

Isolation and Partial Purification Maltase Isoenzymes From Urine of Type 2 Diabetic Patients

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Abstract:

This study was performed on 50 urine specimens of patients with type 2 diabetes, in addition, 50 normal specimens were investigated as control group. The activity rate of maltase in patients (6.40 ± 2.17) I.U/ml and activity rate of maltase in normal (0.44 ± 0.20) I.U/ml. The results of the study reveal that maltase activity of type 2 diabetes patient's urine shows significant increase ($P < 0.01$) compare to normal.

Key word: Maltase, Type 2 diabetes mellitus.

Introduction:

Diabetes mellitus is the most common illness due to hormonal causes (1,2). Type 2 diabetes is characterized by decline in insulin action due to the resistance of tissue cells to the action of insulin. The problem is intensified by the inability of the beta cells of the pancreas to produce enough insulin to counteract the resistance. Thus Type 2 diabetes is a disorder of both insulin resistance and relative deficiency of insulin(3).

Type 2 diabetes is often considered a polygenic disorder with multiple genes located on different chromosomes being associated with this condition (4).

(α - D- Glucoside glucohydrolase; EC 3.2.1.20):

Alpha- glucosidase is also known as maltase it catalyzes the hydrolysis of maltose to glucose units (5,6), Mammalian intestinal mucosa secretes disaccharidases such as maltase, lactase and sucrase. Alpha- glucosidase (maltase) is used for assaying the activity of alpha- amylase and for the determination of maltase in brewing alpha- glucosidase catalyzes (7).

Maltase has been found in pancreas(8), small intestinal mucosa(9,10,11), liver, kidney (12), human seminal plasma(5,13) and blood serum of animals(14), in addition maltase detected in plants, molds, yeasts and fungi(15).

Determination of maltase activity might be helpful to evaluate functions of the epididymis and particularly to exclude epididymal obstruction(5). Deficiency of maltase should be considered in the diagnosis of rigid spine syndrome(16), and determination of maltase activity in mixed leukocytes helpful to diagnosis of glycogen storage disease type 2 (Pompe's disease), (17,18).

The aim of this study was to measure maltase activity and partially purification, isolation isoenzymes of maltase from patients urine with diabetes type 2.

Materials and method:

Specimen:

Fifty urine samples obtained from normal individuals (25) men and (25) women, age (40-90) years, and (50) patient's urine with type 2 diabetes (30) men and (20) women, age (40-90) years. The patients were diagnosed by specialist physicians in Samaraa hospital.

1- Measurement activity of maltase in urine :

Maltase activity was assayed by measurement of the glucose released by the tris/ glucose oxidase/ peroxidase procedure of Dahlqvist (1964), the increase in absorbance at 340 nm, caused by the reduction of NADP is measured as the catalytic activity of maltase(19).

2- Protein determination:

Protein concentration was determined according to the method of Lowry (1951) (20).

3- Isolation of maltase :

Isolation of maltase from urine depending on Dahlqvist (1964) method, and inhibitors removed using two steps(21):

A- Gel filtration:

5.0 ml of the fresh urine sample was passed through a column of sephadex gel G-25 (fine)(20x1.5 cm). Ten fractions each of 5.0 ml, were collected by passing phosphate buffer, pH 7.4 through the column. Entire operations were carried out inside a refrigerator. And the flow rate ($50 \text{ cm}^3/30 \text{ min}$).

B- Dialysis:

It was one of important methods used in enzymes purification, visking dialysis tubes (3/4 diameter HMC Gloucester) were used for dialysis of 10 ml of fresh urine against two liters of phosphate buffer pH (7.4) inside refrigerator. The volume of urine after 18 hours of dialysis was measured and enzyme activity determined in this. 4- Isolation and partial purification of maltase isoenzymes from patient's urine with type 2 diabetes:

2.0 ml of the fresh filtered urine sample was passed through a column of sephadex ion exchange chromatography (DEAE- sephadex A-50) pre-equilibrated (50 mM/dm^3 phosphate buffer, pH 7.4). The sephadex ion exchange column of the size of ($20 \times 1.5 \text{ cm}$). Collection (11) fractions each one contain (2)ml and complete isolation by phosphate buffer pH(7.4) containing gradient concentration of sodium chloride ($0.1-0.4 \text{ mol/Liter}$), Entire operations were carried out inside a refrigerator, at flow rate of ($53 \text{ cm}^3/\text{hr}$).

Result and Discussion:

The Results showed that the maltase activity in patients urine with type 2 diabetes mellitus is higher

than that of normal. figure(1) (22).

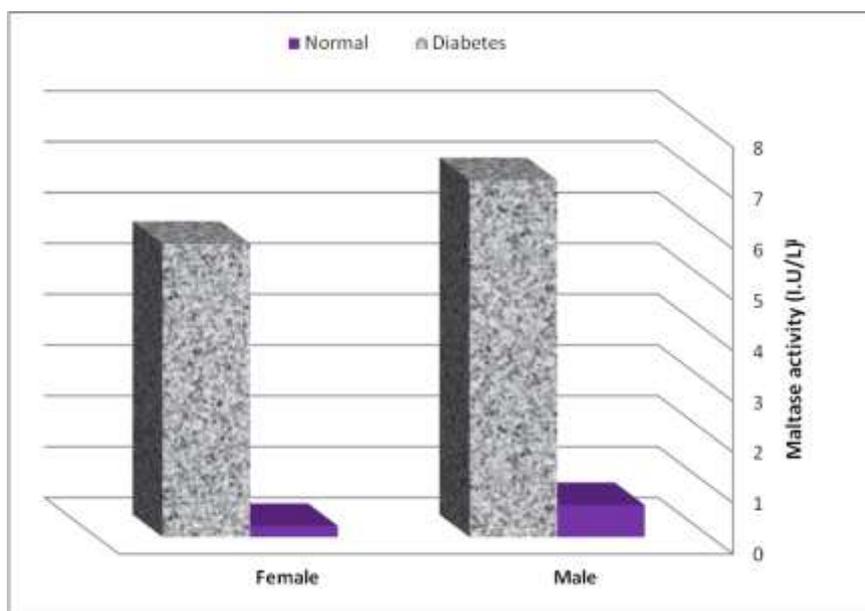


Fig (1): illustrate value of maltase activity in urine of normal and patients (male and female) with type 2 diabetes.

Table (1) Illustrate the comparison between the mean levels of urine maltase activity of the normal individuals (0.44± 0.20)I.U/L with patients with type 2 diabetes mellitus, (6.40± 2.17) I.U/L, significant increase (P<0.01). Also table (1)refers to comparing mean levels of urine maltase activity in patients (male and femal) with type 2 diabetes (7.02± 2.50) I.U/L, (5.77± 1.83) I.U/L respectively, and significant increase (P<o.01). While mean levels of urine maltase activity in normal (male and female), (0.63± 0.32) I.U/L, (0.22± 0.07) I.U/L respectively, significant increase (P<0.01). Tandon (1975) &

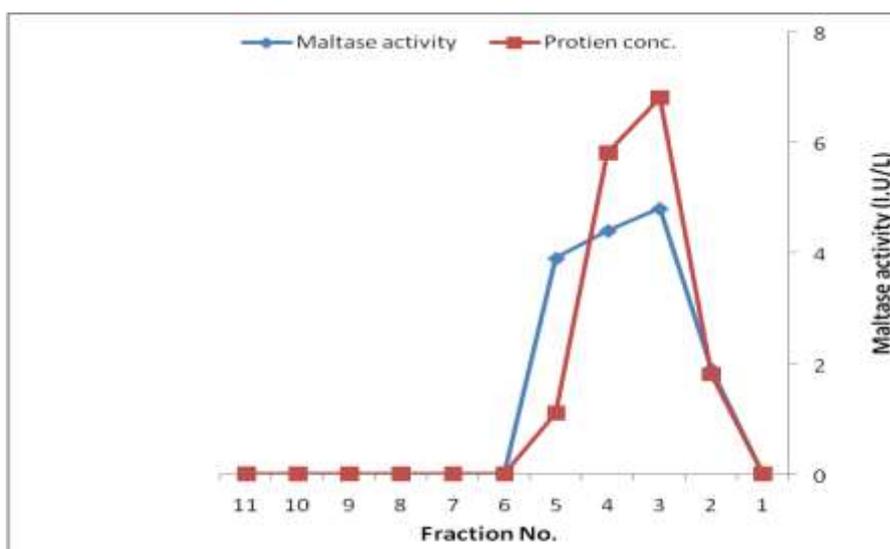
Mehler (1976) referd to increased maltase activity in urine of patients because disaccharidase activity has been shown to increased human diabetics (22, 23). The previous research referd to urinary maltase activity in the kidney attributed to localized maltase in the brush broder of the proximal tubules and diabetes effect of kidney function, in addition when cell necrosis occurs, fragments of the brush border membren with bound maltase can be shown to be present in the 100,000 g sediment of the urine. Therefore maltase activity increased in urine (24,25).

Table(1): illustrates values of maltase activity in urine of normal and patients with type 2 diabetes

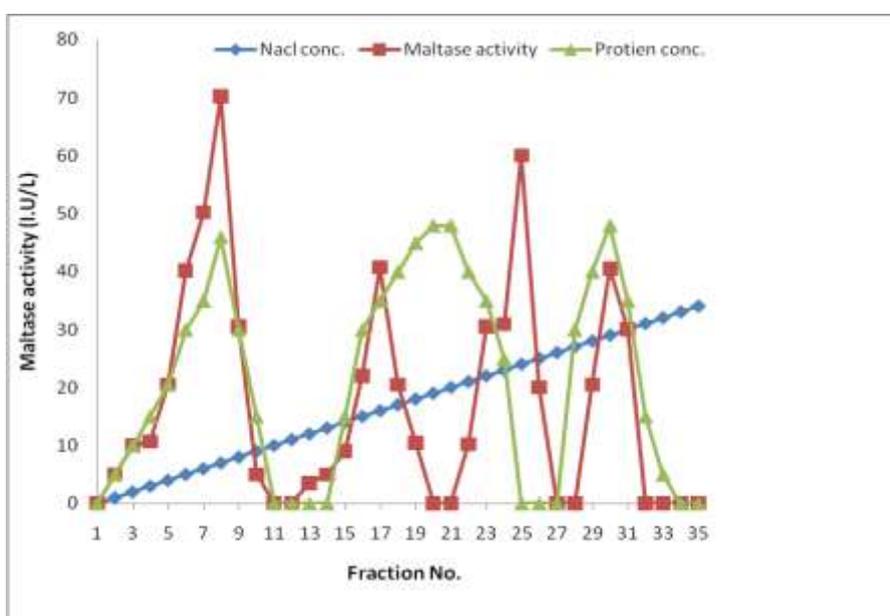
specimen	Normal			Type 2 diabetes			P<
	No. of cases	Age (years)	Maltase activity (I.U/L) mean±S.D	No. of cases	Age (years)	Maltase activity (I.U/L) mean±S.D	
Male	25	40- 90	0.63±0.32	30	40-90	7.02±2.50	0.01
Female	25	40- 85	0.22±0.07	20	40- 75	5.77±1.83	0.01
Total	50	40- 90	0.44±0.2	50	40- 90	6.40±2.17	0.01

The enzyme was purified by using sephadex G-25 column chromatography. A 5.38 fold purification of maltase from patient's urine with type 2 diabetes was achieved, and this enzyme showed single peak(26) see figure (2).While purification degree increased to 10.7 fold by using dialysis then isolation of purified maltase by using ionic- exchange column (sephadex

DEAE A-50) to four isoenzymes vary in purification degree, figure (3). Table (2) illustrates purification steps, observed activity of maltase increase in patient 's urine with type 2 diabetes after process of purification which remove inhibitors such as (urea, amino acids, and ammonia) in urine which dcreased the activity of maltase (21).



Fig(2): maltase Isolation from patient's urine with type2 diabetes by using gel filtration



Fig(3): Isolation and partial purification of isoenzymes of maltase from urine of patients with type 2 diabetes.

Table (2): illustrates isolation and purification steps of isoenzymes of maltase from patient's urine with type 2 diabetes.

Step	Elute (ml)	protein conc. (mg/ml)	Total protein (mg)	Activity (mu/ml)	Specific activity (mu/mg)	Degree of Purification (fold)
Crude urine	10	83	830	1.11	0.0013	1
Sephadex G-25	5	78	390	3.07	0.007	5.38
Dialysis	5	64	320	4.5	0.014	10.7
DEAE sephadex A-50						
Isoenzyme I	2	46	92	7.2	0.07	53.84
Isoenzyme II	2	48	96	4.7	0.04	30.76
Isoenzyme III	2	48	96	6.0	0.06	46.15
Isoenzyme IV	2	47	94	4.5	0.047	36.15

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فصل وتنقية جزئية لمتناظرات أنزيم المالتيز من أدرار المرضى المصابين بالداء السكري النوع الثاني

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الملخص:

شملت الدراسة 50 عينة من ادرار المرضى المصابين بداء السكري النوع الثاني بالإضافة الى 50 عينة من ادرارالأصحاء كمجموعة ضابطة. أظهرت نتائج الدراسة ارتفاعا معنويا ($P < 0.01$) بنشاط أنزيم المالتيز في ادرارالمرضى المصابين بالداء السكري النوع الثاني مقارنة بالأصحاء، أذ بلغ معد الفعالية (0.44 ± 0.20) وحدة عالمية/لتر في حين كان معدل الفعالية (6.40 ± 2.17) وحدة عالمية/لتر في حالة الداء السكري النوع الثاني وتم أيضا تنقية أنزيم المالتيز في ادرار المرضى المصابين بالداء السكري النوع الثاني بأستعمال كروماتوغرافيا الترشيح الهلامي (-Sephadex G-25) ومن ثم فصل الأنزيم المنقى جزئيا بأستعمال كروماتوغرافيا التبادل الأيوني (DEAE-Sephadex A-50) الى أربع متناظرات أنزيمية تختلف في درجة تنقيتها.