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**Research Article**

# Acetylcholinesterase: biochemical characterization in individuals with autism spectrum disorder

**Ako Saber Kaky[1], Sayran Sattar Saleh[1], Mustafa Elias Abdulqader[2]**

[1] Kirkuk University, College of Science, Chemistry Department, Kirkuk, Iraq
[2] Ministry of Health – Kirkuk health directorate, Kirkuk, Iraq.
*Email: ako.kaky@uokirkuk.edu.iq

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## Abstract

The present study aims to evaluate the levels of acetyl cholinesterase activity, specific activity, and total protein in blood samples obtained from persons who have received a diagnosis of autism spectrum disorder. Furthermore, the objective of this study is to determine the most favourable parameters for acetyl cholinesterase concentration, substrate concentration, pH level, temperature, and duration. In addition, the present study aims to ascertain the Km and Vmax values of acetyl cholinesterase in blood samples obtained from patients diagnosed with autism spectrum disorder. A complete collection of 100 samples was procured from individuals who have been diagnosed with autism spectrum disorder (ASD). The study's results suggest that persons diagnosed with ASD demonstrate reduced levels of enzyme activity in comparison to the control group. Moreover, the enzymatic activity in individuals with ASD is shown to be significantly elevated compared to the control group. In contrast to the control group, individuals diagnosed with ASD exhibit decreased amounts of total protein. persons with ASD exhibit slight deviations in the thermodynamic characteristics of enzymes, including the activation energy, ΔG, ΔS, Pz, and ΔH*, when compared to persons in the control group.

**Keywords**: acetylcholinesterase, enzyme activity, total protein, autism spectrum disorder

## Introduction

As a hydrolase enzyme, acetylcholinesterase is accountable for converting acetylcholine into choline and acetic acid. It is exclusively present in animal tissues and is essential for nerve tissue function. There are two primary classifications of cholinesterase enzymes, namely acetyl

cholinesterase and butyryl cholinesterase. The understanding of acetylcholine's role as a neurotransmitter and its hydrolysis by cholinesterase is well-established in academic literature. The primary enzyme utilized for this objective is acetyl cholinesterase, which is sometimes referred to as choline esterase I or erythrocyte cholinesterase. It is predominantly located inside chemical synapses and erythrocyte membranes (Xie and Gross 2022). The membrane of the human erythrocyte contains a considerable number of enzymes, with acetyl cholinesterase being the primary enzyme that consistently exhibits altered activity in response to pathological circumstances. The precise physiological activities of erythrocyte acetyl cholinesterase are not yet fully understood. However, its positioning at or close to the cell surface makes it particularly important in investigations concerning cellular membranes and the changes in activity observed in various illnesses (Calas et al. 2020).

A neurodevelopmental illness known as autism spectrum disorder (ASD) is typified by brain abnormalities that result in a variety of impairments. The condition under consideration is a neurological and developmental illness that exerts an impact on individuals' social interactions, communication abilities, learning processes, and behavioral patterns (Thorsen 2020). Autism spectrum disease is classified as a "spectrum" condition due to the considerable diversity observed in the nature and intensity of symptoms shown by individuals. Certain individuals diagnosed with autism (ASD) have identifiable distinctions, such as genetic anomalies, but the etiology of other cases remains undetermined (Zainal, Safaa, and Obead 2013). The prevailing consensus among experts is that autism spectrum disorder (ASD) is a multifaceted disorder that is impacted by a confluence of circumstances. These factors combined contribute to deviations from the customary trajectories of human development (Zainal, Safaa, and Obead 2013). Autism is characterized by symptoms that encompass difficulties in social communication and interaction, as well as the presence of limited or repetitive activities and interests (Leader et al. 2021; Tseng et al. 2020). Individuals diagnosed with Autism (ASD) may display heterogeneity in their learning modalities, motor proficiencies, and attentional capacities. (Tseng et al. 2020). Individuals diagnosed with autism often encounter difficulties in social communication and interaction skills. These challenges may manifest in behaviors such as avoiding or failing to maintain eye contact, not responding to their name being called, or exhibiting a limited range of facial emotions, including but not limited to happiness, sadness, anger, and surprise (Mundy and Bullen 2022). It is noteworthy that individuals without autism spectrum disorder may also exhibit these symptoms, albeit to a lesser extent. However, for those diagnosed with autism spectrum disorder, these

specific traits might provide significant challenges in their daily lives (Mundy and Bullen 2022). Autism spectrum disorder (ASD) is prevalent across many racial, cultural, and socioeconomic groups, with a notable gender disparity as it affects males more than girls by a factor of over four (Yavari et al. 2022). Every individual diagnosed with ASD has a distinct set of behavioral patterns and varying levels of severity, ranging from low to high functioning (Tseng et al. 2020). Certain individuals diagnosed with autism may encounter challenges in the realm of acquiring knowledge, while others may exhibit indications of below-average cognitive abilities (Mundy and Bullen 2022). Research has indicated that children diagnosed with autism have a higher prevalence of sleeplessness, anxiety, and depression compared to their neurotypical counterparts. Furthermore, there is a heightened probability that these individuals may be diagnosed with attention deficit hyperactivity disorder (ADHD) (Schott, Tao, and Shea 2022).

The determination of autism spectrum disorder is reliant upon the identification of specific symptoms, as there is currently no diagnostic procedure of a medical nature available for this condition. The management of autism spectrum condition may encompass a multifaceted approach, incorporating several therapeutic modalities such as behavioral, speech, and occupational interventions, as well as pharmacological interventions (Al-Beltagi 2021). The mitigation of the effects of co-occurring illnesses can be achieved by the provision of appropriate services, encompassing the aforementioned options, as well as psychotherapy and/or prescription treatment (Al-Beltagi 2021). Recent research conducted on both humans and animal models indicates that there is a potential association between autism-related behavioral symptoms and malfunction of the cholinergic system. Dysregulation of cholinergic neurotransmission has been documented in persons diagnosed with autism spectrum disorder (Chien et al. 2020). Nicotinic acetylcholine receptors (nAChRs) have been implicated in the pathogenesis of autism spectrum disease, as shown by available evidence. The phenomenon of diminished expression of the nicotinic α4β2 subtype of acetylcholine receptors (nAChRs) has been documented in persons diagnosed with autism. The aforementioned receptors have been recognized as prospective biomarkers and therapeutic targets in relation to autism spectrum disease (Zou et al. 2021). In addition, Aberrations in cholinergic activity within the cerebral cortex and basal forebrain have been observed in persons diagnosed with autism spectrum condition, as indicated by many studies. Although the cholinergic enzyme indicators seem to be within normal range, deviations in the nicotinic and muscarinic receptors have been detected (Kramer et al. 2022). Furthermore, the dysregulation of the interaction between neuronal nicotinic acetylcholine receptors and cholesterol

is being examined. Recent research has indicated that there exists a correlation between autism spectrum disorder and the disturbance of cholesterol metabolism. This disturbance has the potential to impact the communication between neuronal nicotinic acetylcholine receptors and cholesterol. The imbalance described has the potential to play a role in the pathogenesis of autism spectrum disease (Wang, Ding, and Wang 2020).

Ultimately, it is plausible that deviations within the cholinergic system, encompassing modifications in nicotinic acetylcholine receptors and imbalances in cholinergic functioning, might potentially contribute to the etiology and manifestations of autism spectrum disease. Additional investigation is required in order to comprehensively comprehend the processes that underlie this association and to examine prospective treatment strategies that focus on the cholinergic system in persons diagnosed with autism spectrum disorder (Persico et al. 2021).

Recent articles about autism spectrum disorder and acetylcholinesterase suggest that the cholinergic system may play a role in the development and symptoms of autism, and that acetylcholinesterase inhibitors may have potential therapeutic implications for treating autism. Here are some key findings from the articles (Lee et al. 2021):

- Individuals diagnosed with ASD exhibit various deficiencies in the generation of acetylcholine (ACh) and the functioning of ACh receptors. Acetylcholinesterase, an enzyme responsible for the breakdown of acetylcholine, is implicated in these processes.
- There is a growing body of research indicating that disruption in the cholinergic system is implicated in the phenotypic manifestations of behavioral traits associated with autism.
- The potential role of reduced acetylcholine levels in the manifestation of autistic symptoms has been subject to investigation.
- Abnormalities of cholinergic neurotransmission in autism spectrum disorders may be involved in the development and symptoms of autism, and targeting nicotinic acetylcholine receptors may have therapeutic implications for treating autism.
- Reduced acetylcholinesterase activity in the fusiform gyrus, a brain region involved in face recognition, has been observed in adults with autism spectrum disorder, suggesting a deficit in cholinergic innervations of this brain region.

In general, the aforementioned articles posit that the cholinergic system and acetylcholinesterase may exert significant influence on the aetiology and manifestations of autism. Consequently, additional investigation is warranted to comprehensively elucidate the mechanisms that underlie

this association and to investigate potential therapeutic interventions that target the cholinergic system in individuals diagnosed with autism. (Bougeard et al. 2021; Persico et al. 2021).

## Materials and methods

### Study design and Subjects

This study involved the selection of a sample of one hundred blood samples from kids diagnosed with autism. The patients included in the sample ranged in age from 5 to 12 years. The patients sought medical care at the Pediatric Hospital located in Kirkuk City. The diagnoses of the cases were conducted by specialist medical professionals between August 2022 and May 2023. Each patient underwent a personal interview utilizing a specifically created questionnaire format that encompassed a comprehensive medical history and thorough information. The control group consisted of 50 individuals who were deemed healthy and matched the age range of the patients. Any individuals with conditions such as diabetes mellitus, liver disorders, hypertension, and anemia were excluded from the research.

### Acetylcholine esterase extraction

A volume of about 5 millilitres of venous blood was collected from both control participants and patients with a disposable syringe. The blood was obtained using venipuncture and collected into Jel-tubes. Following that, the blood samples that had been gathered were subjected to centrifugation at a magnitude of 1500 times the acceleration due to gravity for a period of 15 minutes. This centrifugation process facilitated the separation of serum from the cellular components. Finally, the obtained serum samples were kept at a temperature of -20 degrees Celsius until they were utilized for further analysis.

### Acetyl cholinesterase assay

The activity of acetylcholinesterase was assessed using the methodology described by reference (Birman 1985). The quartz cuvette holds a reaction mixture of 3 mL with a path length of 1 cm. The mixture consists of 0.075 M of ATI, 0.01 M of DTNB, and 0.1 M phosphate buffer at a pH of 7.4. The optical density was assessed at a wavelength of 412 nm during a period of 3 minutes, with measurements taken every 30 seconds. The enzyme activity was determined by employing the extinction coefficient $\Delta A \times D.F \times 106 / 13600 \times 3 \times 103$ μmol/min/ml and was quantified as $\mu$moles of acetylthiocholine hydrolyzed $mL^{-1}$ $min^{-1}$ or units (U). The enzymatic activity was quantified and reported in units per milligram (U $mg^{-1}$).

### Protein estimation

The estimation of protein content was conducted using the Lowry technique and Ciocalteu's Phenol

reagent. Additionally, protein measurement was performed using the Folin phenol reagent. The standard employed in this study was bovine serum albumin (Brodrick et al. 1978). The measurement of absorbance was conducted at a wavelength of 620 nm for the blue-colored complex.

**The optimum conditions**

The optimum conditions for the acetylcholine esterase were determined. To estimate the optimum concentration of the acetylcholine esterase, different volumes of serum ranging from (20 to 65) µL were taken, optimum concentration of the substrate determined using different concentrations (0.03 to 0.5) M. The kinetic factors, which are the Michaelis-Menten constant (Km) and the fastest speed (Vmax), were found by checking how active the enzymes were. This was achieved by conducting assays with different doses of the substrate acetylthiocholine iodide, ranging from 0.03 M to 0.5 M, the optimum pH and temperature were determined using different values from pH (5.8 to 9.0) and temperatures (20 to 40) $C_o$ respectively. To determine the optimum incubation time for acetylcholine esterase activity, different incubation times were taken, ranging from (2 to 8) minutes.

**Thermodynamic studies on the partially purified enzyme include the following**

The experimental procedure described in reference (Birman 1985) was employed, which involved conducting enzyme activity measurements on partially purified fractions at a temperature of 37°C. These measurements were carried out to determine various energy parameters, including the equilibrium constant “Keq”, Gibb's energy change “ΔG”, enthalpy change “ΔH”, entropy change “ΔS”, activation energy “Ea”, stereo-frequency collision factor “Pz”, and heat of activation “ΔH*”. The equilibrium constant, Keq, was determined at a temperature of 37°C using the equation provided in reference (Xie and Gross 2022):

$$Keq=1/Km \quad (1)$$

The following equation was used to calculate energetic parameters:

$$\Delta G= 2.303\ RT\ \log(Keq) \quad (2)$$

The integral Van't Hoff equation was used to calculate the change in enthalpy ΔH between the limits of Keqat 37 C˚ temperatures:

$$\Delta H=2.3R\ (\log(Keq))\ T \quad (3)$$

The entropy change was calculated using Gibb's Helmholtz equation:

$$\Delta G=\Delta H-T\Delta S \quad (4)$$

The activation energy (Ea) of the reaction was determined by use the integrated version of the Arrhenius equation:

$$\text{Log}(K) = Ea/2.303\ R*T \quad (5)$$

The (Pz) factor was calculated using the following formula:

$$\text{Ln}(Pz) = (Ea/RT) + \ln(Keq) \quad (6)$$

The variable Z represents the rate of molecular collisions between enzymes and reacting molecules, expressed as the frequency per unit volume per unit time.. The stearic potency, denoted as P, represents the effectiveness of a percentage of molecules that are able to acquire sufficient energy during collisions. On the other hand, the term (Ea/RT) corresponds to the frequency factor, which quantifies the likelihood that any given molecule possesses the energy to undergo a reaction. The following equation was used to calculate the heat of activation (H*):

$$Ea = \Delta H^* + RT \quad (7)$$

**Statistical analysis**

The statistical analysis was conducted using GraphPad Prism Version 8, a software application developed by GraphPad Software in San Diego, California, United States. The findings were thereafter examined. The findings were presented using the mean ± standard deviation (SD). The term "notation" refers to a system of symbols or characters used to represent and communicate. The statistical technique employed for comparing means in this study was the analysis of variance (ANOVA) test. The means were presented as the mean ± standard deviation (SD). The operationalization of the idea of statistical significance involved the establishment of a threshold of ($P < 0.5$) and the subsequent examination of the correlation between the parameters.

## Results and discussion

Tables (1) and (2) represented the activity and specific activity of erythrocytes extract AChE expressed as (Mean ±SD) (U/ml and U/mg) of the autism spectrum disorder as compared to control group.

**Table 1.** Activity, specific activity and total protein of serum AChE of autism spectrum disorder respectively as compared to control group.

| Parameters | mean± SD | | |
|---|---|---|---|
| | Control(n=50) | Patient(n=100) | P value |
| Acetyl choline esterase activity µmole/ml/min | 5.02 ± 0.867 | 4.22 ± 1.160 | 0.0369 ± |
| Total Protein (gm/dl) | 7.06 ± 0.792 | 7.41 ± 0.899 | 0.508 ± |
| Specific activity of enzyme U/mg | 0.72 ± 0.150 | 0.57 ± 0.145 | 0.254 ± |

Significant ($p \leq 0.5$).

The results presented in Table 1 indicated that the activity and specific activity of the enzyme in patients were significantly ($p \leq 0.5$) lower than in the control group, these findings were consistent with the prior studies conducted by the researchers (Sponchiado, Liao, and Reznikov 2022; Vallés and Barrantes 2021), The researchers investigate the factors related to the brain and blood of adult individuals who have had ovariectomy, as well as those diagnosed with autism spectrum disorder. The observed effect can be attributed to lipid peroxidation, which functions as a signal of oxidative stress. Acetylcholinesterase has been documented to have associations with stress reactions and is implicated in inflammatory processes. Furthermore, the findings align with prior research that has suggested a correlation between the enzymatic activity and the modulation of metabolic precursors, the regulation of enzymes involved in the synthesis of active acetylcholine from its precursors, the facilitation or inhibition of acetylcholine release by nervous system cells, and the downregulation of acetylcholine esterase expression. These effects may be linked to lipid peroxidation, which serves as an indicator of oxidative stress (Fang et al. 2020). The decrease in enzyme activity can be attributed to a variety of causes, including memory, concern, boredom, and dissatisfaction. This provides more evidence that this condition is associated with psychiatric health and psychiatric problems (Dennison et al. 2021). A statistically significant decrease ($p \leq 0.5$) was observed in the specific activity. The decline in specific activity can be ascribed to the existence of certain chemicals in the form of impurities. The specific activity, which is determined by dividing the enzyme activity by the concentration of total protein in the serum extract, is commonly used as an indicator of enzyme purity (Zhou et al. 2021). The findings of the study revealed a statistically significant increase ($p \leq 0.1$) in the total protein content within the patients group as compared to the control group. Proteins serve several functions in biological systems, including acting as enzymes, hormones, and antibodies. Additionally, they play a role in regulating osmotic pressure in the blood. The results displayed in Table 2 indicate that there is no statistically significant disparity ($p \geq 0.1$) in the overall protein levels observed among persons diagnosed with autism and those belonging to the control group. The present study examines the disparity in total protein levels between individuals diagnosed with autism and a control group. Total protein is considered as the most abundant components of human serum. The possible cause of increased serum TP secondarily increased the synthesis of protein by the liver (Lagerwaard et al. 2021). The results of total protein concentration agreed with (Smedler et al. 2021), the studied the above parameter in autism patients.

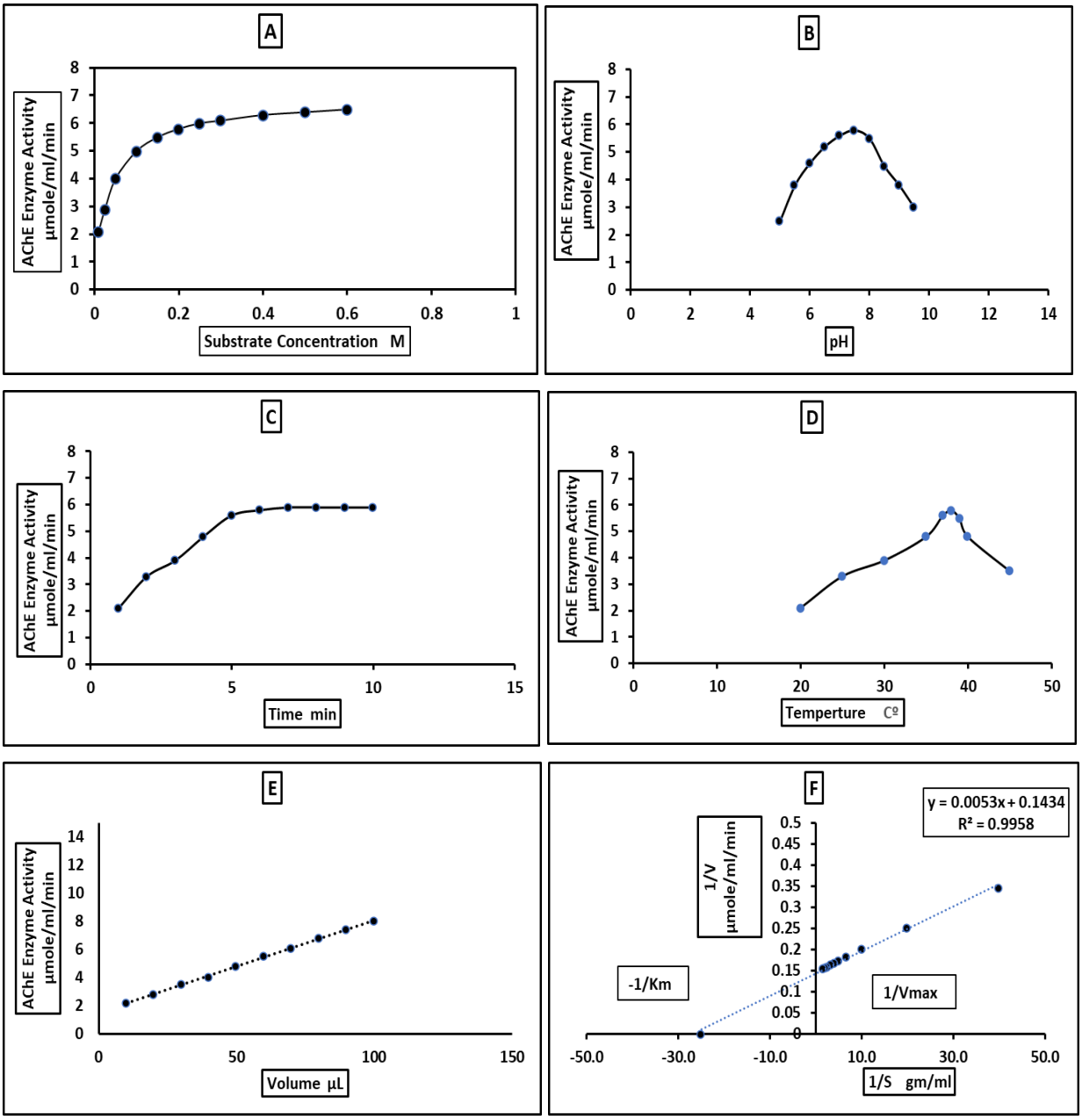


**Figure 1.** A) The effect of different concentrations of substrate on the serum of enzyme activity of autism spectrum disorder patients; B) the effect of pH on enzyme activity in autism spectrum disorder patients serum; C) The effect of incubation time on the activity of enzyme in serum with autism spectrum disorder; D) the effect of pH on enzyme activity in autism spectrum disorder patients serum; E) The effect of volume serum on enzyme activity the effect of temperature on the activity of enzyme inserum with autism spectrum disorder patients; F) Lineweaver – Burk Plot for Km and Vmax values of enzyme.

In order to investigate the impact of an enzyme on the rate of an enzymatic reaction, varying quantities of serum were employed. Based on the observations depicted in Figure (E), taking into account the data that is now available, it is possible to draw the conclusion that there is a direct

connection between the pace of the enzymatic reaction and the concentration of the enzyme. This is evident as the rate of the enzymatic reaction demonstrates an upward trend in patients diagnosed with autism spectrum disorder, in response to an elevated concentration of the enzyme. It is important to note that this relationship is observed under the condition where the substrate remains at a constant concentration within the reaction medium. According to the findings of this study, the rate at which the enzymatic reaction, which is responsible for the transformation of the substrate into a product, occurs is influenced by the concentration of the enzyme that is present within the parameters of the reaction (Persico et al. 2021). In Figure (A), the relationship between substrate concentration and the activity of AChE in patients with autism spectrum disorder was demonstrated. The experimental results revealed that the most favorable concentration of substrate was determined to be 0.2 M. It is noteworthy that the concentration of the substrate is a crucial determinant in estimating the rate of enzymatic reaction within the saturation range, leading to the formation of a substrate enzyme complex [ES]. As the substrate concentration continues to increase, the rate of enzymatic reaction experiences a deceleration until it reaches a constant speed, irrespective of further increases in substrate concentration. The user's text does not contain any information to rewrite (Whitaker 2018). The data shown in Figure (F) illustrates the impact of varying substrate concentrations on the velocity of acetyl cholinesterase in serum. The results indicate that there exists an optimal substrate concentration that corresponds to the maximum velocity (Vmax) of the enzyme. The results obtained from this study suggest that the enzyme achieved saturation with the substrate when the concentration of S-Acetyl thiocholine iodide reached 0.35 M. After doing an analysis of the plot pertaining to the Line Weaver-Burk, it was ascertained that the Vmax value exhibited a magnitude of 0.11 U/ml, while the Km value was revealed to be 0.035 M. The findings from prior investigations have exhibited incongruity. For instance, (Shivalingu et al. 2016) reported that The Km and Vmax values of the enzyme were discovered to be 0.037 mM and 0.192 U/ml, respectively. These values correspond to the Km and Vmax for acetylcholine esterase, which were found to be 0.128 mM and 0.073 mM sec-1, respectively. The researchers investigated the characteristics of rat brain acetyl cholinesterase as a biomarker for cadmium-induced neurotoxicityThe determination of the ideal pH of the enzyme involved testing its activity over a pH range of 5 to 9.5. The enzyme activity was assessed under optimal circumstances, and it was noted that the maximum level of enzyme activity was achieved at a pH of 7.4 in blood. The enzymatic reaction exhibits an increase in speed as the pH of the buffer solution is elevated. This increase continues until it achieves its maximum velocity, at which point

the pH is referred to as the optimal pH. The optimum pH corresponds to the highest level of enzyme activity. Subsequently, the enzyme's activity diminishes as the pH continues to rise (Fincan et al. 2021).

The pH has an impact on certain locations of enzymes. The ionic characteristics of the amino and carboxylic groups of the enzyme are of interest in understanding their role in enzymatic activity. The present work investigates the ionic characteristics shown by the side groups of amino acid units. Extensive characterization has been conducted on the ionic characteristics of the amino acid residues situated inside the active site of the enzyme, as well as the particular areas accountable for catalytic activity (Nothling et al. 2018). The enzymatic activity was assessed by subjecting the reaction mixture to various incubation temperatures, namely 20°C, 25°C, 27°C, 30°C, 35°C, 37°C, 40°C, and 45°C. By employing the optimal circumstances of acetylcholinesterase (AChE) and its substrate, together with the optimal pH level. The findings depicted in Figure (D) demonstrate that the optimal temperature for acetylcholinesterase in serum among individuals with autism spectrum disorder is 37°C. Enzymes exhibit optimal bonding with substrates and achieve maximum efficiency at their respective optimum temperatures. This phenomenon arises from the positive correlation between temperature and the speed of enzymatic reactions. The underlying mechanism can be attributed to the heightened kinetic energy of enzymes at higher temperatures, which subsequently enhances their affinity for substrates. However, it is crucial to note that this temperature-induced enhancement should not surpass the threshold that leads to enzyme denaturation (Bray et al. 2021). The impact of temperature on the pace of enzymatic reactions may be attributed to two main factors: the alteration of enzyme-substrate affinity and the influence on enzyme stability or complex dissolution (Li et al. 2020). A reduction in enzymatic activity was seen when temperatures exceeded the optimal range. The cause of this phenomenon may be attributed to a disruption in the three-dimensional conformation of the protein that plays a crucial role in enzyme catalysis. This disruption arises from distortions occurring inside certain enzyme molecules, thereby resulting in alterations within the enzyme's active sites (Rodrigues et al. 2018). To determine the ideal incubation time for the enzymatic reaction of the partially purified enzyme in patients diagnosed with autism spectrum disorder, the enzyme's activity was measured at various time intervals ranging from 1 to 10 minutes. This assessment was conducted under the optimal conditions for the enzyme, as previously established (Halloy et al. 2021). Figure (C) illustrates that the optimal incubation time for achieving maximum enzymatic activity of the serum enzyme in patients with autism spectrum disorder is 5 minutes. This observation suggests a decline in the

reaction rate over time, which can be attributed to various factors, as mentioned in reference (Shivalingu et al. 2016). Enzyme inhibition can occur when the reaction products bind to the enzyme, leading to a drop in the rate of the reverse reaction. This phenomenon becomes apparent when there is a rise in the concentration of the product that is generated as a result of the enzymatic process. As the reaction progresses, the reaction rate steadily decreases due to a decrease in the concentration of the substrate (Dermiki and FitzGerald 2020).

**Thermodynamic studies on acetylcholine esterase activity of autism spectrum disorder patients**

The determination of the enzyme activation energy (Ea) serves as a valuable metric in the computation of the thermodynamic obstacle that necessitates surmounting within the catalytic cores. The utilization of Ea has been employed in the examination of conformational alterations that occur during catalysis, as well as in the comparison of enzymes derived from diverse purified samples of patients with retinopathy. Consequently, the parameter Ea has emerged as a topic of significant relevance within the field of enzymology. To the best of our knowledge, there is currently a lack of research on the energetic characteristics of enzyme-catalyzed reactions, despite the numerous in vitro studies conducted on isolated enzymes from different sources. This study aimed to evaluate the equilibrium constant (keq) and other energetic characteristics associated with a human enzyme. These parameters include the Gibbs free energy (ΔG), enthalpy change (ΔH), heat of activation (ΔH*), entropy change (ΔS), activation energy (Ea), and the number of collisions (Pz). The initial part of the experimental procedure involved assessing the enzymatic activity under optimal conditions, namely at a temperature of 37°C, while varying the amounts of the substrate (ranging from 0.01 M to 0.6 M). Table 3 displays the values of several thermodynamic parameters, including the equilibrium constant (Keq), the change in Gibbs free energy (ΔG), the activation energy (Ea), the enthalpy change (ΔH), the entropy change (ΔS), the partial pressure (Pz), and the activation enthalpy (ΔH*) (Najia, Salehb, and Taherc 2018).

**Table 2.** For autism spectrum disorder children, energetic parameters of partial putrefaction enzyme reaction.

| | **Ea (KcaL.mol)** | **ΔH (kcal. mol)** | **ΔG (kcal.mol )** | **ΔS (kcal.mol.deg)** | **Keq mM** | **Pz (mM)** | **ΔH* (kcal.mol)** |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Control** | 10.89 | -602 | -2960 | -7.6 | 0.982 | 1.396 | -204.7 |
| **Patients** | 9.52 | -604 | -3254 | -8.5 | 0.985 | 1.208 | -116 |

Table (1) showed that there were decreases in Ea of acetylcholine esterase in fraction of autism spectrum disorder patients. The fraction had Ea lower, the observed findings suggest the existence of a modification in samples of individuals with autism spectrum disorder, which leads to an elevation in the thermodynamic barrier. Consequently, this delay in the disintegration of the [ES] complex and the subsequent generation of products occurs (Nordin et al. 2021). Furthermore, the data show that the energy barrier in fraction is higher due to the lack of a cofactor or other substances required for acetylcholine esterase activity.

In addition, the results showed that for autism spectrum disorder patients, $K_{eq}$ values of acetylcholine esterase increased in fraction. The Keq values expressed as a percentage were found to be (0.985) mM. This suggests that the enzymatic reaction in this particular fraction is more inclined towards proceeding from right to left compared to the other fractions. This observation implies that the concentration of the enzyme-substrate complex ([ES]) is relatively low at equilibrium, as indicated by the following equation.


The calculation of ΔG yields negative fractional values, which indicate that The formation of the activated complex [ES] occurs spontaneously throughout the manufacturing process. This reaction's change in free energy value is affected slightly by molecular pathway mechanism transformation. The change in acetylcholine esterase enthalpy values was negative charge in fraction, indicating that the enzymatic reaction is exothermic. The randomness in acetylcholine esterase structure was indicated by the negative entropy value. The presence of a negative ΔS value signifies the development of a transition state between cholinergic esterase molecules and substrate molecules. This ΔS value represents the intrinsic likelihood of the transition state occurring, regardless of energy factors. The collision factor, Pz, for acetylcholinesterase was

determined to be 1.208 mM for the interaction between S-Acetyl thiocholine iodide and acetylcholinesterase in the fraction. This value suggests that the collision potency of the partially purified enzyme is influenced by the favourable orientation and potential energy distribution of the colliding molecules. For fraction the change in heat activation was calculated as (9.52) kcal.mol. The observed high values of ΔH* suggest significant molecular bond stretching, compression, and potential bond breakage occurring during the development of the transition state (Nordin et al. 2021). The biological significance of the table (2) data can be explained as follows: For fraction each of the thermodynamic parameters (ΔG, Ea) increased dramatically, indicating that the partially purified acetylcholine esterase was lacking in metals or substancesthat aid the enzyme in overcoming the energy barrier. Simultaneously, the acetylcholine esterase, which was partly purified and in its unbound state, had the highest probability of adopting a favorable substrate orientation. Moreover, the thermodynamic activation state diagram (ASD) of the acetylcholine esterase reaction exhibited an increase, and the significant alterations in ΔG, Ea, ΔH, and ΔS were consistent with the impact of the pathological condition on the mechanism and activity of the enzyme (Nabeel, Zainal and Zainal 2019). In addition, the results showed that for beta thalassemia patients, Keq values of acetylcholine esterase increased in fraction. The values of Keq in fraction were (1.208) mM, The observation suggests that the enzymatic process in this particular fraction has a higher propensity for proceeding from right to left compared to other fractions. This implies that the concentration of the enzyme-substrate complex ([ES]) was relatively low at equilibrium, as depicted in the equation provided.


When calculating the equilibrium constant (Keq) and the change in Gibbs free energy (ΔG), It is observed that all fractions yield positive numerical values. This implies that the generation of the activated compound [ES] is an energetically unfavourable process. This reaction's change in free energy value is unaffected by molecular pathway mechanism transformation. The change in acetylcholine esterase enthalpy values was negative charge in fraction, indicating that the enzymatic reaction is exothermic. The randomness in AchE structure was indicated by the negative entropy value. The occurrence of a transition state between AChE molecules and substrate molecules is suggested by a negative ΔS value, which quantifies the intrinsic likelihood of the

transition state development regardless of energy factors. The number of collisions Pz factor for AChE, on the other hand, indicated the observed concentration of sterically and energetically favorable collisions between S-Acetyl thiocholine iodide and AChE in the fraction was determined to be 1.208 mM. The aforementioned discovery indicates that the main factor influencing the strength of collisions involving the partly purified enzyme is the formation of a suitable arrangement and distribution of potential energy among the molecules involved in the collision. The observed high values of ΔH* suggest significant molecular bond stretching, compression, and potential bond breaking occurring during the development of the transition state (Nordin et al. 2021). The biological meaning of the findings shown in Table 2 can be elucidated in the following manner: The observed results indicate a significant rise in the thermodynamic parameters (ΔG, Ea) for each fraction, suggesting a deficiency of metals or other chemicals that facilitate the enzyme's ability to surmount the energy barrier in the partly purified AChE. At the same time, it can be shown that the partly purified acetylcholinesterase (AChE) in its unbound state had the highest likelihood of adopting a favorable substrate orientation. Furthermore, the significant alterations in ΔG, Ea, ΔH, and ΔS were consistent with the impact of the diseased condition on the mechanism and activity of the enzyme (Alexopoulos 2021).

## Conclusions

- Autism spectrum disorder patients were having a lower acetylcholine esterase activity compared to the control groups.
- Autism spectrum disorder patients were having a lower total protein and specific activity compared to the control groups.
- It was concluded that the optimum conditions for the enzyme are the optimum concentration of the substrate is 0.2M, the optimum temperature for the enzyme is 37 C$^{o}$, the optimum pH is 7.4, and the optimum fermentation time is 5 minutes
- It was concluded that the value of Km for the enzyme is 0.034M and the value of $V_{max}$ is 6.785 µmole/ml/min.
- Each of the fraction's thermodynamic parameters (G, Ea, Pz) exhibited a minor decrease, while (H*) exhibited a slight increase.

## Recommendations

- The possibility of using the enzyme activity (E.A), specific activity (S.A) and total protein (T.P), as biochemical parameters to follow the severity of the disease.

- Participating in research projects pertaining to autism in order to determine more biochemical markers and investigate the relationship between autism and developmental impairment.

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