Evaluation of alpha- amylase activity in serum and saliva of normal individuals and patients with jaundice

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ABSTRACT

Enzymes are very important components in the biological system. α-Amylase is one of these enzymes, it is one of the hydrolytic enzymes that breaks down the dietary starch and glycogen to glucose and maltose. The present study is conducted to evaluate α-Amylase activity in serum and saliva of patients with jaundice in comparison it with the enzyme activity in the serum and saliva of healthy individuals. That is, to provide further information about the relationship between jaundice and α-Amylase activity. Estimation of α-Amylase activity in serum and saliva was made by Amyloclastic (iodometric) method.

A 120 samples of serum and saliva were collected; 50 samples represented the control group whose ages range between (20–55) years and 70 samples were the patient with jaundice group whose ages range between (15–55) years. Alpha-amylase activity appeared significantly higher in normal individuals (both in serum and saliva in comparison with patients with jaundice.

تعيين فعالية انزيم الفا – اميليز في مصل ولعاب الطبيعيين والمصابين باليرقان

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المستخلص

للانزيما ت دور جوهري في الحياة حيث تعتبر مكونات مهمة جدا في النظام الحيوي،أنزيم ألفا-اميليز (أنزيم النشا) هو أحد هذه الأنزيمات فهو أحد الأنزيمات ألحاله التي تحلل النشا الغذائي والكلايكوجين (مولد سكر العنب) إلى الكلوكوز (سكر النشا) والمالتوز (سكر الشعير). أجريت الدراسة الحالية لغرض تقدير فعالية أنزيم ألفا- اميليز في مصل ولعاب المرضى المصابين باليرقان ومقارنتها مع فعالية هذه الأنزيمات في مصل ولعاب الأشخاص الأصحاء، وذلك لتوفير معلومات أوفر ومعرفة أوسع حول العلاقة بين فعالية أنزيم ألفا-أميليز ومرض اليرقان. أجري تقدير فعالية أنزيم ألفا-اميليز في المصل واللعاب بوساطة طريقة النشا.

جمعت مائة وعشرون عينة من المصل واللعاب، خمسون عينة منها كانت تمثل المجموعة الضابطة والتي تتراوح أعمارها بين (20–55) سنة والمجموعة الثانية متكونة من سبعين عينة وهي مجموعة المرضى المصابين باليرقان والتي تتراوح أعمارها بين (15–55) سنة.

KeyWords: α -Amylase, Serum Saliva, Normal Individuals, Patients with Jaundice

Introduction

The pattern of enzyme release is largely related to the tissue of the origin of the enzyme, thus enzyme determination aiding in the diagnosis; so the study of enzyme has immense practical importance ,because regulation of enzyme activity contributes mainly in preserving homeostasis (1,5,6). α-Amylase is an enzyme secreted by the pancreas and salivary glands; α-Amylases (-1,4-D-glucan 4-glucanohydrolase EC3.2.1.1), are end-acting hydrolyses (7,8) that catalyze the hydrolysis of internal α (1—4) glycoside linkages of starch and glycogen (8-10) . Amylase from amylum (starch) that acting on starch (11), is a stable enzyme(5), and has (3914) (Carbon, Oxygen, Hydrogen and Nitrogen), (511) amino acids molecules of a single polypeptide chain, one activator chloride ion, one structural Ca^{+2} ion and (393) water molecules (10-13). X-ray crystallographic studies have shown that α-Amylase has three domains, a central α/β barrel called domain A from the core of the molecule and contain the three active sites residues (Asp 23, Glu 261 and Asp 328), domains B and C are located roughly at opposite sides of this α/β barrel. There are three kinds of amylase (α, β and γ) amylase, β and γ are exoglycosidase that cleaves from the non reducing end of the polymer. α-Amylase is an endoglycosidase which can hydrolyze, a glycosidic linkage anywhere along the chain to produce glucose and maltose (14-16). Total amylase activity of normal plasma originates from the salivary and the pancreas, the contribution to the plasma activity from the two sources being roughly equal, The amylases normally occurring in human plasma are small molecules with molecular weights varying from (55-60)KDa .The enzyme is thus small enough to pass through the glomeruli of the kidney and amylase is the only plasma enzyme normally found in urine (17,18) ; α-Amylase in human can be fractionated into two major forms called pancreatic (P) or salivary (S), three to six minor (P) designed (P1) and (P2) and four or five minor (S) isoamylase designed (S1) to (S5) (19).

Saliva is the fluid secreted by numerous glands in the oral cavity. The salivary glands produce 1500 ml of fluid per day. There are three pairs of major salivary glands usually found in the oral cavity and it has been found that changes in the physiology and molecular biology of saliva are important factors involved in the initiation and development of diseases (20-22).

Jaundice is a frequent feature of liver diseases which refers to yellowish discoloration of the skin, sclera and mucous membrane resulting from an increase bilirubin concentration in the body fluids because there is excess circulating bilirubin(23,24), and only as clinically apparent when plasma bilirubin concentration reaches about 35 mmol/L (2-3mg/dl), normally bilirubin concentration is(5.1-17mmol/L) (0.3-1.0mg/dl) (25,26). In fact, detailed observations about jaundice and its underlying cause were not recorded until the 17th and 18th centuries when outbreak of jaundice was noted in association with camping (27,28).

Amylase levels may be low in severe liver diseases including hepatitis and jaundice as reported by Bhutta and Rahman (29,30).

The major goal of this study is to evaluate the α-Amylase activity in serum and saliva in patients with jaundice regardless its cause.The present study is planned to fulfill the following objectives:

To estimate the activity of α-Amylase in the serum and saliva and patients with jaundice. To compare the enzyme activity in serum and saliva between normal and jaundiced individuals.
Material and Methods

Equipments
Spectrophotometer (Cecil 1021 Cambridge England), Water bath, (Thermoline Scientific Equipment), pH meter (Jenway), Centrifuge (Sigma 3- E -1 Germany), Glass Column, Balance (Memmeret).

Chemical substances
Starch powder ,Anhydrous disodium phosphate (Na2HPO4), Benzoic acid (C6H5COOH), Sodium chloride (NaCl), Potassium iodate (KIO3), Potassium iodide (KI) Concentrated hydrochloric acid (HCl),Distilled water. Cellulose-DEAE Phosphate buffer (pH 7.0).

Serum and Saliva Samples
Serum and saliva samples were to be collected from (120) individuals, aged (15-55) years, (50)of them were normal persons and (70) of them were patients with jaundice, who were admitted to Ibn-Senna Teaching Hospital from December 2003 to June 2004 .The patients were selected according to their clinical presentations and laboratory results, so, the study populations was divided into two groups:-
Group I: 50 normal persons or individuals, control group, who are free of jaundice with no history of salivary or pancreatic diseases.
Group II: 70 jaundiced patients regardless to the underlying cause or pathology but with the exclusion of patients with salivary and pancreatic diseases, blood samples were collecting during the onset of the jaundice i.e. (within few days from the appearance of the jaundice).

Collection of serum:
After collecting of venous blood samples . Blood was centrifuged at (3000) rpm for (5) min to isolate the serum from the whole blood then the serum draw to another disposable plane tube and stored in deep freeze at (-20)C° till the time of analysis.
Collection of saliva:
Saliva sample were collected simultaneously with the blood samples . The patients were asked to wash their mouths with 30ml of distilled water, several times to ensure complete removal of any remnants food or debris, then the patient was asked to accumulate the saliva in his mouth and then spite this saliva into a disposable plain tube, about (2-3)ml of saliva sample was collected from each patient. The saliva sample was placed immediately in a cool box and transferred to the laboratory where it centrifuged at 3000rpm for 5 minutes to separate any remnants, debris or contaminating materials. The clear part of the saliva sample was draw to another disposable plain tube and stored in deep freeze at (-20)C° as described by Edger (31) till the time of analysis after ensuring complete sealing of the opening of the tube.

Method
Measurements of α-Amylase activity in normal and abnormal serum and saliva:
α-Amylase activity in serum and saliva of normal and jaundiced individuals was determined by the Amyloclastic DEAE- method, by which the determination of α-Amylase activity depends upon the ability of this enzyme to hydrolyze the starch (substrate) to its
simple sugar.(32) The color spectrophotometically after addition of iodine solution and comparing it with

iodine solution and comparing it with control, the quality of unhydrolysed starch can be determined and so the activity of α-Amylase can be calculated, the decrease in color is proportional to the amylase concentration, the absorbance was measured at (660nm) and the enzyme activity was expressed as S.U/dl

Reagents

1. Buffered starch substrate (0.4g/L, pH 7.0):

(13.3)g of anhydrous disodium phosphate (Na2HPO4) and (4.3)g of benzoic acid (C6H5COOH) were dissolved in approximately (250)ml of distilled water in a (600)ml beaker, the solution was brought to boil and (0.2)g of soluble starch which had been suspended previously in (5)ml of cold water was added, the container was rinsed with additional water so as to transfer all the starch, the boiling of the solution was continued for one minute after the addition of starch, the solution was transferred qualitatively to a 500ml volumetric flask and the volume was made (500)ml with distilled water and let to cool at room temperature, kept at (4)C° and prepared freshly each month.

2. Normal Saline:

Dissolve (9)g of NaCl in (1)L of distilled water.

3.Stock iodine solution (0.1N):

(3.56)g of potassium iodide (KIO_3) and 45gm of potassium iodide (KI) were dissolved in approximately 800 ml of distilled water in one volumetric flask, (9) ml of concentrated hydrochloric acid was slowly dissolved with mixing, the diluted to (1)L with water. This solute was stored in the refrigerator in brown bottle at (4)C° stable for (12) months.

4. Working iodine solution (0.01N):

(50)ml of stock iodine solution was diluted to (500) ml with distilled water, fresh working iodine solution was prepared for each individual assay.

Procedure:

α-Amylase enzyme has an optimum pH of (6.5-7), it is activated by chloride so dilution of serum and saliva has to be made in normal saline.

TEST: Dilute serum (1:10) with normal saline, pipette (1)ml of buffered starch substrate into a test tube and place in a water bath at (37)C° for (3)min, add (0.1)ml of diluted serum (or 0.1ml of diluted saliva in case of salivary sample), mix gently and incubate for exactly (15) min, remove the tube from the bath, add (0.4)ml of working iodine solution, mix well and then add (8.5) ml of distilled water and mix well again.

Control: Mix 1ml of buffered substrate, (0.4) ml of iodine and (8.6)ml of distilled water, measure immediately the absorbance of the blue color spectrophotometerically.

Materials	Test, T	Control, C
1-buffered substrate	1.ml	1.1ml
2- place in water bath for 3minuts at 37c		
3-sample (diluted 1:10)*	0.1ml	-
4- place in water bath for 15minuts at 37C°		
5-iodine	0.4ml	0.4ml
6- Mix well		
7- D.W.	8.5ml	8.6ml

*Saliva sample was diluted 1:1000 with water. C= control ,T= Test

The control tube contains 0.4mg of starch , the amount of starch which has been digested is therefore:-

$$= \frac{C-T}{C} \times 0.4mg$$

$$= \frac{C-T}{C} \times \frac{0.4}{5} S.U$$

so amylase activity in S.U/dl**

$$= \frac{C-T}{C} \times \frac{0.4}{5} \times \frac{100}{0.01***}$$

$$= \frac{C-T}{C} \times 800$$

**The somogyi unit (S.U):- It is the amount of amylase that digests 5 mg of starch under optimum condition, in 15 minutes at 37C° and pH 6.5-7.0.S.U % = 1.85 IU/L

*** The amount of enzyme present in 0.01 ml of serum. C= control, T= Test.

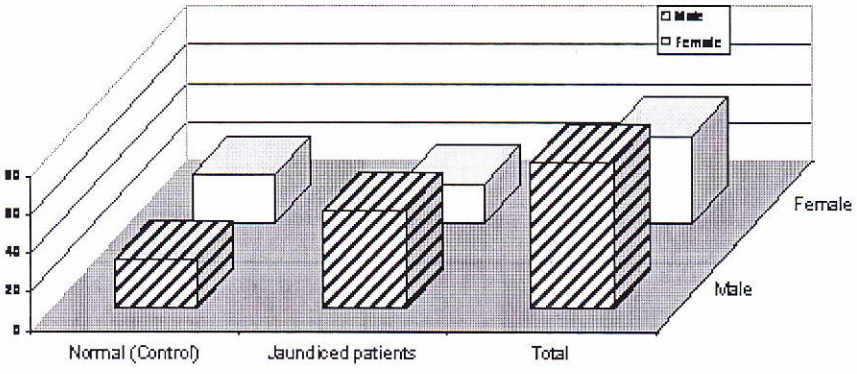
RESULTS

α- Amylase study: In the present study, α-Amylase activities were measured, in the serum and saliva of normal individuals and patients with jaundice. Detailed information of all individuals included in this study are summarized in table(1).The demographic distribution of those individuals is shown in fig.(1).

α-Amylase Activity measurements in serum and saliva samples:

α-Amylase activity was studied in serum and saliva of individuals who are diagnosed to have jaundice clinically and laboratory regardless their underlying causes whether prehepatic, hepatic or post hepatic. The mean values of serum and saliva α-Amylase activities both normal and for the patients with jaundice are shown in table(2) and table(3) .

The mean values of α-Amylase activity in serum and saliva of control group was (371 S.U/dl) ranged from (28-758 S.U/dl) and (54059.06 S.U/dl) ranged from (2670-95246 S.U/dl) respectively. The mean α-Amylase activity level in the serum of jaundiced group is lower than that in the normal group,($P < 0.001$). The mean α-Amylase activity level in saliva of the jaundiced group is also significantly ($P < 0.05$) .The mean value of α-Amylase activity in serum was (177.6 S.U/dl) in saliva was (38860.6 S.U/dl) for the jaundiced patients.



Fig(1): Demographic characteristics of study population

Table (1): Demographic characterization of study population for serum and salivary amylase.

Range of age (years)	No. of subjects			Group
	Female	Male	Total	
(20-55)	25	25	50	Normal (control)
(15-55)	20	50	70	Patients (jaundiced)
	45	75	120	Total

Table (2): Mean ± SD, and Range of α-Amylase activity in serum of normal individuals and jaundiced patients.

α-Amylase activity (S.U/dl)		Diagnosis
Range	Mean ± SD	
(28-758)	371.04 ± 168.34	Normal (control)
(13-754)	177.60 ±168.10	Patients (jaundice)

Table (3): Mean values, SD and Range for α-Amylase activity in saliva of normal individuals and jaundiced patients.

α-Amylase activity (S.U/dl)			Diagnosis
Range	SD.	Mean	
(2670-95246)	±21042.9	54059.06	Normal (control)
(800-75344)	±29396.5	38860.65	Patients (jaundiced)

Discussion:

Although assays of amylase activity in serum and urine are largely of use in the diagnosis of pancreatic diseases and in the investigation of pancreatic function [19] . Amylase activity may alter in some pathological condition other than that of pancreas . Our goal in this research is to provide a clear knowledge about the relation between amylase activity and jaundice.

In this study , α-Amylase activity was determined in the serum and saliva in normal individuals and jaundiced patients. With concerned to norm,al individuals, 50 healthy persons of both sexes aged (15-55) years and regarded as normal (control) group. α-Amylase activity was determined in this group in order to provide a useful informations about the enzyme activity in normal individuals and to compare it with the enzyme activity that was observed in jaundiced patients . on other hand , 70 patients with jaundiced were considered in this study as diseased group who aged (15-55) years.

These results of decline of serum and saliva α-Amylase activity in jaundiced patients were agreed with the results observed by Bhutta [32] who found that serum amylase values in patients with liver diseases including : jaundice , infective hepatitis and cirrhosis, were significantly less than in normal individuals ($p<0.01$) . Furthermore, he had concluded from his study, that the severity of hepatic disease appeared to be important in the

diminution of serum amylase levels and that patients with severely impaired hepatic function had lower serum amylase activity through demonstrating that serum amylase had been decreased as serum bilirubin had been increased, in another ward, he had found an inverse relationship between serum amylase values and serum bilirubin values which could be due to competition between amylase and bilirubin for binding to albumin which will transport them in the blood . In addition , decreased the activity of amylase that accompanied liver diseases and jaundice might be attributed to impaired synthesis of serum proteins. Although Otsuki [33] found no amylase activity in extracts of human liver ,but Fridhander [34] reported that amylase activity in liver extracts is too low to be a significant source of human serum and urine amylase . Lech [34] reported a significant increase in serum amylase in patients with acute viral hepatitis, in particular hepatitis B and hepatitis non A non B and suggested that pancreatic injury might be behind the increase in serum amylase in such cases.

Holzel [35] reported a significant increase in serum amylase activity in patients with chronic liver diseases and he supposed the possibility of coincident pancreatitis which could be the cause of high amylase activity.

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