Study the activity Of ALT&AST in Serum of diabetic Patients Type II

Tagreed U.Mohammd
Chemistry department , College of Education Ibn AL- Haytham , Baghdad university , Baghdad , Iraq
Email: tagreedaloom@yahoo.com

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Abstract

Alanine aminotransferase and Aspartate aminotransferase were purified from serum of patients with type 2 diabetes (Non insulin dependent diabetes mellitus) in a 2 steps procedure involving dialysis bag and sephadex G-25 gel filtration (column chromatography). ALT and AST were purified 4.82 fold with 65.38% yield and 3.46 fold with 71.42% yield in serum of patients with type 2 diabetes respectively . The purified enzyme showed single peak . The results of this study revealed that ALT and AST activity of type 2 diabetes serum increased significantly ($p<0.001$) compared with control group .

Key word : Alanine aminotransferase, Aspartate aminotransferase, Purifiction, Diabetes .

Abbreviation : ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Diabetes Mellitus , D.M , BSA, Bovine Serum Albumin .

Introduction

Type 2 diabetes mellitus accounts for 80%- 90% of the diagnosed cases of diabetes .

It usually occurs in middle- aged to older obese people and is characterized by hyperglycemia,often with hypertriglycerid-emia and other features of the metabolic syndrome. Obesity often precedes the development of type 2 diabetes and is the major contributing factor. Obese patients are usually hyperinsulinemic and have high levels of free fatty acids, which impair insulinaction [1] . Alanine aminotransferase (ALT) [EC 2.6.1.2., formerly glutamate-pyruvate transaminase], is a pyridoxal enzyme catalyzing reversible transamination between alanine and 2- oxoglutarate to form pyruvate and glutamate. Through producing those four important intermediary metabolites, ALT plays an important role in gluconeogenesis and amino acid metabolism, particulary during fasting and exercise where glucose is made from pyruvate converted from alanine from alanine through de- transamination by ALT [2,3] .

An ALT test measures the amount of this enzyme in the blood. ALT is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT is measured to see if the liver is damaged or diseased. Low level of ALT are normaly found in the blood . But when the liver is damaged or diseased, it releases ALT into the blood stream, which makes ALT levels go up. Most increases in ALT levels are caused by liver damage[4]. High serum ALT levels have also been proposed as a marker of risk for type 2 diabetes [5] .

Aspartate aminotransferase is a pyridoxal-5-phosphate dependent enzyme that catalyses the reversible transfer of an amino group from aspartate to 2-oxoglutarate in order to form oxaloacetate and glutamate [6] .

Aspartate :2-oxoglutarate aminotranferase (EC2.6.1 .1, aspartate aminotransferase, AST) is the best studied of aminotransferase. AST is found in many body tissue including the heart, muscle, kidney, pancreas, brain and lung. It is also present in the liver [7] . Low levels of (AST) are normally found in the blood ,when body tissue or an organ such as the heart or liver is diseased or damaged , additional (AST) as released into the blood stream.The amount of (AST) in the blood is directly related to the extent of the tissue damage.After sever damage ,AST levels rise in (6 to 10) hours and remain high for about four days[8,9] .

The aim of our study is to comparied (ALT&AST) activities in patients with type 2 diabetes with that of partially purified enzyme .

Expermintal

Chemicals

K_2HPO_4, KH_2PO_4, were obtained from Fluka-Switzerland company, Sephadex G-25from Sigma chemicals company and (ALT& AST) Kit from bio labo (France) .

Assay of serum ALT activity

ALT activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4- Dinitrophenyl hydrazine, by using kit supplied by Bio labo- France [10] .

Assay of serum AST activity

AST activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4- Dinitrophenyl hydrazine, by using kit supplied by Bio labo- France [10] .

Purification of (ALT & AST) from serum patients with type 2 diabetes

Step 1: Dialysis

Visking dialysis tube (3/4 diameter HMC Glouchester) were used for dialysis of 10 mL of fresh serum against two liters of phosphate buffer pH (7.4) inside refrigerator. The volume of urine or serum after 18 hours of dialysis was measured and enzyme activity determined in this .

Step 2: Gel filtration

The dialyzed serum from step 1 was applied directly to sephadex G-25 column (20x1.5 cm) and developed at a few rate of 50mL/h and 5mL fractions were out inside a refrigerator .

Protein determination

Protein was determined by the procedure of Lowry et al. (1951), with crystalline BSA as standard[11].

All statistical analyses in studies were performed using SPSS version 15.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability P< 0.05 = significant, P> 0.05 = non-significant .

Results and Discussion

Figure (1) and Figure. 2 showed thatALT& AST activities in serum of patients with type 2 diabetes is higher than that of normal and they also exhibited significantly increased in p value (p<0.001) .

The mean levels of serum ALT activity (156.44±11.46)U/L and serum AST activity (75.2±11.7) U/L of the patients with type 2 diabetes revealed distinct increase (p<0.001) table (1), (2) with no significant difference between (male & femal) patients with type 2 diabetes. In agree with our results, Debasis et al, (2010) observed increasing in diabetes[12]. While Andallu noticed that the activity of AST was enormously elevated (p<0.01) by (243%) in uncontrolled diabetes from that of normals [13] .

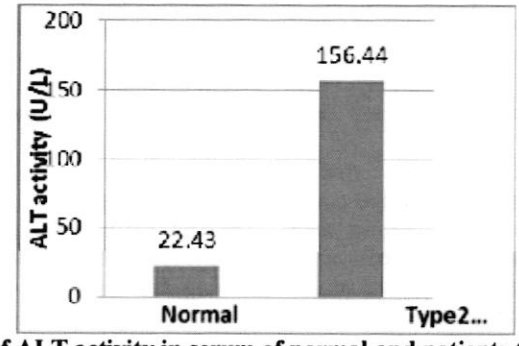


Fig (1): Illustrate values of ALT activity in serum of normal and patients (male& female) with type 2 diabetes

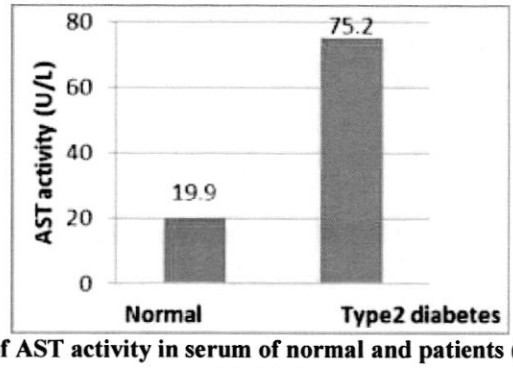


Fig (2): Illustrate values of AST activity in serum of normal and patients (male& female)with type 2 diabetes

The mean levels of serum ALT activity (156.44±11.46)U/L and serum AST activity (75.2±11.7) U/L of the patients with type 2 diabetes revealed distinct increase (p<0.001) table (1),(2) with no significant difference between (male & femal) patients with type 2 diabetes. In agree with our results, Debasis et al, (2010) observed increasing in diabetes [12] . While Andallu noticed that the activity of AST was enormously elevated (p<0.01) by (243%) in uncontrolled diabetes from that of normals [13] .

Table (1): Illustrates values of ALT activity in sera of normal and patients type 2 diabetes

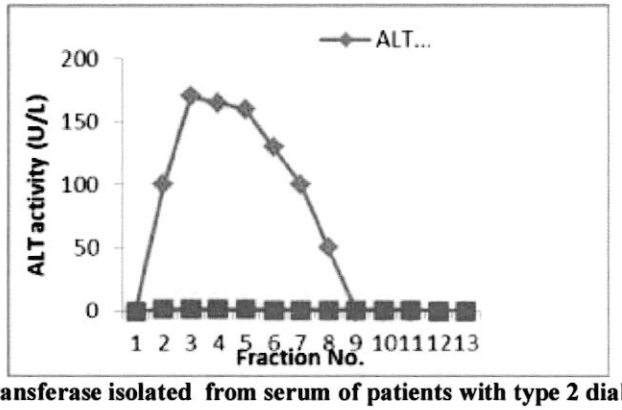
Normal				Type 2 diabetes			
Specimen	No. of cases	Age (years)	ALT activity(I.U/L) mean ± S.D	No. of cases	Age (years)	ALT activity (I.U/L) mean ± S.D	P<
Male	14	40-80	22.42±12.0	19	40-75	154.57±12.75	0.001
Female	16	40-80	18.93±11.19	16	40-80	150.81±11.96	0.001
Total	30	40-80	22.42±11.37	35	40-80	156.44±11.46	0.001

Table (2): Illustrate values of AST activity in serum of normal & patients with type 2 diabetes

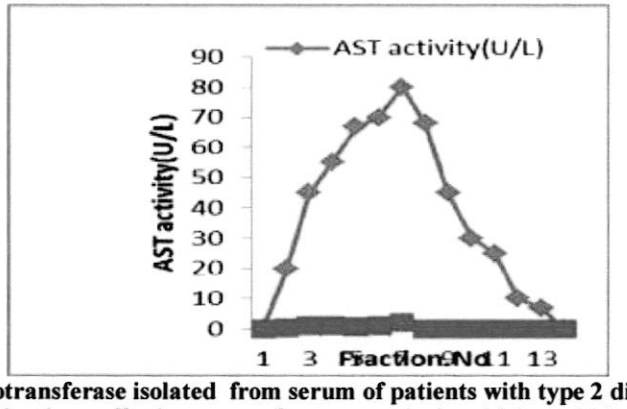
Normal			Type 2 diabetes				
Specimen	No. of cases	Age (years)	AST activity (U/L) mean ± S.D	No. of cases	Age (years)	AST activity (U/L) mean ± S.D	P <
Male	20	40- 70	19.4±6.1	20	40-75	80.1±10.8	0.001
Female	20	40- 65	20±6.2	15	40-70	71.5±8.7	0.001
Total	40	40- 70	19.9±6.1	35	40-75	75.2±11.7	0.001

The rising ALT levels in patients with type 2 diabetes, because ALT was associated with reduced insulin sensitivity and glucose tolerance as well as rising free fatty acid and triglycerides, a study showed a significant relationship between ALT activity and insulin resistance [14], and Andrad (1998) refer to high serum ALT activity have also been proposed as a marker for type 2 diabetes [15], our results are in agreement with reported data [16,17] .

In this experiment there was an apparent rise in serum AST levels in diabetic patients, which could relate to excessive accumulation of amino acid (glutamate) in the serum of diabetic patients as a result of amino acid mobilization from protein stores [18]. These excessive amino acid are then converted to ketone bodies (α-keto- glutaric) for which the enzyme AST are needed, leading to increase in enzyme activity [19] . The enzymes in serum of patients with type 2 diabetes were purified with dialysis followed by sephadex G-25 gel filtration, and these enzymes showed a singl peak fig (3),(4). The purification procedures of the ALT & AST are summarized in table(3),(4) . The results showed that the ALT & AST were purified by dialysis and showed 1.55-fold with a specific activity of 28.07 U/mg protein, and 2.11-fold with a specific activity of 610.16 U/mg of serum. The enzymes were then purified with sephadex G-25 and showed 4.82-fold enzyme purification with a specific activity of 87.17 U/mg protien and 3.46-fold, 1000 U/mg protien of serum patients with type2 diabetes .



Fig(3): ALanine aminotransferase isolated from serum of patients with type 2 diabetes by gel filtration



Fig(4): Aspartate aminotransferase isolated from serum of patients with type 2 diabetes by gel filtration

These results indicated the effectiveness of purification method applied in this research confirmed by the high results of purification by sephadex G-25 which were 65.38% and 71.42% respectively table (3,4) . Inspite of the low yield of purification by dialysis 61.53% and 32.14% respectively which could be due to the autolysis of the enzyme leading to loss in enzyme activity during dialysis for such along duration . Low activities could also caused by the presince of unidentified high molecular weight inhibitors or it possibly related to the dialysis of its coenzyme [20] .

Table (3): Purifiction of Alanine aminotransferase from serum of patients with type 2 diabetes.

Purifiction step	Volume (ml)	protein (mg/ml)	Enzyme activity (u/ml)	Total enzyme activity (U)*	Specific activity(u/mg)	Purifiction (fold)	Yield%
Crude serum	10	7.2	130	1300	18.05	1	100
Dialysis	5	5.7	160	800	28.07	1.55	61.53
Sephadex G-25	5	1.95	170	850	87.17	4.82	65.38

One unit of ALT activity was defined as the amount of enzyme producing 1µmol oxaloacetate (pyruvate) per h under standard assay condition .

Table (4): Purifiction of Aspartate aminotransferase from serum of patients with type 2 diabetes

Purifiction step	Volume (ml)	Protein (mg/ml)	Enzyme activity (U/ml)	Total enzyme activity (U)*	Specific activity (U/mg)	Purifiction (fold)	Yield (%)
Crude(serum)	10	0.194	56	560	288.6	1	100
Dialysis	5	0.118	36	180	610.16	2.11	32.14
Sephadex G- 25	5	0.08	80	400	1000	3.46	71.42

One unit of ALT activity was defined as the amount of enzyme producing 1µmol oxaloacetate (pyruvate) per h under standard assay condition

In diabetes, the causes and site of intervention in biochemical process are diverse (Larner, 1985) [21], and high level of AST and urea have been implicated [22] . Observation from this study correlate with the reports from previous studies, in that, aspartate aminotransferase (ALT&AST) is released in to the serum especially when there is damage to the hepatic cells as a result of chemical assault. Serum levels of this enzyme there are significant diagnostic value in assessing the level of hepatic damage [12] .

Since liver dysfunction is frequently associated with D.M, many clinical reports have indicated that serum enzyme activity derived from the liver such as (ALT& AST) are elevated [23]. The levels of enzyme increased in D.M is ametabolic result already treatable with pancreas hormons . Befor the availability of sensitive pancreas hormone analysis, increased serum enzyme levels were considered important evidence supporting the diagnosis of D.M [24] .

In the present study , the serum ALT activity increased significant compared with serum AST . Alanine aminotransferase measurements are used in the diagnosis and treatment of certain liver disease (e. g., viral hepatitis and cirrhosis) and heart disease. Elevated levels of the transaminases can indicate myocardial infraction, hepatic disease, muscular dystrophy, or organ damage .

Serum elevations of ALT activity are rarely observed except in parenchymal liver disease, since ALT is a more liver- specific enzyme than aspartate aminotransferase table (5) .

Table(5): Illustrate values of(ALT& AST) activity in serum patients with type 2 diabetes

	ALT			AST			
Specimen	No. of case	Age (years)	ALT activity(I.U/L) mean ± S.D	No. of cases	Age (years)	AST activity(I.U/L) mean ± S.D	P<
Male	19	40-75	154.57±12.75	20	40-75	80.1±10.8	0.001
Female	16	40-80	150.81±11.96	15	40-70	71.5±8.7	0.001
Total	35	40-80	156.44±11.46	35	40-75	75.2±11.7	0.001

Conclusion

Levels of ALT in patients serum with type2 diabetes were higher than in AST. 4.82 fold with 65.38% yield for ALT, but AST was 3.46 fold with 71.42% yield .

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دراسة فعالية الانزيمات الناقلة لمجموعة الامين (AST, ALT) من مصل المرضى المصابين بالداء السكري النوع الثاني

تغريد علوم محمد

قسم الكيمياء ، كلية التربية أبن الهيثم ، جامعة بغداد ، بغداد ، العراق

Email: tagreedaloom@yahoo.com

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الملخص

تضمنت الدراسة الحالية دراسة مقارنة نشاط كل من أنزيم ALT وAST من أمصال المرضى المصابين بالداء السكري النوع الثاني (غير المعتمد على الانسولين) ومن ثم تم تنقية كلا الأنزيمين من خلال خطوتين : الاولى باستخدام الديلزة والثانية باستخدام الترشيح بالهلام Sephadex G-25 (كروموتوغرافيا العمود) . نقي الأنزيمان ALT وAST 4.82 مرة مع الناتج 65.38% و3.46 مرة مع الناتج 71.42% من امصال المرضى المصابين بالداء السكري النوع الثاني على التوالي. أعطى كلا الأنزيمين المنقاة بالترشيح الهلامي قمة واحدة. تشير نتائج هذه الدراسة الى الأنزيم ALT المنقى من امصال المرضى المصابين بالداء السكري النوع الثاني يكون أكثر تحسسا للتلف الحاصل في خلايا الكبد كما انه أعطى تغيراً معنوياً ملحوظاً ($P<0.001$) مقارنة بالانزيم AST المنقى من امصال المرضى المصابين بالداء السكري النوع الثاني .