

## ESTIMATION ACTIVITY OF LAP IN PATIENT'S WITH TYPE 2 DIABETES BY USING LEUCINE AMIDE AS SUBSTRATE

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### Abstract

This study was performed on 50 serum specimens of patients with type 2 diabetes, in addition, 50 normal specimens were investigated as control group. The activity rate of LAP in patients ( $560.46 \pm 10.504$ ) I.U/L and activity rate of LAP in healthy ( $10.58 \pm 4.39$ ) I.U/L. The results of the study reveal that Leucine aminopeptidase (LAP) activity of type 2 diabetes patient's serum shows a high significant increase ( $p < 0.001$ ) compare to healthy subjects. Addition preparation leucine amide as substrate of LAP, identification melting point and spectra by FTIR.

**Keyword:** Leucine Aminopeptidase(LAP), Diabetes mellitus.

### Introduction

Diabetes mellitus (sugar diabetes) is the most common illness due to hormonal imbalance. Symptoms include: sugar in the urine; 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.[1]. The ability of B-cells to adapt to insulin resistance depends on various genetic factors that determine the total B-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 B-cell failure [2]. Leucine aminopeptidase (LAP) (a-amino acylpeptide hydrolases cytosol, E.C. 3. 4. 11.1) is a proteolytic enzyme which 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.[3-6]. It has a molecular weight 326,000 [7]. LAP was detected in human tissues, animals, plants and bacteria[8-10]. High activities are seen in the small intestinal mucosa, pancreas[8], stromal cells of the uterus, and hepato cyets [3-11].

Determination of microsomal leucine aminopeptidase activity in serum is of clinical significance, since LAP levels are elevated in obstructive jaundice, liver cirrhosis, liver carcinoma and also during the late part of pregnancy[12]. The serum LAP level may be a potential activity indicator for systemic lupus erythematosus[13].

L-leucinamide, a more specific substrate was synthesized [14]. Leucinamide was preferable substrate for the measurement of activities of serum LAP in the clinical examination [15].

The aim of study preparation and purification substrate (Leucinamide) of enzyme LAP, in addition measurement activity of LAP in patient's serum with type 2 Diabetes.

### Material and Methods

#### Preparation of leucine amide:

##### 1-Preparation of ester leucine amide

Ester leucine amide was prepared by dissolved (0.1) mole of leucine as amino acid in absolute ethanol (50-100)ml, added (5ml) of concentration sulfonic acid gradually with stirring and then reflux for (5-6hr) (during refluxing added 5 ml Toluene). Continued reflux for (2hr) and distillation for (30min). Added (6-7ml) of ethanol and repeated the reflux with adding (3ml) Toluene.

After completed reaction and freed mixture, equilibrated acid by used  $\text{NaHCO}_3$

(Saturated solution). When precipitated substance or separated by funnel concentrated, dried and measured melting point. Then added bicarbonate to filterate [16].

## 2-Preparation of Amide from esters:

Added(0.1mole) of ester leucine (prepared in step 1) to (25ml) of conc.ammonium solution in conical flask it's capacity (100ml) . Closed the vent of flask and then continued stirring within (10-15min).Left the mixture to stable for (24hr), filtered crystal of amide and washed in the fewer amount of cooled distilled water(0-5)°C. Recrystallization using by hot distilled water and dried by air or hot oven. Then measured melting point [16].

## Specimen:

Fifty serum samples obtained from normals (20) men and (30) women, age (40-70) years, and (50) patient's serum with type 2 diabetes (25 men and 25 women), age (42-75) years. From each patients a detailed history was taken concerning the illness, (age at which the patient consults his physician),

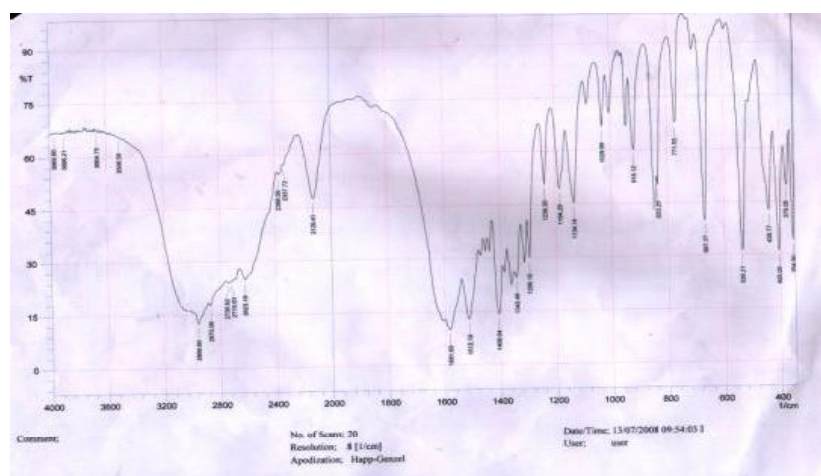
complication of the disease other associated diseases residency and their jobs or whether taking any drugs, and smoking. The patients were diagnosed by specialist doctors in Al-Yarmok hospital (Diabetic center).

## Measurement activity of LAP in serum:

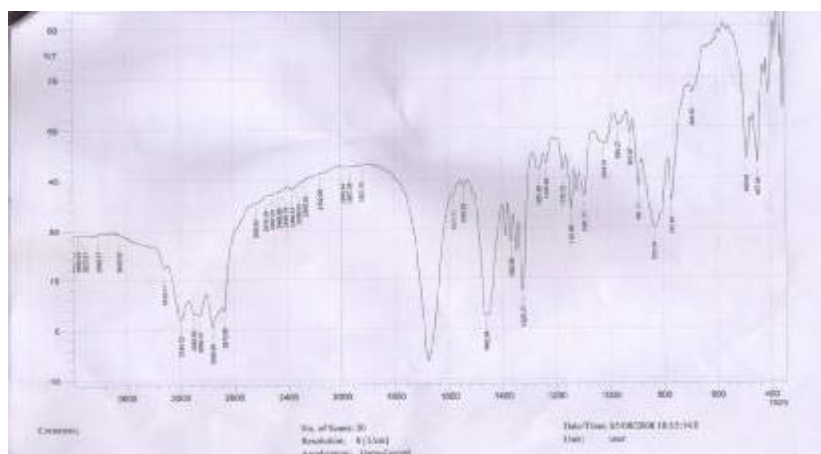
The measurement method is based on the described by Binkley and Torres (1960)[17]. The rate of decrease in absorbance at (238nm) resulting from the hydrolysis of L-leucine amide is proportional to the catalytic activity of the enzyme. Determination activity of LAP by compared absorbance of sample with standard curve for different concentration of ammonia.

## Result and Discussion

Leucinamide was obtained (m.p 250 °C), the formation of Leucinamide is characterized by using in Fourier Transformation (FTIR) spectrum which showed, strong bonds for  $\nu$  C=O and  $\nu$  NH<sub>2</sub> of the amide , at 1678 cm<sup>-1</sup> and 3313-3194 cm<sup>-1</sup> combined with the disappearance of  $\nu$  OH for the leucine Fig.(1), (2). [18], [19].



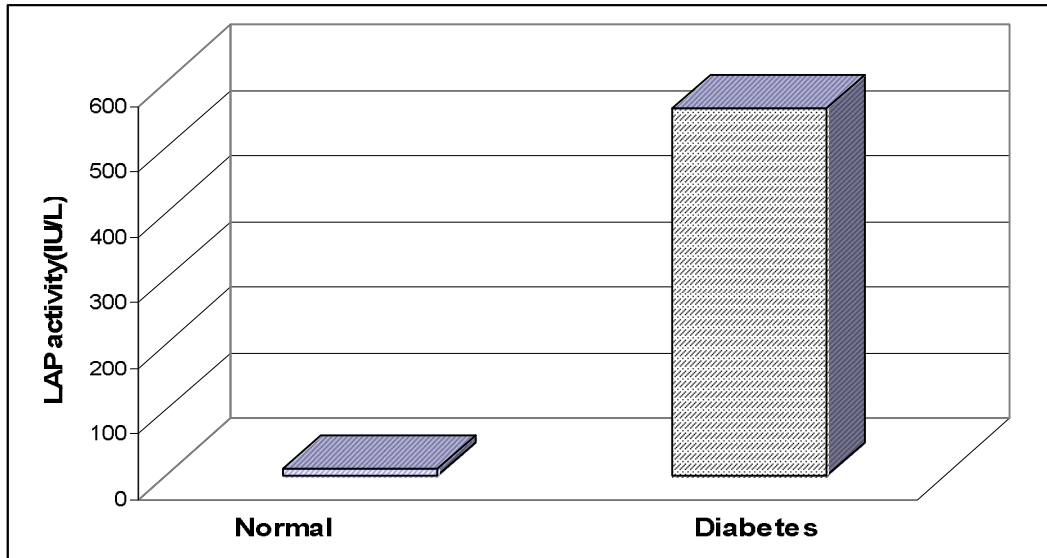
**Fig.(1) : Illustrate spectra FTIR of LAP.**



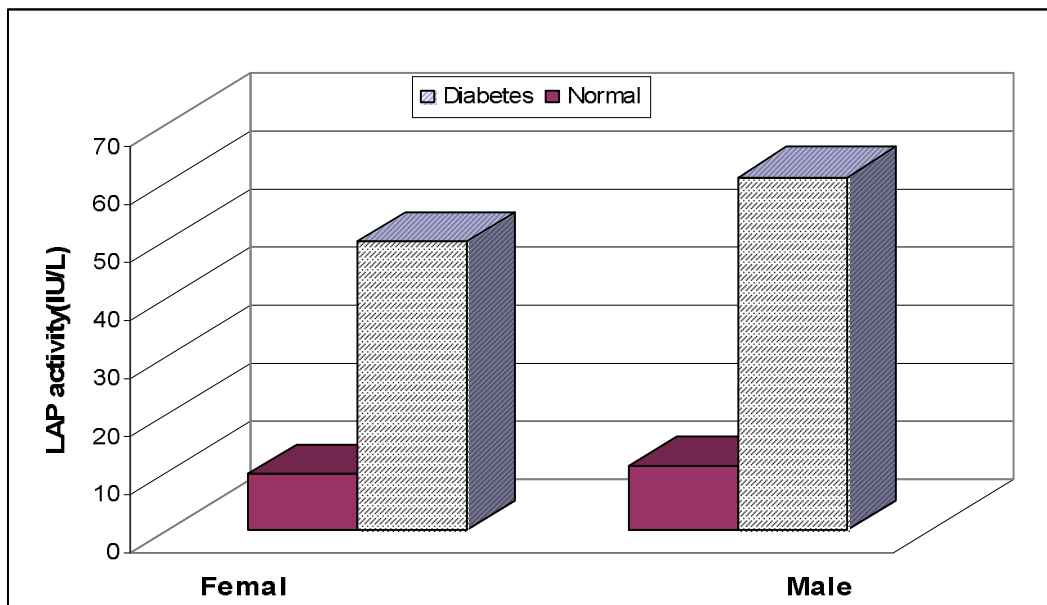
**Fig.(2): Illustrate spectra FTIR of leucine amide.**

Results this study illustrate difference in levels of LAP activity in (50) patients' serum with type 2 diabetes with (50) normals as shown Fig. (3) and the results, as shown in

Fig.(4) refers to LAP activity in serum of normal and patients with type 2 diabetes (male) was slightly higher than that of femal serum of normal and patients with type 2 diabetes [14].



**Fig.(3):** Illustrate value of LAP activity value in serum of normal and patients with type 2 diabetes.



**Fig.(4):** Illustrate value of LAP activity in serum of normal and patients (men and women) with type 2 diabetes.

These results suggest that LAP has sensitivity and diagnostic significance. Elevated LAP activity in serum usually indicates diseases of liver, pancreas and bile ducts and elevation is less affected by damage of liver parenchyma than 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 [14].

Table (1) illustrate comparing the mean levels of serum LAP activity of the normals (10.58  $\pm$  4.39) I.U/L with patients with type 2 diabetes, (560.46  $\pm$  10.5) I.U/L, significant increased ( $p < 0.001$ ). Also Table (1) illustrate comparing mean levels of serum LAP activity

in patient's men with type (2) diabetes (61.12  $\pm$  17.94) I.U/L and normals men (11.09  $\pm$  4.94) I.U/L and significant increase ( $p < 0.05$ ).

Mean levels of serum LAP activity in patients' women with type 2 diabetes (49.8  $\pm$  34.06) I.U/L and normals women (9.73  $\pm$  4.38) I.U/L, significant increase ( $p < 0.001$ ) [21]. These results also suggested by the higher levels of LAP activity in patients and normal (male) than in patients and normal (femal) [14].

**Table (1)**

*Illustrate value of LAP activity in serum of normal and patients with type 2 diabetes.*

Normal				Diabetes			
Specimen	No. of cases	Age (years)	LAP activity (I.U/L) mean $\pm$ S.D	No. of cases	Age (years)	LAP activity (I.U/L) mean $\pm$ S.D	P <
Male	20	38-70	11.09 $\pm$ 4.94	25	40-75	61.12 $\pm$ 17.94	0.05
Femal	30	42-65	9.73 $\pm$ 4.38	25	42-70	49.8 $\pm$ 34.06	0.01
Total	50	38-70	10.58 $\pm$ 4.39	50	40-75	560.46 $\pm$ 10.5	0.001

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### الخلاصة

شملت الدراسة (50) عينة مرضية من أمصال المرضى المصابين بالداء السكري النوع الثاني بالإضافة الى (50) عينة من ادرار الأصحاء كمجموعة ضابطة. وقد أظهرت النتائج ارتفاع نشاط الانزيم في أمصال المرضى مقارنة بالأصحاء في أمصال المرضى المصابين بالداء السكري و النوع الثاني. كما أظهرت نتائج الدراسة ارتفاع معنوي ملحوظ بنشاط أنزيم الليوسين أمينو بيتايديز في أمصال المرضى المصابين بالداء السكري النوع الثاني مقارنة بالأصحاء. بالإضافة الى تحضير Leucine amide كمادة أساس للأنزيم إذ تم تشخيصها بتعيين درجة الانصهار وطيف FTIR.