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Partial Purification of Cholinesterase Enzyme and Study of Some Biochemical Variables in Patients with Type 2 Diabetes, Along with Analysis of the Linear Correlation between Enzyme Levels and Clinical Variables

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Abstract: Diabetes is a metabolic disorder characterized by high blood sugar levels. This increase occurs due to a reduction in insulin secretion or a decrease in the body's ability to use insulin effectively, leading to the accumulation of glucose in the blood. Insulin regulates blood sugar levels by facilitating the entry of glucose molecules into cells for storage or energy production[1]. **Objectives** The current study aimed to achieve molecular purification of the enzyme acetylcholinesterase and analyze the linear relationship between enzyme levels and clinical variables in diabetic patients while measuring the levels of C-PEP, GLP-1, R.B.S, F.B.S, Hb1Ac, Urea, and Creatinine. **Materials and Methods** This study included 90 samples, comprising 60 diabetic patients and 30 healthy individuals as a control group, with ages ranging from 30 to 75 years. Blood samples (3-5 ml) were collected, and serum was obtained. Necessary tests were performed on all samples. **Results** The results showed a significant decrease in the level of the enzyme acetylcholinesterase in diabetic patients compared to the control group ($p<0.01$). Additionally, there were statistically significant differences in enzyme levels between genders and age groups. The study indicated statistical significance at ($p>0.05$) in enzyme levels, while no significant difference was found in GLP levels at the probability level ($P\leq0.01$). A decrease in the concentration of C-peptide hormone was also observed in diabetic patients compared to healthy individuals at the level ($p>0.05$). The results further indicate a significant increase in glucose concentration in diabetic patients at the probability level ($P\leq0.01$). The statistical results also showed a highly significant increase in urea levels in diabetic patients compared to the control group, with this increase being statistically significant ($p\leq0.001$). However, the results indicate no significant increase in creatinine levels in diabetic patients compared to the control group at the same probability level ($p\leq0.001$). **Conclusion** The study concluded a decrease in the level of the enzyme acetylcholinesterase in diabetic patients compared to healthy individuals.

1. Introduction

Diabetes mellitus is one of the most common and influential endocrine disorders globally, affecting more than 100 million people and constituting approximately 6% of the total population. This disease arises as a result of a deficiency or ineffective production of insulin from the pancreas, which leads to an increase or decrease in the concentration of glucose in the blood[2]. The disease causes many problems and damage to the body's systems, including blood vessels, eyes, kidneys, heart, and nerves. Medications in diabetes are used to save lives and relieve symptoms. In addition, secondary treatments aim to prevent long-term complications and increase life span by eliminating various risk factors. Insulin replacement therapy supported by nutrition and a healthy lifestyle is essential for patients with type 1 diabetes. As for patients with type 2 diabetes, dietary modifications and lifestyle changes form the basis of treating and managing the disease. There are also various

types of drugs used to lower blood sugar levels, such as biguanides and sulfonylureas, but these drugs do not have an ideal effect due to the presence of toxic side effects and their effectiveness sometimes decreases after a long period[3].

Diabetes is classified by the World Health Organization (WHO) into three different types:

- Insulin-dependent diabetes mellitus (Type 1) (IDDM)
- Non-insulin dependent diabetes mellitus (Type 2) (NIDDM)
- Gestational diabetes mellitus

2. Materials and Methods

This study included collecting 60 blood samples from patients with type 2 diabetes. The ages of the patients ranged between 30 and 75 years, and 29 male samples and 31 female samples were included. The samples were collected from Al-Shirqat General Hospital after careful diagnosis by specialist doctors, based on the clinical symptoms and questionnaires filled out by the patients. The study also included a control group consisting of normal, healthy people for comparison. They numbered 30 samples, including 15 males and 15 females. They were selected from among people who undergo routine examinations at Al-Shirqat General Hospital.

2.1 Blood Collection and Serum Separation

The blood sample was collected from a vein using a 5 ml syringe needle and placed in new gel-containing plastic tubes. The sample was left for 15 minutes, then the serum was separated from the clotted fraction using a centrifuge at 5000×g for 10 minutes. The blood serum was drawn and divided into three parts and stored in the freezer at a temperature of -20°C. Part of the serum was used for the purpose of tests (C-PEP, GLP, R.B.S, F.B.S, Hb1Ac, Urea, and Creatinine) using ELISA and manual methods, and another part of the serum was used to purify the cholinesterase enzyme.

2.2 Separation and purification of Enzyme from serum of choline sterase Diabetic Patient

10 ml of crude enzyme was saturated with ammonium sulphate (NH4)2SO4) by adding 3.265 gm, in successive stages of salt, under refrigeration, by stirring for two hours. It was then deposited at 8000 rpm for 30 min at 4 °C.

The precipitate formed was then dissolved in a small amount of Tris-HCl at a concentration of 10 mM, and the activity of each enzyme was determined and the scientific group and experimental activity were calculated.

The resulting precipitation solution was then cleaned with 10 ml of 1 M Tris-HCl, pH 7.2, for 24 h at 4 °C, by stirring to purify it from inactive materials.

Detection was determined after each step and protein strengthening[4]

3. The linear correlation between the level of acetylcholinesterase enzyme and the studied clinical variables in type 2 diabetic patients.

To find the relationship between the acetylcholinesterase enzyme and a number of studied clinical variables in both diabetic patients and the control group, the linear correlation coefficient (r) was calculated between the enzyme and these variables. The correlation coefficient (r) serves as a measure of the degree of correlation or relationship between two independent variables and is used to describe the extent and strength of the relationship between different measured variables. The results are presented as follows:

1. The linear correlation coefficient (r) reflects the strength and direction of the relationship between the acetylcholinesterase enzyme and each of the studied variables.
2. If the correlation coefficient is close to +1 or -1, this indicates a strong relationship between the variables.

3. If the correlation coefficient is close to 0, this indicates a lack of significant relationship between the variables.

Through this method, data can be analyzed accurately to determine the extent of the effect of various clinical variables on the level of acetylcholinesterase enzyme in patients.

3.1 The correlation between acetylcholinesterase enzyme levels and glucose levels (F.B.S, R.B.S,HbA1c) in diabetic patients

- The results showed that the relationship between acetylcholinesterase enzyme levels and F.B.S glucose levels was a significant positive correlation, with a correlation coefficient value of (r=0.182) in diabetic patients.
- The results showed that the relationship between acetylcholinesterase levels and HbA1c levels was an insignificant negative correlation, with a correlation coefficient value of (r=-0.1217) in diabetic patients.
- The results showed that the relationship between acetylcholinesterase levels and R.B.S glucose levels was an insignificant negative correlation, with a correlation coefficient value of (r=-0.6832) in diabetic patients.

3.2 The correlation between acetylcholinesterase levels and urea and creatinine levels in diabetic patients.

- The results showed that the correlation between acetylcholinesterase levels and urea levels was a non-significant inverse relationship, with a correlation coefficient value (r = -0.7006) in diabetic patients.
- There was a significant positive correlation between acetylcholinesterase levels and creatinine levels, with a correlation coefficient value (r = -0.01321) in diabetic patients.

3.3 The correlation between acetylcholinesterase levels and the concentration levels of C-PEP and GLP-1 in diabetic patients.

- The results showed a positive correlation between acetylcholinesterase levels and C-pep levels, with a correlation coefficient (r = 462.9) in diabetic patients.
- There was a significant positive correlation between acetylcholinesterase levels and GLP levels, with a correlation coefficient (r = 17.22) in diabetic patients.

3.4 Statistical methods

The data obtained during the current study were statistically analyzed to determine the importance of different parameters by analysis of variance (ANOVA). Comparisons between means were made using least significant differences, and data are presented as mean ± standard deviation.

4. Results

The results represent statistical values of clinical parameters measured in serum by BMI

The clinical data measured in serum using the Body Mass Index (BMI) were analyzed. The results presented in Table (1-1), and according to previous studies, showed that an increase in serum glucose concentration in diabetic patients with a probability level ($P \leq 0.01$) is consistent with previous studies by Alsancak, Haffiner, and Shahad.I. M2021A.D. These studies indicated that the glucose level in patients is always higher than 120 mg/dl, as another study (wasn.A.H et al., 2020) indicated an increase in blood sugar levels in diabetic patients compared to the control group. This increase in glucose level is attributed either to a disturbance in insulin production and secretion or due to a disruption in the functions of cell receptors contributing to glucose consumption. It is also believed that genetic or acquired factors may play a role in this disorder and insulin deficiency, leading to an increase in blood sugar levels. The results in Table (1-1) also indicate a significant increase in urea levels in diabetic patients compared to the control group, and this increase is statistically significant ($P \leq 0.001$). This increase is attributed to the elevation of blood sugar levels, leading to increased urination and pressure on the kidneys, which can cause damage to their function and impairment. Additionally, the results indicate no significant increase in creatinine

levels in diabetic patients compared to the control group at the same probability level ($P \leq 0.001$). The results in Table (1-1) demonstrated a significant decrease in the concentration levels of the C-peptide hormone in diabetic patients compared to healthy individuals due to differences in insulin production ($P<0.05$) in Type 1 Diabetes Mellitus (T1DM). The C-peptide level was notably lower compared to healthy individuals. Type 1 diabetes is characterized by the immune system's attack on beta cells producing insulin in the pancreas, leading to decreased insulin secretion and consequently reduced C-peptide levels. Conversely, individuals with Type 2 Diabetes Mellitus (T2DM) may also have low levels of C-peptide, but this is not a definitive indicator of diabetes, as other factors can cause a decrease in C-peptide levels, such as low blood sugar levels. The decrease in C-peptide concentration in diabetic patients compared to healthy individuals is attributed to excessive insulin use, leading to increased insulin levels and decreased C-peptide levels, consistent with a study by (Venugopal, Mowery, & Jialal, 2023). C-peptide is considered an indicator of endogenous insulin secretion, with levels sharply rising in patients with Type 2 diabetes. Measuring C-peptide levels is essential to assess the body's ability to secrete insulin in diabetic patients. The C-peptide test can help differentiate between Type 1 and Type 2 diabetes by measuring insulin levels. Overall, low C-peptide levels in diabetic patients reflect impaired insulin production, which is crucial for regulating blood sugar levels and metabolic processes. There is also a significant decrease in the level of cholinesterase enzyme activity in diabetic patients compared to healthy individuals ($P<0.001$), due to various factors associated with the disease. The cholinesterase enzyme breaks down acetylcholine, which acts as an important neurotransmitter in transmitting nerve signals. Autonomic neuropathy is considered one of the possible reasons for the decreased activity of cholinesterase enzyme in individuals with diabetes, as this neuropathy affects the nerves responsible for regulating involuntary body functions, including digestion. Autonomic neuropathy can disrupt the normal function of the cholinesterase enzyme, leading to a decrease in its activity. Regarding GLP, there were no significant differences at ($P<0.01$) in patient groups compared to the control group. GLP-1 analogs have shown promising results in treating neuropathic conditions and do not affect blood sugar levels in non-diabetic individuals.

5. Discussion

Glucose is a simple sugar that contains six carbon atoms and is one of the most well-known types of sugar found in nature. Carbohydrates in food are broken down into glucose molecules in the digestive system before being absorbed into the bloodstream. When glucose levels in the blood rise, the pancreas is stimulated to release insulin, a hormone that helps lower blood sugar levels. Insulin works by promoting the absorption of glucose from the blood and storing it in cells for current or future use. Additionally, glucose plays a role in the formation of amino acids, fats, and nucleotides, which are essential components of proteins, lipids, and DNA[5].

Blood urea is a chemical compound that results from the breakdown of proteins in the body. It is an organic, non-toxic form of nitrogen that is produced when nitrogen combines with ammonia molecules. Blood urea is produced when ammonia, the final metabolic product of proteins, is converted to urea in the liver. It is then excreted in the urine by the kidneys[6]. In the case of diabetes, patients suffer from elevated blood sugar levels due to a deficiency in insulin secretion or a lack of cellular response to insulin. This elevation impacts protein metabolism in the body. When there is a deficiency in insulin secretion or an inadequate response to insulin, the body's ability to use glucose as an energy source is compromised. As a result, cells rely on converting fatty acids and proteins into energy[7].

C-peptide, a byproduct of insulin synthesis, has multiple functions in managing diabetes. C-peptide levels can be measured to differentiate between type 1 and type 2 diabetes, with low levels indicating type 1 diabetes and insulin dependence[8]. Additionally, studies have shown that C-peptide improves nerve and kidney function in animal models of diabetes, as this peptide has biologically active properties that influence microvascular blood flow and tissue health. GLP-1 is a peptide hormone that plays a key role in maintaining post-meal glucose homeostasis, insulin secretion, regulating food intake, and bowel movements[9]. GLP-1 receptor agonists are approved

treatments for type 2 diabetes, as they enhance insulin secretion and improve blood sugar control[10]. They also inhibit glucagon production and promote the growth of beta cells in the pancreas. Cholinesterase is classified within the category of enzymes known as hydrolases, specifically within the esterase family, which includes a group of enzymes found in the central nervous system. Enzymes are classified based on the Enzyme Commission Number system, where numerical codes are assigned according to their chemical function and activity[11].

Regarding the relationship between cholinesterase and diabetes, there is no direct link between them. Diabetes is a chronic disorder characterized by high blood sugar levels due to a deficiency in insulin production or the body's resistance to it. However, cholinesterase can have an indirect effect on diabetes through its influence on the central nervous system and the sympathetic nervous system, which contributes to the regulation of blood sugar levels and metabolism. Some research suggests that changes in cholinesterase activity may affect the function of the sympathetic nervous system and blood sugar levels[12].

6. Conclusion

The study showed a decrease in the activity level of acetylcholinesterase enzyme in diabetic patients compared to healthy individuals. The results also indicated no significant differences in acetylcholinesterase enzyme levels when comparing males and females within both the healthy and patient groups. Additionally, the study found no significant differences in enzyme concentration between obese and non-obese patients, nor among different age groups included in the study. The study also revealed a decrease in C-peptide hormone levels in diabetic patients compared to healthy individuals, with no significant difference in the concentration of GLP-1 hormone. Furthermore, the studies confirmed an increase in blood glucose levels in patients compared to healthy individuals, and an elevated level of urea in diabetic patients, while no effect was observed in creatinine concentration.

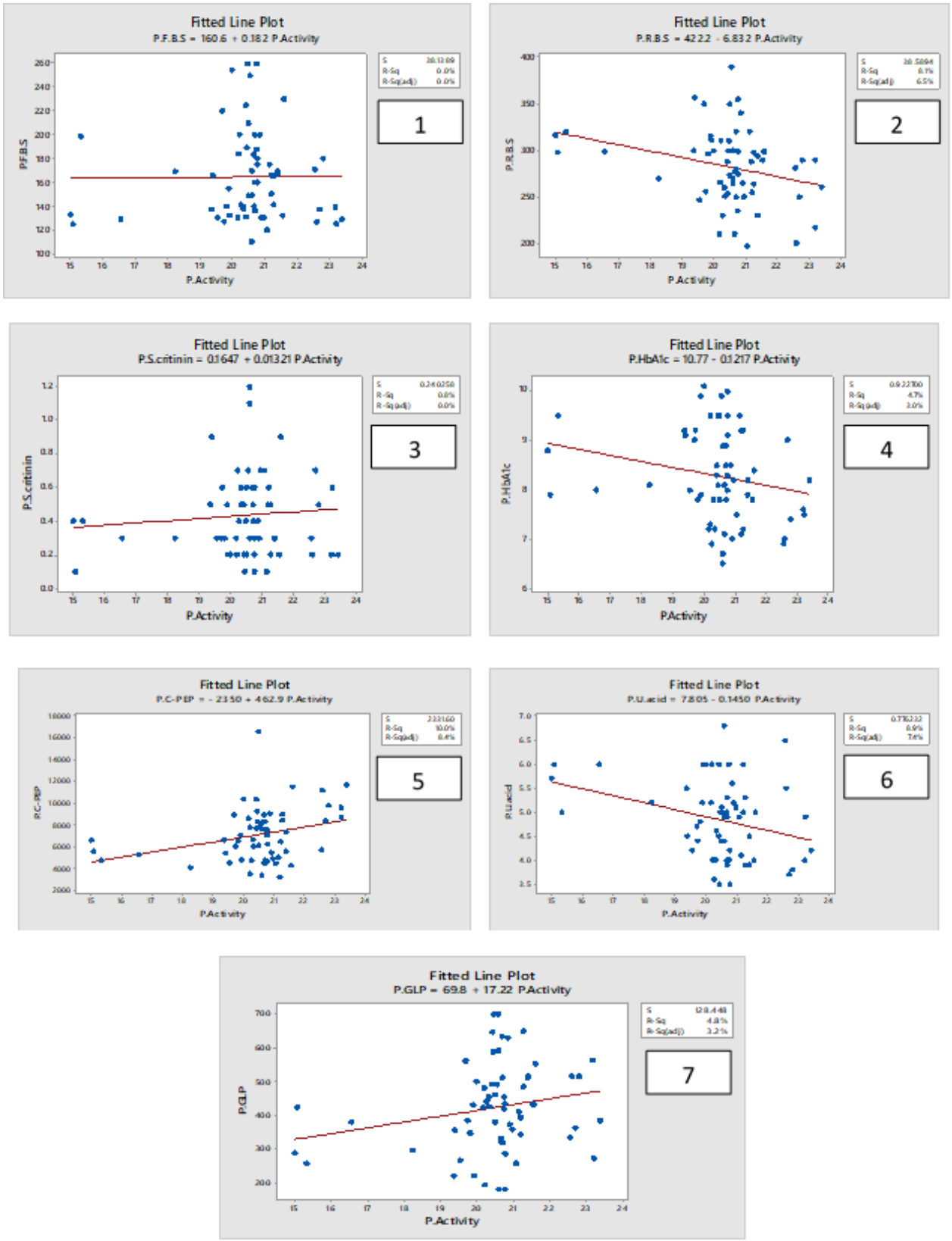
7. Ethical considerations

Permission to conduct this study was issued by the Health Institutional Committee at Al-Shirqat Hospital in Saladin Governorate/Iraq, and samples were taken from patients under the supervision of professional health care workers.

8. Tables and figures

Table (1-1): Levels of the hormones C-peptide, GLP-1, F.B.S, R.B.S, H b A 1 c, Urea, Creatinine and cholinesterase enzyme activity in the blood

Groups Parameter									
GLP (pmol/L)	**C - PEP (ng/ml)**	**Creatinine mg/dl**	**Urea mg/dl**	**H b A 1 c mg/dl**	**R.B.S mg/dl**	**F.B.S mg/dl**	**CHE µmol/ml/min**	**Mean ± SD**	
545 ns 81.7±	**11144 * 140.2±**	**0.397 ns 0.187±**	**27.52** **4.91±**	**4.728 0.440±**	**116.3 9.41±**	**84.63 9.92±**	**23.505** **0.851±**	**30**	Control
494 61.3±	**7124 243.7±**	**0.435 ns 0.239±**	**32.38** **8.87±**	**8.278** **0.937±**	**282.4** **19.9±**	**164.3** **17.8 ±**	**20.46 1.66±**	**60**	Patient
0.74 0.465	2.56 0.030	**0.83 0.407**	**3.34 0.001**	**24.44 0.0007**	**30.56 0.0008**	**15.30 0.0007**	11.47 0.0005	T-Value P-Value	



Linear Correlation between the activity level of acetylcholinesterase enzyme and biochemical variables

9. References

1. Phieler, J., et al. *The role of the complement system in metabolic organs and metabolic diseases*. in *Seminars in immunology*. 2013. Elsevier.
2. Ismail, M.Y.M. and M. Yaheya, *Clinical evaluation of antidiabetic activity of Trigonella seeds and Aegle marmelos leaves.* World Applied Sciences Journal, 2009. **7**(10): p. 1231-1234.

3. Tattersall, R.B. and D.R. Matthews, *The history of diabetes mellitus.* Textbook of diabetes, 2024: p. 1-21.
4. Kummari, R. and K. Bose, *Gel Filtration Chromatography*, in *Textbook on Cloning, Expression and Purification of Recombinant Proteins*. 2022, Springer. p. 199-219.
5. Alberts, B., et al., *Molecular biology of the cell*. Vol. 3. 1994: Garland New York.
6. Haslett, C. and S. Davidson, *Davidson's principles and practice of medicine*. 1999: Churchill Livingstone.
7. Kachmar, J. and D. Moss, *Fundamentals of clinical chemistry.* WB Saunders and Company, Philadelphia, 1976: p. 604.
8. Landreh, M. and H. Jörnvall, *Biological activity versus physiological function of proinsulin C-peptide.* Cellular and Molecular Life Sciences, 2021. **78**(3): p. 1131-1138.
9. Gribble, F.M. and F. Reimann, *Metabolic messengers: glucagon-like peptide 1.* Nature metabolism, 2021. **3**(2): p. 142-148.
10. Cornell, S., *A review of GLP-1 receptor agonists in type 2 diabetes: a focus on the mechanism of action of once-weekly agents.* Journal of Clinical Pharmacy and Therapeutics, 2020. **45**: p. 17-27.
11. Gupta, V.K., et al., *Acetylcholinesterase from human erythrocytes as a surrogate biomarker of lead induced neurotoxicity.* Enzyme research, 2015. **2015**.
12. Colovic, M.B., et al., *Acetylcholinesterase inhibitors: pharmacology and toxicology.* Current neuropharmacology, 2013. **11**(3): p. 315-335.