findings Alsharnoubi, *et al*. (47). The amount of copper consumed, the use of Desferal, and intestinal absorption were all counted.

In patients with thalassemia, a very high significant rise in the level of GPT and GOT was identified at the level of probability ($P \leq 0.001$) when compared to the control group, which is consistent with Abdulla (48). It's caused by a drop in haemoglobin and an increase in iron, and the high proportion means liver and heart damage. One of the side effects of thalassemia is liver damage.

The Relationship Between Clinical Parameters and Beta-HydroxyButyrate Dehydrogenase in Patient Group; The linear correlation coefficient was used to investigate the association between the measured clinical characteristics in Table 3 and the activity of the enzyme in the blood serum of the group of patients (Correlation Coefficient "r").

In the group of patients with thalassemia, it was discovered that there was a significant direct link between MPO enzyme and BHBDH enzyme at the probability level ($P \leq 0.001$), as shown in Table 4 repeated blood transfusions resulted in a rise in white blood cells, which in effect resulted in an increase in MPO due to the presence of MPO in white blood cells (49).

Also, as indicated in Table, there was a significant direct link between BHBDH enzyme and globulin at the probability level ($P \leq 0.01$), as shown in Table 4 The level of globulin in the blood reduced as a result of failed liver function or malnutrition (39).

Table 4. The Relation Between Clinical Parameters had been Measured and Beta-HydroxyButyrate Dehydrogenase in Patient Group.

Clinical variables	Pearson Correlation	Probability level
MPO	0.474**	≤ 0.001
Globulin	0.249**	≤ 0.01
Ca	- 0.226*	≤ 0.05
HDL	- 0.191*	≤ 0.05

As indicated in Table, there was a significant inverse connection between HDL with BHBDH enzyme at the probability level ($P \leq 0.05$), as shown in Table 4 Because of the dilution of plasma as a result of anaemia, and the lack of fat in the blood due to increased cholesterol requirements associated with erythrocyte hypertrophy, as well as an increase in cholesterol absorption by the reticuloendothelial system, there is no fat available for transport, so HDL decreases, and excess iron depletes antioxidants and HDL levels. Low HDL levels have been linked to an increased risk of coronary heart disease (50). Excessive clearance of HDL by activated macrophages causes low HDL cholesterol levels (51), and calcium with BHBDH enzyme at the probability level ($P \leq 0.05$), as shown in Table 4.

Isolation of β-HydroxyButyrate Dehydrogenase from Serum

The results in Figure (1) illustrate the appearance of two protein bands, with the following spindle diameters for the tops of these bundles:

The first package of elution has a capacity of 96 ml and a molecular weight of 110,600 ± 500 D, while The second elution packet has a volume of 150 ml with a molecular weight of 55, 100 ± 500 D.

The absorption was monitored at 280 nm and 340 nm, and the activity of the BHBDH enzyme in the separated fractions was determined. It produced a package with a clear BHBDH enzyme activity and a clear 340 nm absorbance. At 86 ml, this package proved effective for the enzyme, which had a molecular weight of 125,800 ± 500 D.

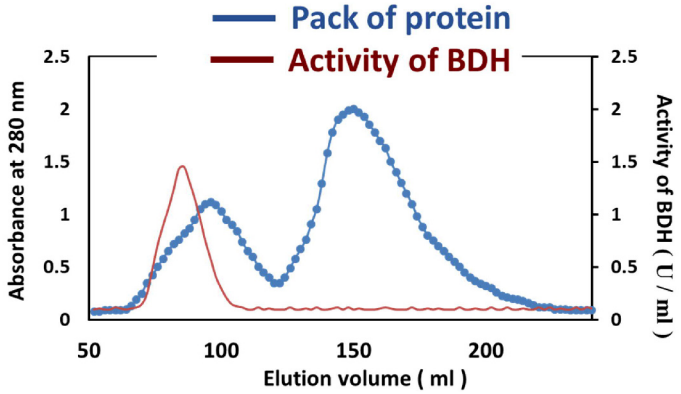

Figure 1. Shows the profile of the protein precipitate solution using gel filtration technique using a column of dimensions (60 x 2.5 cm) containing the gel material (Sephadex G-100) at a height of 53 cm. The proteins were recovered at a flow rate of (60 ml/h).

Table 5 summarizes the findings of the several phases of purification of the BHBDH enzyme from a solution of ammonium sulfate precipitate. It should be noted that the number of times BHBDH enzyme was purified from serum was (51.7) times. After the gel filtering process, the specific activity of the BHBDH enzyme is (Sephadex G-100). For the blood serum, it climbed to (0.09816 U/mg).

Table 5. Summary of the results of the stages of purification of BHBDH enzyme from blood serum.

No.	steps	Volume ml	Protein concentration mg/ml	Total protein mg	Activity U/ ml	Total activity U*	Specific activity ** U/ mg × 10^{-2}	% recovery	Active Enzyme Pack
1	**Serum**	20	64.5	1290.0	0.123	2.460	0.190	100.0	1.0
2	**Protein precipitate solution**	12	67.3	807.6	0.196	2.352	0.291	95.6	1.5
3	**Solution after dialysis**	18	21.8	392.4	0.124	2.232	0.569	90.7	3
4	**Solution after lyophilization**	8.8	44.3	389.8	0.252	2.217	0.568	90.1	3
5	**Enzyme activity pack**	94.5	0.218	20.6	0.0214	2.022	9.816	82.2	51.7

*Enzymatic unit (U): the amount of enzyme required to convert one micromole of the starting material into the final product in one minute.
* *Specific efficiency refers to the number of enzyme units found in one milligram of protein.

Determination of Molecular Weight of Beta-Hydroxybutyrate Dehydrogenase by Gel Filtration Chromatography

Table 6 shows the relationship between the molecular weight and the volume of Elution materials used to estimate the approximate molecular weight by gel filtration chromatography technique. Figure (2) show the standard curve for molecular weight determination by gel filtration technique.

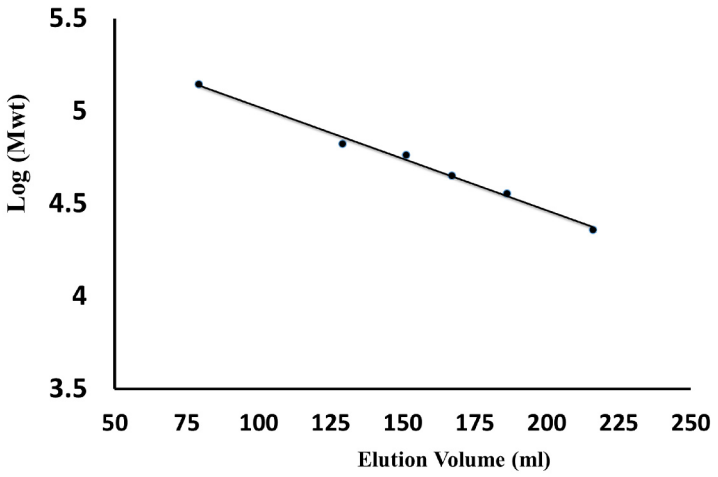

Figure 2. Standard curve for molecular weight determination by gel filtration technique.

Determination of Molecular Weight of Sub Unit for β-HydroxyButyrate Dehydrogenase

Using the technique of electrophoresis containing a substance to determine the approximate molecular weight of the purified BHBDH enzyme subunit from the separation column (SDS - PAGE). For this purpose, several standard

chemicals with known molecular weights ranging from (45000 - 14300 D) were utilized, as indicated in Table 7 Distances traveled by standard substances with known molecular weights A straight line occurs when graphing the traveled distance for each substance of known molecular weight against the logarithm of its molecular weight, by which the approximate molecular weight is obtained, as illustrated in Figure (3).

Table 6. Relationship between the molecular weight and the volume of elution materials used to estimate the approximate molecular weight, gel filtration chromatography technique.

Materials	Molecular weight (D)	Elution volume(ml)
Blue dextran	2,000,000	60
ALP	140,000	79
B.S.A	67,000	129
α - AMYLASE	58,000	151
Egg	45,000	167
Pepsin	36,000	186
Trypsin	23,000	216
Tryp.	207	306

Table 7. Molecular weight and distance of materials used in the electro-migration technique containing SDS.

Materials name	Distance cm	molecular weight D
Egg albumin	3.4	45000
Pepsin	3.8	36000
Trypsin	4.6	23,000
Lysozyme	5.4	14300

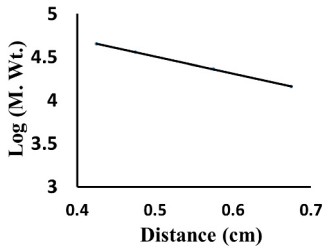

Figure 3. Standard curve for molecular weight determination using SDS based electro-migration technique.

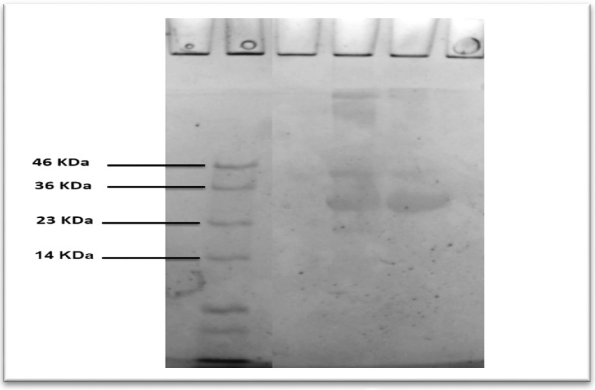

Figure 4. Image of electrophoresis using polyacrylamide/SDS, showing the protein bands of the duplicate sample that passed on the gel.

The distance traveled by the BHBDH enzyme is 4 cm, and the Rf coefficient is 0.5, as shown in Figure (4). The BHBDH enzyme component has an approximate molecular weight of 32,100 ± 500 D, according to the PAGE-SDS method of estimating molecular weight, This is comparable to the researcher's conclusion Marks *et al*. (52). The molecular weight was close to the estimated weight for the enzyme's activity package. 32,1 D × 4 = 128, 4 D

Conclusion

We conclude that thalassemia patients have very high catabolic processes, that a lack of oxygen causes them to activate anaerobic decomposition processes, causing them to resort to catabolism, and that when fats are not available, they resort to the destruction of ketone bodies to provide the necessary energy, and if the two are not available, they resort to protein catabolism, resulting in most patients being underweight. As a result, high-carbohydrate foods are recommended as a supplement.

Acknowledgments

We express our gratitude and admiration for the administration, doctors, nurses, at Ibn Al-Atheer Hospital and the Thalassemia Center, in particular, excellent collaboration and facilitation of our research. We want to thank the Thalassemia patients in particular for their kindness and cooperation in collecting samples, and we wish them a rapid recovery.

Conflict of Interest

The authors declare that no conflict of interest exists for this research.

Adherence to Ethical Standards

The study was approved by the Medical Research Ethics Committee at the University of Mosul. The study approval number and date UOM/COS/2020 (22) on 18/08/2020.

References

1. Galanello R, Origa R. Beta-thalassemia. Orphanet journal of rare diseases. 2010 Dec;5(1):1-5. https://doi.org/10.1186/1750-1172-5-11
2. Olivieri NF. The β-thalassemias. New England journal of medicine. 1999 Jul 8;341(2):99-109. https://doi.org/10.1056/NEJM199907083410207
3. Authored by Dr Colin Tidy, Reviewed by Dr Adrian Bonsall | Last edited 22 Jan 2018 | Meets Patient's editorial guidelines
4. Angastiniotis M, Lobitz S. Thalassemias: an overview. International Journal of Neonatal Screening. 2019 Mar;5(1):16. https://doi.org/10.3390/ijns5010016
5. Żołnierowicz S, Świerczyński J, Żelewski L. β-Hydroxybutyrate dehydrogenase activity in human placenta. Placenta. 1984 Mar 1;5(2):183-7. https://doi.org/10.1016/S0143-4004(84)80061-2
6. Ali BA, Mahmoud AM. Frequency of glomerular dysfunction in children with beta thalassaemia major. Sultan Qaboos University Medical Journal. 2014 Feb;14(1):e88. https://doi.org/10.12816/0003341
7. Kohgo Y, Ikuta K, Ohtake T, et al. Body iron metabolism and pathophysiology of iron overload. International journal of hematology. 2008 Jul;88(1):7-15. https://doi.org/10.1007/s12185-008-0120-5
8. Sharma DC, Arya A, Kishor P, et al. Overview on thalassemias: a review article. Medico Research Chronicles. 2017 Jun 30;4(03):325-37.
9. Rasool M, Malik A, Jabbar U, et al. Effect of iron overload on renal functions and oxidative stress in beta thalassemia patients. Saudi medical journal. 2016 Nov;37(11):1239. https://doi.org/10.15537/smj.2016.11.16242
10. Papanikolaou G, Tzilianos M, Christakis JI, et al. Hepcidin in iron overload disorders. Blood. 2005 May 15;105(10):4103-5. https://doi.org/10.1182/blood-2004-12-4844
11. Stillwell, William (2016). An Introduction to Biological Membranes || Lipid Membrane Properties. , 181–220. doi:10.1016/B978-0-444-63772-7.00010-5

12. Żołnierowicz S, Świerczyński J, Żelewski L. β-Hydroxybutyrate dehydrogenase activity in human placenta. Placenta. 1984 Mar 1;5(2):183-7. https://doi.org/10.1016/S0143-4004(84)80061-2
13. Akil O, El Kebbaj Z, Latruffe N, et al. D-3-hydroxybutyrate oxidation in mitochondria by D-3-Hydroxybutyrate dehydrogenase in Tetrahymena pyriformis. African Journal of Biochemistry Research. 2009 Mar 31;3(3):029-36. https://doi.org/10.5897/AJBR.9000069
14. Otsuka H, Kimura T, Ago Y, et al. Deficiency of 3-hydroxybutyrate dehydrogenase (BHBDH1) in mice causes low ketone body levels and fatty liver during fasting. Journal of inherited metabolic disease. 2020 Sep;43(5):960-8. https://doi.org/10.1002/jimd.12243
15. Lawlor DA, Day IN, Gaunt TR, et al. The association of the PON1 Q192R polymorphism with coronary heart disease: findings from the British Women's Heart and Health cohort study and a meta-analysis. BMC genetics. 2004 Dec;5(1):1-2.https://doi.org/10.1186/1471-2156-5-17
16. Romani A, Trentini A, van der Flier WM, et al. Arylesterase activity of paraoxonase-1 in serum and cerebrospinal fluid of patients with Alzheimer's disease and vascular dementia. Antioxidants. 2020 May;9(5):456. https://doi.org/10.3390/antiox9050456
17. Hussein GM. Evaluation of Paraoxonase1 Activities and Lipid Profiles Concentration in Sera of β-Thalassemia Major Patients. Evaluation. 2017;4(1):14-22.
18. Kabaroglu C, Mutaf II, Boydak B, et al. Association between serum paraoxonase activity and oxidative stress in acute coronary syndromes. Acta cardiologica. 2004 Dec 1;59(6):606-11.
19. Panachan J, Chokchaichamnankit D, Weeraphan C, et al. Differentially expressed plasma proteins of β-thalassemia/hemoglobin E patients in response to curcuminoids/vitamin E antioxidant cocktails. Hematology. 2019 Jan 1;24(1):300-7. https://doi.org/10.1080/16078454.2019.1568354
20. Khan AA, Alsahli MA, Rahmani AH. Myeloperoxidase as an active disease biomarker: recent biochemical and pathological perspectives. Medical sciences. 2018 Jun;6(2):33. https://doi.org/10.3390/medsci6020033
21. Arnhold J. Properties, functions, and secretion of human myeloperoxidase. Biochemistry (Moscow). 2004 Jan;69(1):4-9. https://doi.org/10.1023/B:BIRY.0000016344.59411.ee
22. Liu WQ, Zhang YZ, Wu Y, et al. Myeloperoxidase-derived hypochlorous acid promotes ox-LDL-induced senescence of endothelial cells through a mechanism involving β-catenin signaling in hyperlipidemia. Biochemical and biophysical research communications. 2015 Nov 27;467(4):859-65. https://doi.org/10.1016/j.bbrc.2015.10.053
23. Klebanoff SJ. Myeloperoxidase: friend and foe. Journal of leukocyte biology. 2005 May;77(5):598-625. https://doi.org/10.1189/jlb.1204697
24. Le, Tao (2021). First Aid for the USMLE Step 1 (2021 ed.). New York: McGraw Hill. p. 109. ISBN 9781260467529.
25. Heinecke JW, Li W, Francis GA, et al. Tyrosyl radical generated by myeloperoxidase catalyzes the oxidative cross-linking of proteins. The Journal of clinical investigation. 1993 Jun 1;91(6):2866-72. https://doi.org/10.1172/JCI116531
26. Davies, Michael Davies; Hawkins, Clare L. (2020). The role of myeloperoxidase (MPO) in biomolecule modification, chronic inflammation and disease.. Antioxidants & Redox Signaling, (), ars.2020.8030–. doi:10.1089/ars.2020.8030.
27. Tal S, Smirnoff P, Okon Y. Purification and characterization of D (—)-β-hydroxybutyrate dehydrogenase from Azospirillum brasilense Cd. Microbiology. 1990 Apr 1;136(4):645-9. https://doi.org/10.1099/00221287-136-4-645
28. Kumar P, Pai K, Pandey HP, et al. NADH-oxidase, NADPH-oxidase and myeloperoxidase activity of visceral leishmaniasis patients. Journal of medical microbiology. 2002 Oct 1;51(10):832-6. https://doi.org/10.1099/0022-1317-51-10-832
29. Tomás M, Sentí M, Garcí́a-Faria F, et al. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. Arteriosclerosis, thrombosis, and vascular biology. 2000 Sep;20(9):2113-9. https://doi.org/10.1161/01.ATV.20.9.2113
30. Fischbach F. (2000) ."A Manual of Laboratory and Diagnostic Test"." 6th ed. Lippincott Williams and Wilkins, U.S.A. ,p: 472
31. Richterich R.(1969)." Clinical Chemistry Theory and Practice " .S. Karger A.G. Basel, Switzerland, pp: 245-249
32. Scimone J. and Rothstein R (1978)." Laboratory Manual of Clinical Chemistry" . AVI Puplishing company . Inc., U.S.A., pp: 60,63.
33. D'haese PC, Lamberts LV, Vanheule AO, et al. Direct determination of zinc in serum by Zeeman atomic absorption spectrometry with a graphite furnace. Clinical chemistry. 1992 Dec 1;38(12):2439-43. https://doi.org/10.1093/clinchem/38.12.2439

34. Plummer, T.D.(1978) "An Introduction of Practical Biochemistry". 2nd ed. McGraw-Hill Book Co.U.K.: pp: 48,53,174,270,274.
35. Khawaji MM, Hazzazi AA, Ageeli MH, et al. Clinical and hematological features among β-thalassemia major patients in Jazan region: A hospital-based study. Journal of family medicine and primary care. 2020 Jan;9(1):412. https://doi.org/10.4103/jfmpc.jfmpc_1007_19
36. Kadhem Abeid A. Physiological study for some blood parameters in children with major B-Thalassemia in Al-Najaf governorate/Iraq. journal of kerbala university. 2014 Apr 1;10(1):170-8.
37. Asif M, Manzoor Z, Farooq MS, et al. Status of oxidant, antioxidantand serum enzymes in thalassaemic children receiving multiple blood transfusions. J Pak Med Assoc. 2015 Aug 1;65(8):838-43.
38. Tripathi P, Agarwal S, Tewari S, et al. Status of Catalase, Glutathione Peroxidase, Glutathione S-Transferase, and Myeloperoxidase Gene Polymorphisms in Beta-Thalassemia Major Patients to Assess Oxidative Injury and Its Association with Enzyme Activities. Journal of Pediatric Genetics. 2021 Apr 12. https://doi.org/10.1055/s-0041-1723961
39. Abd IK, Zainal IG. Assessment of biochemical parameters and study its correlation in ß-Thalassemia major patients and healthy controls in Kirkuk City, Iraq. Medical Journal of Babylon. 2020 Apr 1;17(2):172. https://doi.org/10.4103/MJBL.MJBL_77_19
40. Ghone RA, Kumbar KM, Suryakar AN, et al. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Indian Journal of Clinical Biochemistry. 2008 Oct;23(4):337-40. https://doi.org/10.1007/s12291-008-0074-7
41. El-Kareem SA, Ebied SA, Sadek NA. Serum tartrate resistant acid phosphatase 5b in beta Thalassemia Egyptian patients: promising biomarker of iron overload oxidative stress and bone disease. Ann Clin Lab Res. 2019;7(2):303. https://doi.org/10.21767/2386-5180.100303
42. Smesam Hasan NK, Albuthabhak Hasan AQ, Arjmand Sareh, et al (2020). Evaluation of Erythroferrone, Hepcidin, and Iron Overload Status in Iraqi Transfusion-Dependent Î²-Thalassemia Major Patients. Hemoglobin, (), 1–6. https://doi.org/10.1080/03630269.2020.1794888
43. Ghorban K, Shanaki M, Mobarra N, et al. Apolipoproteins A1, B, and other prognostic biochemical cardiovascular risk factors in patients with beta-thalassemia major. Hematology. 2016 Feb 7;21(2):113-20. https://doi.org/10.1179/1607845415Y.0000000016
44. Bordbar M, Haghpanah S, Afrasiabi A, et al. Genotype–phenotype correlation related to lipid profile in beta-thalassemia major and intermedia in southern Iran. Journal of clinical lipidology. 2012 Mar 1;6(2):108-13. https://doi.org/10.1016/j.jacl.2011.12.005
45. Karim MF, Ismail M, Hasan AM, et al. Hematological and biochemical status of Beta-thalassemia major patients in Bangladesh: A comparative analysis. International journal of hematology-oncology and stem cell research. 2016 Jan 1;10(1):7.
46. Mehdizadeh M, Zamani G, Tabatabaee S. Zinc status in patients with major β-thalassemia. Pediatric hematology and oncology. 2008 Jan 1;25(1):49-54. https://doi.org/10.1080/08880010701773738
47. Alsharnoubi J, Nassef Y, Fahmy RF, et al. Using LIBS as a diagnostic tool in pediatrics beta-thalassemia. Lasers in Medical Science. 2021 Jul;36(5):957-63. https://doi.org/10.1007/s10103-020-03117-9
48. Abdulla AA. Evaluation of serum antioxidant enzymes in β-thalassemia major patients. International Journal of ChemTech Research. 2018;11(7):323-8.http://dx.doi.org/10.20902/IJCTR.2018.110738
49. Kargapolova Y, Geißen S, Zheng R, et al. The Enzymatic and Non-Enzymatic Function of Myeloperoxidase (MPO) in Inflammatory Communication. Antioxidants. 2021 Apr;10(4):562. https://doi.org/10.3390/antiox10040562
50. Balcı YI, Ünak Ş, Gümrük F. Serum Lipids in Turkish Patients with β-Thalassemia Major and β-Thalassemia Minor. https://doi.org/10.4274/tjh.2015.0168
51. Kumar Tarun, Basu Surupa, Kundu Ritabrata, et al (2020). Lipid Profile in Children With Thalassemia: A Prospective Observational Study From Eastern India. Indian Pediatrics, 57(11), 1072–1073. https://doi.org/10.1007/s13312-020-2040-2
52. Marks AR, McIntyre JO, Duncan TM, et al. Molecular cloning and characterization of (R)-3-hydroxybutyrate dehydrogenase from human heart. Journal of Biological Chemistry. 1992 Aug 5;267(22):15459-63. https://doi.org/10.1016/S0021-9258(19)49556-2