Purification of (AST) From Sera of Type Π Diabetic Patients

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Abstracte

This study was performed on (35) serum specimen of patients having type 2 diabetes in addition , (40) normal specimens were investigated as control group . The activity of (AST) in diabetic patients was reached to (75.2 ± 11.7) IU/L as compared with normals (19.9 ± 6.1) IU/L . Purification of (AST) from sera of diabetic patients was performed by dialysis and gel filteration (Sephadex G 25) the results of study reveal that Aspartate aminotransferase (AST) activity of type 2 diabetes patient's serum show ahigh significant increase ($p< 0.001$) compare to normal subject.

Key word: Aspartate aminotransferase (AST) , Type 2 Diabetes .

تنقية أنزيم اسبارتيت امينو ترانسفيريز في أمصال مرضى السكري من النوع الثاني

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الخلاصة

شملت الدراسة (35) عينة مرضية من أمصال المرضى المصابين بالداء السكري النوع الثاني فضلا عن (40) عينة من أمصال الاصحاء كمجموعة ضابطة . وقد أظهرت النتائج ارتفاع معنوي بنشاط الانزيم (AST) في أمصال المرضى المصابين بالداء السكري النوع الثاني مقارنة بالاصحاء . كما تم تنقية الانزيم باستعمال كيس الديلزة وكروموتوغرافيا الترشيح يالهلام (Sephadex G – 25)

Introduction

Aspartate aminotransferase (AST2.6.1.1) also known as Glutamate Oxaloacetate transaminase (GOT) catalyzes the following reaction:[1].

L-Aspartate +2-Oxaglutarate ←→ Oxaloacetate +L-Glutamate

Enzyme reaction involving inter molecular transfer of amino groups are important in metabolic processes [2]. An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood [3]. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas and kidneys[4-6], also AST is found in alkalophilic Bacillus circulans [7].

Low levels of (AST) activity are normally found in the blood ,when body tissue or an organ such as the heart or liver is diseased or damaged , additional (AST) are released into the blood stream [8].

The amount of (AST) in the blood is directly related to the extent of the tissue damage .After sever damage ,AST activity levels rise in(6 to 10) hours and remain high for about four days[9].

Aspartate aminotransferase , in which pyridoxal phosphate act as a cofactor , exists as two isozymes ,one mitochondrial (m-GOT) and the other from cytosol (s-GOT)(10). Though differing makedly in primary structure chemical and physical properities ,both catalyze the same reaction with subtly different catalytic steps[11- 13].

Diabetes mellitus is the most common illness due to hormonal imbalance . Symptoms include: glucose uria , frequent ,copious urination , abnormal thirst , poly phagia which is excessive eating rapid weight loss ,general weakness ,drowsiness and fatigue , itching of the genitals and skin , visual disturbances and blurring and skin disorders ,such as boils carbuncles and infection [14].

The ability of β- cells to adoptto insulin resistance depends on various genetic factors that determine the total β – cell mass ,rates of replication and apoptosis of the cells , and the activity of key biochemical components of cells .Environmental factors can probably aggravate the genetic predisposition leading to β – cell failure (15) . Serum glutamate oxaloacetate transaminase (SGOT) activity which is increased in diabetes was restored by the extract [16].

The aim of the presented study is purification of enzyme (AST) patients having from sera of type 2 diabetes and compared with normal .

Material and Methods

Specimens:

Fourty serum sample obtained from normals (20) males and (20) females , age (40 - 75) years ,and serum of type 2 diabetic patients (20) males and (15) females age (40 - 75) years , from each patient adetailled history was taken concerning the illness , (age at which the patient consults his physician) , complication of the disease the associated diseases residency and their jobs or whether taking any drugs , and smoking . The patients were diagnosed by specialist doctors in AL-Azade Hospital from Kirkuk .

Measurement AST activity in serum :

The aminotransferase enzyme (AST) activity was measured colorimetrically according to the method of (Reitman & Frankel , 1957) , using kit purchased from (Bio labo / France [17] .

Results and Discussion

Results obtained illustrate differences in level of AST activity in (35) patients type 2 diabetes and (40) normal as shown in fig.(1) and fig.(2) illustrate defferences in levels of AST in patients (male and femal) having type 2 diabetes and normal (male and femal).

Table (1) illustrate comparing the mean levels of serum AST activity of the normal subject (19.9± 6.1) IU/L and patients type 2 diabetes (75.2± 11.7) IU/L which found that there were significant increase ($p < 0.001$) .In agree with our results , Debasis etal (2009) observed serum (AST) activity increased in diabetes [16]. Andallu referd to activity of(AST) was enormously elevated ($P < 0.01$) by (243%) in uncontrolled diabetes from that of normals [18].

In this experiment there was asignificant rise in serum AST level 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 prtein stores [19] **.** These excessive amino acid are then converted to ketone bodies (α – keto – glutaric) for which the enzyme AST are needed , leading to increased enzyme activity [20].

Fig.(3) shows AST purification by using dialysis bag and column chromatography .In agreement with our results , a number of other studies have referd to AST purification by using (sephadex G – 25) chromatography [21]. A (2. 11) fold purification of AST from serum patients type 2 diabetes by using dialysis and (3. 46) fold by using (sephadex G – 25)chromatography . This enzyme showd a single peak . Tabel (2) illustrates purification steps , observed activity of AST increase in serum patients having type 2 diabetes after process of purification because removed inhibitors which decrease AST activity [22]

Conclusion

AST is sensitive indicators of pancrease damage or injury from different types of disease . therefore elevation activity of this enzyme in serum patients with type 2 diabetes .

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Fig (1) : Illustrate value of AST activity in serum of normals and patients type 2 diabetes.

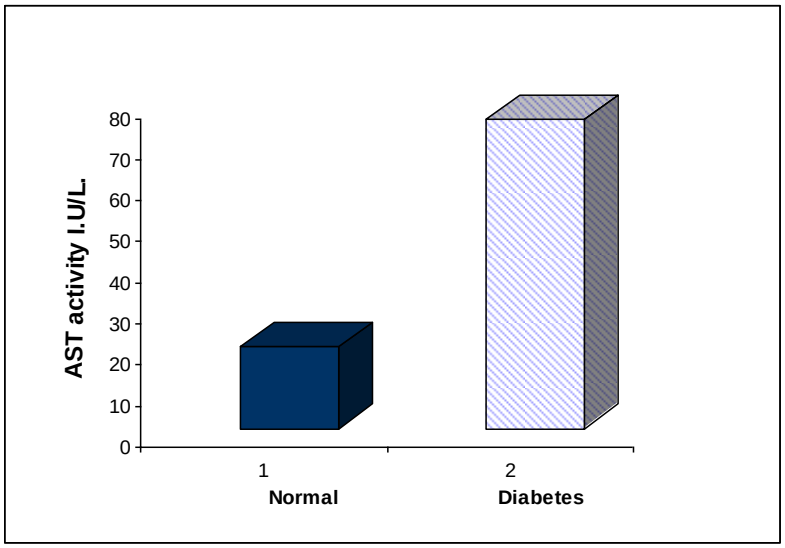

Fig (2) : Illustrate value of AST activity in serum of normals and patients type 2 diabetes (male and female).

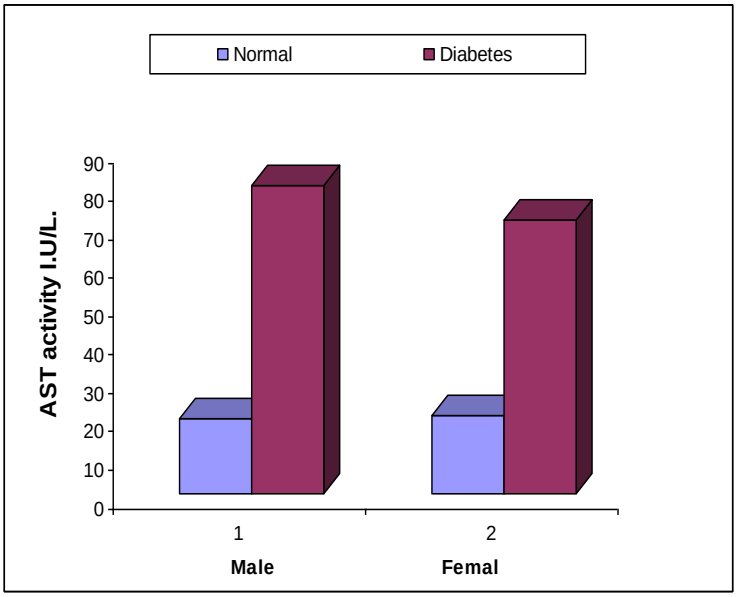

Fig (3) : Illustrate AST purification from patients type 2 diabetes .

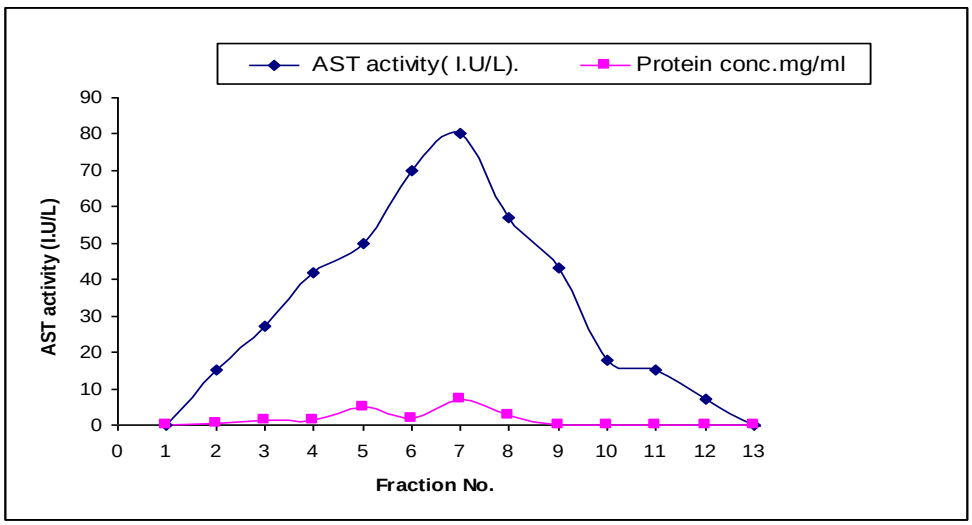

Table (1) :Illustrate value of(AST) activity in serum of normal and patients type 2 diabetes

	Normal			Diabetes			
Specimen	**No. of case**	**Age (years)**	**AST activity (I.U/L) mean±S.D**	**No. of case**	**Age (years)**	**AST activity (I.U/L) mean±S.D**	**P value**
Male	20	40 - 70	19.4±6.1	20	40 -75	80.1± 10.8	P<0.001
Femal	20	40 - 65	20.4±6.2	15	40 - 70	71.5± 8.7	P<0.001
Total	40	40 - 70	19.9±6.1	35	40 -75	75.2± 11.7	P<0.001

Table (2) : Illustrate steps of (GOT) purification from patients serum type 2 diabetes

Step	**Elute (ml)**	**Protein conc. (mg / ml)**	**Total Protein (mg)**	**Activity (Iu/ml)**	**Specific Acivity (Iu/mg)**	**Degree of Purification (fold)**
Crude	10	0.194	1.94	56	288.6	1
Dialysis	5	0.118	0.59	72	610. 16	2.11
Sephadex G – 25	5	0.08	0.40	80	1000	3.46