Estimation Activity And Partial Purification Of Leucine Amino Peptidase (Lap) In Patients Wiith Diabetic Nephropathy

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Abstract:

Leucine aminopepotidase (LAP)[EC:3.4.11.1] activity has been assayed in (50) serum samples of patients with diabeties naphrophathy D.N (non-insulin dependent diabetic (NIDD) , and (50)serum sample of healthy individuals without any clinically detectable diseases have been as control group. The aim of this study is to measure leucine aminopeptidase activity and partially purifying the enzyme from sera of patients with diabetes nephropathy The results of this study revealed that Leucine aminopeptidase (LAP) activity of nephropathy patient's serum shows a high signifiacant increase ($p < 0.001$) compared to that of the healthy subjects.LAP was purified from the serum of patients with diabetes nephropathy by dialysis and gel filtration (Sephadex G-25) (fine) (20 × 1.5 cm) .A (1.37) fold purification of serum LAP from patients serum with diabetic nephropathy was achieved by using dialysis and this enzyme showed single grade increased to (8.33) fold by using gel filtration

Abbreviation: Leucine aminopeptidase=LAP, Diabetes Nephropathy= D.N, Non-Insulin dependent diabetic= NIDD.

Key word : Leucine Aminopeptidase , Diabetes Nephropathy.

Introduction:

Diabetes Mellitus (DM) is agroup of metabolic disorders of carbohydrate metabolism in which glucose in underused producing hyperglycaemia.Different statistics have led to diabetes being described as one of the main threat to human health in the 21 st century [1]. DM is the major cause of renal morbidity and mortality ,and diabetic nephropathy is one of chronic kidney failure [2]. Diabetes nephropathy is the kidney disease that occurs as result of diabetes. Diabetes after many years will destroy the will filtering system in the kidney ,initially becoming leaky to larger blood proteins such as albumin which are then lost in urine .This is more likely to occur if the blood sugar is poorly controlled [3,4].

Leucine aminopeptidase (LAP) (α-amino acylpeptide hydrolases cytosol, E.C. 3. 4. 11.1) is aproteolytic enzyme with a M.wt 326,000 Dailton [5], that hydrolyses the peptide bond adjacent to a free group.It is called leucine aminopeptidase because it rapidly catalyzes the hydrolysis of leucine containing amino peptidase, however, it also catalyzes the hydrolytic release of other amino acids located at the N-terminal end of various proteins.[6-9].

LAP was detected in human tissues, animals, plants and bacteria[7-9]. High activities are seen in the small intestinal mucosa , pancreas[9] ,stromal cells of the uters ,and hepatocyets[10-13]. Determination of microsomal leucine aminopeptidase activity in serum is of clinical

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significance, since LAP levels are elevated in obstructive jaundice, liver cirrhosis , liver carcinoma and also during the late part of pregnancy[14]. The serum LAP level may be apotential activity indicator for systemic lupus erthematosus[15]. The aim of this study is to measure leucine aminopeptidase activity and partially purifying the enzyme from sera of patients with diabetes nephropathy .

Materials and Methods:

Chemicals:

All laboratory chemicals and reagents were of analar grade: Tris(Hydroxy methyl)amino methane, $MgCl_2$, $MnCl_2$, were obtained from Fluka- Switzerland company, and bovine serum albumin(BSA)from Sigma- USA company.

Specimens:

Fifty serum samples collected from healthy subjecit (20) men and (30) women with out any detectable diseases , age (40-70) years ,and (50) patient's serum with diabetic nephropathy (25) men and from (25) women ,age (42-75) years . The disease were diagnosed by specialist doctors in AL-Yarmok hospital (diabetic center).

Measurement of LAP activity :

LAP activity was assayed according to Binky and Torres (1960) [18].The reaction mixture contained 2.5ml 0.5 M Tris- HCl, pH8.5 , 0.4ml 0.025M mangese chloride , 0.1ml enzyme (total volume 3.0 ml)and incubated at 40°C for one hour . After incubation, 0.20 ml Tris buffer, 0.20ml magnesium chloride , and 2.5ml L-Leucinamide . Place cuvette in spectrophotometer at 25°C for 5 minutes. Recored absorbance at 238 nm(blank) . To initiate the reaction , add 0.10 ml of treated enzyme to the cuvette. Follow the reaction by recording the decrease in absorbance at 238nm for 5-8 minutes. The enzyme activity was determined by using the standard curve with ammonia .One unit of aminopeptidase activity was calculated as the amount of enzyme liberated 1µmol of leucinamid per hour under standard assay condition.

Total Protein determination :

Serum protein concentration was determined Lowry et .al. method [19],by using bovine serum albumin (BSA) as a standard protein .

Isolation of LAP

Isolation of LAP from serum according to Binky and Torres (1960) method[18] ,and inhibitors removed using two steps [20].

A-Dialysis **:**

It is one of the important methods used in enzymes purification , visking dialysis tubes c314 diameter HMC Glouchestrs were used for dialysis of 10 ml of fresh serum against two liters of potassium phosphate buffer PH (7.4) inside refrigerator .The volume of serum and enzyme activity was measured after 18 hours of dialysis was measured and enzyme activity determined in this.

B- Gel filtration :

Fresh serum sample (5.0 ml) was passed through a column of Sephadex gel G-25 (fine) (20 × 1.5 cm).Ten fraction each of 5.0 ml were collected by passing potassium phosphate buffer ,pH 7.4 through the column .The entire process was carried out inside the frigerator .and the flow rate was (50 cm^3/30 min).

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:

The results showed that the LAP activity in patients serum with nephropathy was increased significantly ($p < 0.001$) than that of control group . (Fig 1). Also the results showed that LAP activities in sera of femal patients were higher significantiy ($p < 0.001$) than of male patients (Fig 2) .

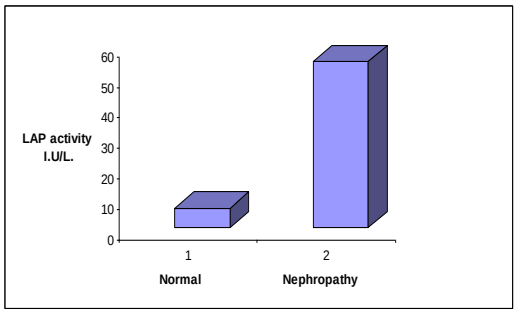

Fig (1): Values of LAP activity in sera of normal and patients with nephropathy .

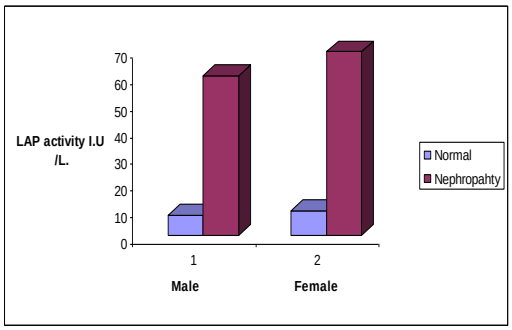

Fig (2): Values of LAP activity in sera of normal and patients (male and female) with nephropathy .

Table (1) : illustrate the comparison between the mean levels of serum LAP activity of the normal individuals (4.6± 4.05) I.U /L with patients diabetic nephropathy (54.43 ± 45.79) I.U / L , significant increase ($p < 0.001$). Also table (1) refers to the mean levels of serum LAP activity in patient's (male and female with diabetic nephropathy (60.68 ± 47.27)I.U/L, (69.2 ± 42.47) I.U/L respectivety ,with significant increase ($p < 0.001$). While mean levels of serum LAP activity in normal (male and female),(9.47± 5.71) I.U/L,(7.8±5.52)I.U/L respectivety ,and significant increase ($p < 0.001$). These results suggest that LAP has sensitivity and diagnostic significance . Elevated LAP activity in serum used to usually indicates diseases of : liver ,pancreas and bile ducts, and the elevation is less affected by damage of liver parenchyma that by active participation of biliary tract in the process [8,20].Further studies may indicate that some or all of these increases in leucine amino peptidase activity are under endocrine control [16].In addition LAP may be increased early in diabetics subjects and may be a more sensitive predictor of incipient nephropathy than microalbumin uria [17]. Miltenyi et al (1985) refered that to tubular dysfunction occurring during diabetic ketoacidosis and poorly controlled diabetics may contribute to the development of diabetic nephropathy [18]. The enzyme was partially purified using dialysis method .A 1.31 purification fold of serum LAP from patients with diabetic nephropathy was achieved .While purification degree increased to 8.33 fold with recovery of (289.4) % from the crude sera by using Sephadex G-25 column chromatography and this enzyme showed single peake (Fig. 3).

The specific LAP activity observed with leucinamide as the substrate at each purification step has been summarized in table (2). The specific activity of LAP was purified from sera patients with diabetes nephropathy by sephadex G-25(56.42)U/mg and other research referd to specific activity of LAP was purified from Fasciola. Gigantic by sephacryl- S-200 column(811.5)U/mg. Observed activity of LAP increased in sera patients with diabetic nephropathy after of purification which remove inhibitors such as urea, amino acids and ammonia that cause decrease in LAP activity .

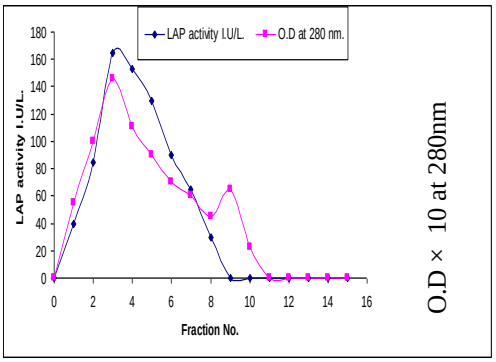

Fig (3) : LAP isolation from patients serum with diabetic nephropathy by gel filtration

Table (1) :LAP activity in sera of normal and patients with diabetic nephropathy .

Healthy subjects				Diabetic Nephropathy			
Specimen	No. of cases	Age (years)	LAP activity (I.U/ml) mean ± S.D	No. of cases	Age (years)	LAP activity (I.U/ml) mean ± S.D	P <
Male	20	38-70	9.47±5.71	25	40-75	60.68±47.27	0.05
Female	30	42-65	7.8±5.52	25	42-70	69.2±42.78	0.01
Total	50	38-70	6.4±4.05	50	40-75	54.43±45.79	0.001

Table (2) : Steps of serum LAP purification from patients with diabetic nephropathy.

step	Elute (ml)	Protein conc. (mg/ml)	Total protein (mg)	Activity (I.U /ml)	Specific activity (I.U/mg)	Total activity I.U	fold purification	Recovery %
Crude serum	10	37.05	370.5	28.5	7.69	285	1	100
Dialysis	5	29.74	148.7	60	10.08	300	1.37	105
Sephadex G- 25	5	14.62	73.1	165	56.42	825	8.33	289.4

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تعيين فعالية انزيم الليوسين امينوببتايد في المرضى المصابين بالسكر الكلوي وتنقيتة جزئيا .

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الخلاصة :

تم قياس فعالية انزيم الليوسين امينو ببتايديز (LAP) في امصال 50 مصاب بالفشل الكلوي السكري و50 شخص سليم (مجموعة قياسية).هدفت الدراسة الحالية بيان تاثير الفشل الكلوي السكري على مستوى انزيم (LAP) وتنقية جزئية لانزيم (LAP) في امصال المرضى المصابين بالفشل الكلوي السكري .لوحظ وجود زيادة احصائية مقبولة في مستوى (LAP) ($p < 0.001$) للمرضى مقارنة بالاصحاء . نقي (LAP) من امصال المرضى المصابين بالفشل الكلوي السكري باستخدام اكياس الفرز الغشائي ، وكروموتوغرافيا الترشيح بالهلام (Sephadex G-25) . (1.37)عدد مرات تنقية انزيم (LAP) من امصال المرضى المصابين بالفشل الكلوي السكري باستخدام اكياس الفرز الغشائي ،اعطى انزيم (LAP) قمة واحدة باستخدام كروموتوغرافيا الترشيح بالهلام (Sephadex G-25)بابعاد (1.5 cm × 20) وبعدد مرات تنقية (8.33) .