Partial purification of Leucine aminopeptidase (LAP) in Acromegalic Sample of Iraqi Patients

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Abstract

Acromagaly is a syndrome caused by increased growth hormone secretion from the frontal lobe of the pituitary gland. A Leucine aminopeptidase (EC 3.4.11.1) activity has been assayed in (30) patient’s sera samples (15 females and 15 males) with acromegaly age range between (30-50) years and (30) sera of healthy as control group (16 females and 14 males) age range between (30-50) years. The goal of the research was partial purified of enzyme from sera patients with acromegaly by dialysis gel filtration by using sephdex G50 and ion exchange chromatography by using DEAE cellulose A50. The results showed a single peak by using gel filtration and the activity was reached to 152 U/L. Two isoenzymes were obtained by using ion exchange chromatography and the purity degree of isoenzyme (I, II) were (125) and (128) fold respectively. The current study found that the enzyme showed no significant difference between the healthy and the patients.

Key words Leucine aminopeptidase (LAP), Acromegaly (Acro), Purification.

1 Introduction

The rise of growth hormone (GH) secretion reasons Acromegaly (Acro) in addition to high levels of insulin like growth factor1 (IGF1). The occurrence of acromegaly is considered to be 34 cases per million for every year (1). For the reason that GH secretion is pulsatile elevated serum IGF1 heights are a useful showing tool for Acro (2). The increase of the release of GH has important metabolic effects; the two most significant effect of GH on metabolism in adipose tissue are insulin resistance (IR) and lipolysis (3). Insulin resistance presenting as diabetes or impaired glucose tolerance is found in most Acro patients and contributes to the improved illness. Growth hormone makes the expression and secretion of IGF1 thus phenotypes associated with Acro may be as a result of either GH signaling IGF1 signaling or a combination of both (4). Acro treatment includes surgical treatment often used for transphenoidal route radiotherapy administration of somatostatin analogs for example octreotide or of dopamine agonists (5). Amino peptidases "EC 3.4.11XX" belongs to the group of proteases which are essential enzymes as they play an important role in many life processes They catalyze the hydrolysis of amino acids located at the Nterminus of pepide and are involved in proteins degradation to free amino acids (6). leucine aminopeptidase "LAP" is well known to be generally spread in organisms from bacteria to humans as well as various cancer cells LAP is normally found in most body fluids and tissues It is mainly abundant in the biliary mucosa and small intestine. Elevated enzyme activity was observed in serum and urine patients with liver pancreatic and biliary diseases Its determination is of significant value in the diagnosis of cancer of the pancreas in addition to metastatic carcinoma of tile liver Malignancies in overall the
nephrotic syndrome and several skin diseases may be related by elevated levels and a physiologic increase happens in the third trimester of pregnancy Animal tests show raises occur with infarction of the small bowel (78).
The aim of this study purification of LAP from serum patients with acromegaly and isolation.

2. Statistical Analysis
The results were analyzed to determine the mean value and standard error of different parameters. The statistical analysis (2012) method was used to study effect of different groups on LAP enzyme test analysis was used to compare a significant between the means values [9].

3. Patients and Methods

3.1. Patients
Thirty people with Acromegaly aged 30-50 years were studied "15 female and 15 males" and thirty controls "16 female and 14 male" aged 30-50 years were recruited at the "National Diabetes Center_ Al_Mustansiria university amidst Dec 2015 and the end of 2016 In all the patients a previous diagnosis of acromegaly had been promptly decided by their typical clinical features and biochemical findings.

- Determinations of protein concentration this method is based on protein interaction with basal solution and then Folin detector is added to a complex product with a blue colour that depends on the intensity on the amount of proteins (10).
- Determination of LAP activity hydrolysis of the peptide bond of leucinamide is measured according to Mitz and Schlueter (1958) wavelength is measured at 238 nm (11).
- Purification of LAP from sera of patients with acromegaly.

3.2. . Precipitation by using Ammonium sulfate (40%)
The method used for precipitation of protein by adding 08 gm of ammonium sulphate was added gradually to 5ml of fresh serum in a beaker with constant stirring at about (4°C) for one hour until the solution became turbid and then it was centrifuge at speed (3500 rpm) for 10 min to split the precipitate. The precipitate was dissolved in a less amount of tris buffer solution pH (87) then the activity of enzyme and total protein concentration were measured in it.

3.3. Dialysis
Two mL of protein solution that prepared in the previous step was put into a tightly wrapped cellophane bag from bottom and from top then the pipe was put into a container which contains tris buffer solution (pH=87). This process was done with constant stirring at 4°C.

3.4. Gel Filtration
Two mL of serum was added slowly on the surface of sephadex (G50) column (20x 2 cm) and left for 5min to be absorbed. Twenty Fractions were collected by passing tris buffer solution pH =87 through the column. The process was carried out inside a refrigerator and the flow rate was (2 ml /min).

3.5. on Exchange Chromatography
Two mL of fresh filtered serum was passed through a column of diethyl amino ethyl cellulose A50 column (20x 2 cm). A syringe pump is commonly used to pump various buffer via the column. One salt
commonly used is sodium chloride and forty five fractions were collected by passing different concentration of sodium chloride solution (0.01-0.04)M The flow rate was (2ml/4 min).

4. Results and Discussion
Table (1) presents the isolation and partial purification of LAP and isoenzymes from sera patients with acromegaly disease. The LAP activity was reached to (72) U/L by using ammonium sulphate salt in concentration of (40%). The enzyme was partially purified by using dialysis method with tris HCl buffer at (pH=7.8) the purity degree of LAP was reached to 26 fold which yields (656%) while the purity degree was increase to 451 fold which yields (95%) by using Sephadex G50 column chromatography. This enzyme shows a single peak in Figure (1). The Purification by ion exchange chromatography technique offered several distinct advantage over other conventional methods of separation serum isoenzymes. This enzyme was purified by using DEAE cellulose A50 two isoenzymes were obtained as mentioned in Figure (2). The purity degree of isoenzyme (I) was 125 fold which yields (20%) and isoenzyme (II) was 128 fold which yields (156%).

Table 1 Steps of LAP Enzyme Partial Purification From Acromegaly Patients

| Step          | Volum (ml) | Activity of enzyme U/L | Total activity (U) | Protein Con (mg/ml) | Total Protein (mg) | Specific activity (U/mg) | Fold Purification | Yield % |
|---------------|------------|------------------------|--------------------|---------------------|--------------------|--------------------------|-------------------|--------|
| Crude serum   | 5          | 653                    | 0032               | 064                 | 3235               | 102                      | 1                 | 100    |
| Ammonium sulphate | 2          | 72                     | 0014               | 053                 | 265                | 1358                     | 133               | 45     |
| Dialysis     | 2          | 109                    | 0021               | 040                 | 08                 | 2725                     | 267               | 656    |
| Sephadex G50 | 2          | 152                    | 003                | 033                 | 6458               | 4606                     | 451               | 95     |
| Ion exchange Iso I | 2          | 32                     | 00064              | 025                 | 05                 | 128                      | 125               | 20     |
| Ion exchange Iso II | 2          | 25                     | 0005               | 019                 | 038                | 1315                     | 128               | 156    |
Several previous studies have indicated the use of leucine aminopeptidase in clinical diagnosis for more than 10 years. The high LAP activity of the serum usually indicates a defect in the liver or bile duct. This rise is minimal influenced by injury of the liver parenchyma than by the active involvement of the
biliary tract in the process. There is likewise a prominent raise in LAP activity in sever pancreatitis. High LAP amounts have been found in addition in advanced pregnancy malignancies in some skin diseases and in cases of nephrotic syndrome [12]. In fact, none of the literature has indicated a relationship between the enzyme and Acromegaly except for a previous source found that the activity of the enzyme is normal in some cases such as Paget's disease [13].

5. Conclusion

Two isoenzymes of the purified LAP were obtained from patients with Acromegaly and there was no clear difference in enzyme activity between healthy and patients. Thus the leucine aminopeptidase enzyme is no evidence of the presence of the disease.

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