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

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Abstract

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 filtration (Sephadex G 25) the results of study reveal that Aspartate aminotransferase (AST) activity of type 2 diabetes patient's serum show a high significant increase (p< 0.001) compare to normal subject.

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

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

\[
\text{Oxaloacetate + L-Glutamate} \rightarrow \text{L-Aspartate + 2-Oxoglutarate}
\]

Enzyme reaction involving intermolecular 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 severe 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 properties, 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, polyphagia 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 adopt to 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:

Fouaty 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 detailed 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 differences in levels of AST in patients (male and female) having type 2 diabetes and normal (male and female).

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 et al. (2009) observed serum (AST) activity increased in diabetes [16]. Andallu refer 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 a significant 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 protein 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 refer 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 showed 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 pancreas damage or injury from different types of disease therefore elevation activity of this enzyme in serum patients with type 2 diabetes.

References


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20- Z Kechrid and Bouzerna. Effect of zinc deficiency and experimental diabetes on glutamate oxaloacetate.


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

![Fig (1): Illustrate value of AST activity in serum of normals and patients type 2 diabetes.](image-url)
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

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. of case</th>
<th>Age (years)</th>
<th>AST activity (I.U/L) mean±S.D</th>
<th>No. of case</th>
<th>Age (years)</th>
<th>AST activity (I.U/L) mean±S.D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>20</td>
<td>40 - 70</td>
<td>19.4±6.1</td>
<td>20</td>
<td>40 - 75</td>
<td>80.1± 10.8</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Femal</td>
<td>20</td>
<td>40 - 65</td>
<td>20.4±6.2</td>
<td>15</td>
<td>40 - 70</td>
<td>71.5± 8.7</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>40 - 70</td>
<td>19.9±6.1</td>
<td>35</td>
<td>40 - 75</td>
<td>75.2± 11.7</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
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Table (2) : Illustrate steps of (GOT) purification from patients serum type 2 diabetes

<table>
<thead>
<tr>
<th>Step</th>
<th>Elute (ml)</th>
<th>Protein conc. (mg / ml)</th>
<th>Total Protein (mg)</th>
<th>Activity (Iu/ml)</th>
<th>Specific Activity (Iu/mg)</th>
<th>Degree of Purification (fold)</th>
</tr>
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<tbody>
<tr>
<td>Crude</td>
<td>10</td>
<td>0.194</td>
<td>1.94</td>
<td>56</td>
<td>288.6</td>
<td>1</td>
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<tr>
<td>Dialysis</td>
<td>5</td>
<td>0.118</td>
<td>0.59</td>
<td>72</td>
<td>610.16</td>
<td>2.11</td>
</tr>
<tr>
<td>Sephadex G–25</td>
<td>5</td>
<td>0.08</td>
<td>0.40</td>
<td>80</td>
<td>1000</td>
<td>3.46</td>
</tr>
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