

## **Study of some Hydrolytic Enzymes in Sera and Saliva of Patients with Oral Epithelial Tumors**

**Hathama R. Hasan**

*Department of Chemistry  
College of Science  
Baghdad University*

**Rukzan M. Dawood**

*Department of Chemistry  
College of Education for Girls  
Mosul University*

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### **ABSTRACT**

The present study was designed to investigate the changes in the activity and specific activity of some hydrolytic enzymes like  $\alpha$ - amylase, alkaline and acid RNase in sera and saliva of patients with oral epithelial tumors (benign and malignant). Then follow up the variation of these enzymes using polyacrylamide gel electrophoresis technique to evaluate the changes of these isoenzymes due to the presence of the tumors.

Thirty three patients with oral tumors (benign and malignant) aged from 15-75 years attending the specialized surgery hospital in Baghdad medical city, were included in the present study. Thirty one healthy individuals of matched age and gender were utilized as control. The results indicated the presence of highly significant increase ( $P < 0.001$ )of sera alk. RNase activity (U/L) in both benign and malignant groups in comparison to that of control. The specific activity results (U/mg) showed also a significant increase ( $P < 0.05$ ) in both benign and malignant groups. The results of acid RNase activity and specific activity reflected a non significant increase in both benign and malignant groups. The salivary amylase activity (U/L) and specific activity (U/mg) showed significant decrease ( $P < 0.001$ ) in malignant group, whereas the sera amylase activity and specific activity revealed a non significant increase in both benign and malignant groups.

**Keywords:** saliva, oral cancer, amylase, RNases, tumors



Human salivary alpha amylase is the only enzyme in saliva capable of degrading oligosaccharides. It has been found that amylase accounts for 40-50% of the total salivary gland-produced protein, mostly synthesized in the parotid gland (Nobel, 2000). Human parotid saliva and submandibular saliva contain about 45 mg and 30 mg of amylase, per 100 mg of protein respectively. It is generally considered to be a reliable marker of serous cell function (Almstahl *et al.*, 2001). Amylase also interacts specifically with certain oral bacteria and may play a role in modulating the adhesion of those species to teeth (Van der Maarel *et al.*, 2002). It has been found that salivary amylase inhibits the growth of *Lgionella pneumophila* and *Neisseria gonorrhoeae* (Rantonen, 2003).

Ribonucleases are enzymes catalyze the breakdown of ribonucleic acid (RNA) which is the chemical material in a cell that codes for different proteins (Raines, 1998). Different types of RNase have been reported to be present in human, the most extensively studied one is the bovine pancreatic ribonuclease (or RNase A; EC 3.1.27.5) because of its ease of purification and small size (14 KDa). It catalyzes the cleavage of the P-O bond of RNA on the 3' side of pyrimidine nucleosides (Jianzhi *et al.*, 2003). The structure of RNase A is stabilized by four disulfide bonds that involve all eight of its cysteine residues. Human ribonuclease is widely distributed in various organs, such as the pancreas and body fluids including serum, urine, saliva, and cerebrospinal fluid (Blank, 1989; Jochen, 2006). RNase has been considered as a possible tumor marker for some tumors e.g. pancreatic tumor (Ester *et al.*, 2000), and an elevation level of this enzyme was reported in sera of patient with benign and malignant uterine tumors (Al-Shamarre, 2002).

The aim of the present study was to investigate amylase and RNases activity, and to detect any changes in these enzymes forms both in sera and saliva of patients with benign and malignant oral tumors.

## MATERIALS AND METHODS

### Subjects

Sixty five individuals were included in the present study; thirty three of them were clinically and histologically diagnosed as patients with oral epithelial tumors (benign and malignant). The samples were collected from patients attending the hospital of Specialized Surgery in Baghdad Medical City. Patients were evaluated by full medical history to exclude any existing of systemic disease or taking any drug that may affect the parameters to be examined. The results were compared with those obtained from a control group of thirty one age and gender matched healthy individuals.

### Samples

**Sera Samples:** Six milliliters of venous blood taken from each individual, collected in plane polyethylene tube, allowed to stand at room temperature for thirty minutes, then samples were centrifuged at (2000xg) for 10 minutes. The obtained sera transferred immediately to another test tubes. These samples were estimated directly for enzymes activities or frozen at -20 °C for subsequent analysis. Hemolyzed samples were discarded.

**Saliva Samples:** about ten milliliters unstimulated (resting) whole saliva were collected after the diagnosis, under resting conditions between 8.0-10.0 A.M. Patients were asked to rinse their mouth with water and to generate saliva in their mouth and to spit into a wide test tube (Mavazesh, 1993). The collection period was twenty minutes. Following the collection, the saliva was centrifuged at (2000 xg) for 10 minutes. The resulting supernatant was stored at - 20 °C in polyethylene tubes until assayed.

### Determination of protein concentration

A modified Lowry method by Hartree (Hartree, 1972) was used to determine saliva and serum total protein concentration using BSA (bovine serum albumin) as a standard.

### Measurement of $\alpha$ - Amylase Activity

Amylase activity was measured according to Caraway method (Caraway, 1969). The samples were incubated with buffered starch substrate at controlled temp. (37°C) for 15 minutes, then subsequently react with iodine to produce a blue color with starch. The decrease in the color compared with that obtained in the absence of amylase (the control), provides a measure of amylase activity.

$$(\text{Amylase activity unit/100ml}) = \frac{C-T}{C} \times \text{conc. of St.} \times \text{dilution factor}$$

T= the absorbance of the test

C= the absorbance of the control

The specific activity of the enzyme was expressed in U per mg of protein.

### Measurement of alkaline RNase activity

Alkaline RNase was estimated according to Bardon and Shugar method (Bardon, 1980) with the following modification:

A volume of 1 ml of Davis buffer was used. A volume of total reaction mixture was 1.1 ml instead of 0.2 ml reported in the original method.

- **Davis buffer** ( Davis,1958): The following weights were dissolved in appropriate amounts of distilled water to prepare one liter of the buffer: Citric acid (21.01gm); Potassium dihydrogen orthophosphate (13.61gm); Sodium tetraborate (19.07gm); Tris (hydroxymethyl aminomethane)(12.11gm); and Potassium chloride (7.46gm) . The pH of the solution was adjusted to (8) using sodium hydroxide (0.4N),

Note: 0.1ml of saliva sample was used instead of 0.05 ml of serum.

### Measurement of acid RNase activity

Acid RNase activity was measured as alkaline RNase activity except that the pH of Davis buffer was adjusted to 5.

Acid andalkaline **RNase** activity was expressed as Unit/L.

$$\text{RNase activity (U/L)} = (\Delta A/t \times V_t/V_s \times 1000)$$

$\Delta A$  = Sample's absorbance – Control's absorbance (at  $\lambda = 260\text{nm}$ )  
Vt = the total volume, Vs = the volume of the sample (serum, saliva),  
t = the incubation time (min)

### **Conventional polyacrylamide gel electrophoresis**

This technique was carried out to analyze sera and saliva samples of the control and benign and malignant groups, employing amylase activity stain to detect the location of amylase on the gel using 7.5% acrylamide gel. According to application note 306 LKB, pre electrophoresis was carried out at 50 mA and 15 v/cm for 30 min. A volume of (10  $\mu$  l) of the samples were applied into the wells in the gel, and concentrated for 5-10 min. with a current of 20 mA. Electrophoresis was continued using 40 mA, until the bromophenol blue dye reached the gel margin. Then the gel was stained for amylase activity.

The method of Laemmli (Laemmli, 1952) using 12.5% acrylamide gel as a separating gel (containing 0.5 ml 10% SDS and 0.5 gm RNA), while 4.5% acrylamide was used as stacking gel (containing 0.3ml 10% SDS) was used to separate alkaline RNase isoenzymes in sera and saliva samples of control and patient groups, according to application note 306 LKB. Then the gel was stained for RNase activity.

### **Amylase activity staining (Merritt *et al.*, 1973)**

- 1- Phosphate buffer (0.02M) pH6.9 containing 0.0067M NaCl
- 2- Starch 1% (w/v) in phosphate buffer
- 3- KI- iodine solution. This solution was prepared as follows:  
5gm KI and 1.0 ml of stock solution (iodin 2gm/100ml and KI 6gm/100ml) then adjusted to final volume of 125ml.

After electrophoresis, the part of the gel which contained saliva samples was incubated in starch solution for 15 min at room temp. followed by immediate staining with KI- iodine solution, the bands which represented amylase activity was rapidly appeared. Gel part which contained serum samples was incubated in starch solution for 2 hours at 37 °C. the gel was brief water-rinsed and stained with KI-iodine solution until resolution of the amylase banding was evident. Photographs were taken immediately after bands appearance.

### **Alkaline RNase activity staining (Bravo *et al.*, 1994)**

- 1- The incubation buffer: Tris- HCl buffer (0.1 M; pH8.5)
- 2- Isopropanol 10%(v/v) in Tris buffer 10mM at pH8
- 3- Staining solution: toluidine blue (0.2% w/v) dissolved in the incubation buffer.
- 4- The SDS was removed from the gel with isopropanol 10 % (v/v) in Tris buffer 10mM at pH8. The gel was then incubated in the activity buffer (Tris- HCl buffer 0.1 M; pH8.5) for an hour to allow enzymatic digestion of the embedded substrate. The gel was stained with the staining solution for 10 min. then the gel was destained with water until the positive bands appeared.

### Statistical Analysis

The findings were expressed as the mean  $\pm$  standard deviation. The data were analyzed with student's independent *t* test. All statistical analyses were performed with the program Statistical Package for the Social Science (SPSS) for windows, version 10.0.

A *P* value of  $<0.05$  was accepted as statistically significant.

### RESULTS

The amylase activity (U/L), and specific activity (U/mg) of sera samples are presented in Table (1) and reveal a non significant difference ( $P>0.05$ ) in both benign and malignant groups incomparison to that of the control. The specific activity results showed a non significant difference in both benign and malignant groups in comparison to that of control.

Table 1: Mean value of amylase activity and specific activity in sera of control and patient groups.

| GROUP     | Sample Number | Age (year)<br>Mean $\pm$ SD | Mean $\pm$ SD       |                        |
|-----------|---------------|-----------------------------|---------------------|------------------------|
|           |               |                             | Activity U/L        | Specific Activity U/mg |
| Control   | 32            | 35.66 $\pm$ 11.64           | 331.69 $\pm$ 70.44  | 4.44 $\pm$ 1.32        |
| Benign    | 14            | 35.78 $\pm$ 15.63           | 336.37 $\pm$ 104.89 | 3.85 $\pm$ 1.31        |
| Malignant | 19            | 46.57 $\pm$ 15.21           | 352.91 $\pm$ 142.46 | 3.62 $\pm$ 1.41        |

The results of salivary amylase activity and specific activity were presented in Table (2), and showed a significant decrease ( $P<0.05$ ) in amylase activity of the malignant group. The specific activity of the same group showed a highly significant decrease ( $P<0.001$ ). Meanwhile the activity and specific activity results of benign group show non significant decrease ( $P>0.05$ ) in comparison to that of control.

Table 2: Mean value of salivary amylase activity and specific activity of control and patient groups.

| GROUP     | Sample Number | Age (year)<br>Mean±SD | Mean±SD                         |                           |
|-----------|---------------|-----------------------|---------------------------------|---------------------------|
|           |               |                       | Activity×10 <sup>3</sup><br>U/L | Specific<br>Activity U/mg |
| Control   | 32            | 35.66±11.64           | 1.46±0.04                       | 0.738±0.216               |
| Benign    | 14            | 35.78±15.63           | 1.34±0.36*                      | 0.646±0.300*              |
| Malignant | 19            | 46.57±15.21           | 1.33±0.24*                      | 0.378±0.284**             |

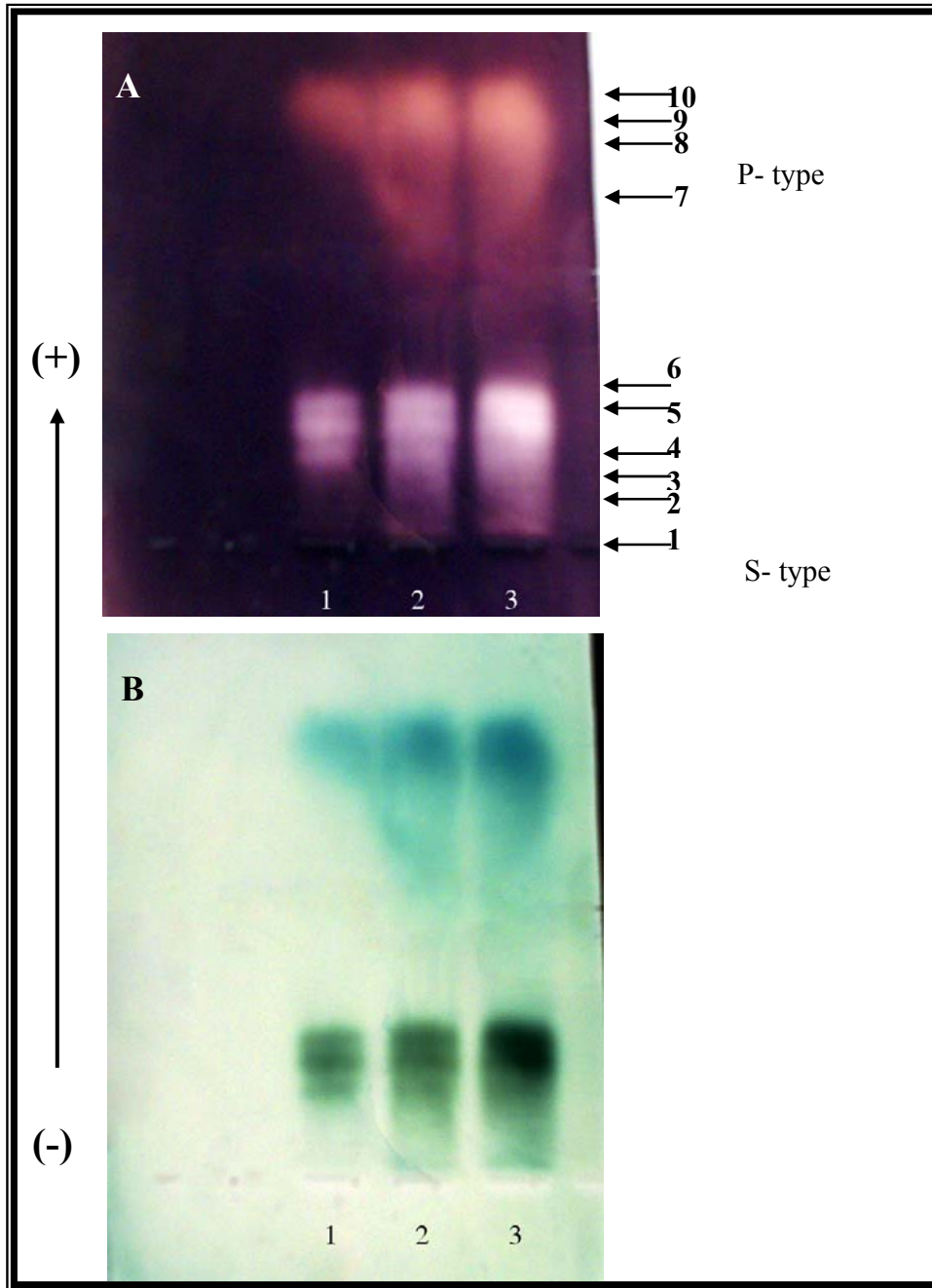
\*\* Highly significant difference in comparison to control at (P<0.001).

\* Significant difference in comparison to control at (P>0.05).

### **α- Amylase Isoenzyme**

Figure (1) and Figure (2), showed the electrophoresis pattern of amylase in sera and saliva samples respectively of control and oral tumor patient groups (benign and malignant). In refer to Figure (1) the serum amylase activity located in two different parts of the gel, one with slow mobility (S-type) and the other with fast mobility (P-type), and each of them in turn consist of more than one band. Upon comparison of the amylase separation pattern there are interesting differences between the three studied groups. The isozymes in benign and malignant groups (Lane 2, 3) respectively began to increase in both the slowest and the fastest fraction (according to their mobility to the anode) i.e. the pancreatic type and the salivary type were increased (number of bands and their intensity) in the sera of benign and malignant oral tumor patients in comparison to the control (Lane 1).

The salivary amylase zymogram in Figure (2) showed several interesting differences between the three studied groups. The electrophoretic pattern for salivary amylase shows an alternating light bands and heavy bands with decreasing intensity toward the anode. Four-five bands were visualized in control group (Lane 1). These bands start in reduction (faint) in benign group (Lane 2), meanwhile, only three – four bands were detected in the malignant group (Lane 3) and they seemed to be heavy bands, i.e. the light bands disappeared in the malignant group.



A- Positive

B- Negative

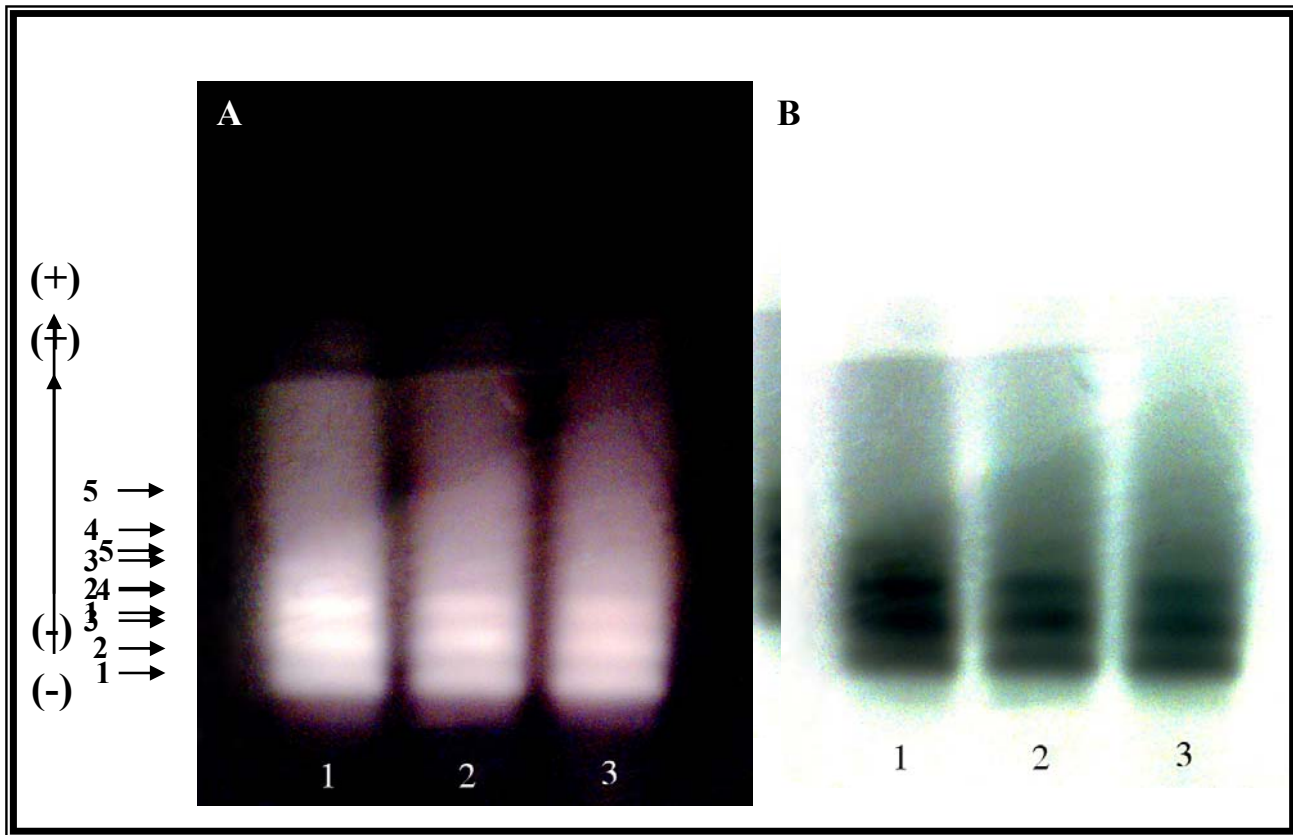
Fig. 1: Conventional poly acrylamide gel electrophoresis (PAGE) 7.0%, using Tris- glycine buffer, pH 8.9 as electrode buffer. Electrophoresis was carried using a constant current of 40 mA and voltage of 15v/cm. The gel was stained for amylase activity. The samples were applied as follows:

1- crude pooled serum (control)

2- crude pooled serum (benign)

3- crude pooled serum (malignant)





A- Positive

B- Negative

Fig. 2: Conventional poly acrylamide gel electrophoresis (PAGE) 7.0%, using Tris- glycine buffer, pH 8.9 as electrode buffer. Electrophoresis using a constant current of 40 mA and voltage of 15v/cm. The gel was stained for amylase activity. The samples were applied as follows:

1- crude pooled saliva (control)

2- crude pooled saliva (benign)

3- crude pooled saliva (malignant)

### Alkaline RNase activity and specific activity

The results of sera alkaline RNase activity (U/L) and specific activity (U/mg) were presented in Table (3). These results indicated the presence of a highly significant increase ( $P < 0.001$ ) in the activity of both benign and malignant groups in comparison to that of control. The specific activity results showed also a significant increase ( $P < 0.05$ ) in both benign and malignant groups.

Table (4) presented the results of alkaline RNase activity and specific activity in saliva samples of control and oral tumor patients groups. The results reflected a non significant difference ( $P > 0.05$ ) in the activity of both benign and malignant groups, while the specific activity show a significant decrease ( $P = 0.01$ ) in malignant group and a non significant decrease ( $P > 0.05$ ) in benign group, in comparison to that of control group.

Table 3: Mean value of alkaline RNase activity and specific activity in sera of control and patient groups.

| GROUP     | Sample Number | Age (year)<br>Mean±SD | Mean±SD                         |                              |
|-----------|---------------|-----------------------|---------------------------------|------------------------------|
|           |               |                       | Activity×10 <sup>3</sup><br>U/L | Specific<br>Activity<br>U/mg |
| Control   | 32            | 35.66±11.64           | 18.85±3.67                      | 0.42±0.34                    |
| Benign    | 14            | 35.78±15.63           | 55.97±24.64**                   | 0.62±0.24*                   |
| Malignant | 19            | 46.57±15.21           | 62.08±17.65**                   | 0.65±0.16*                   |

\*\*Highly significant difference in comparison to control at (P<0.001).

\*Significant difference in comparison to control at (P< 0.05).

Table 4: Mean value of alkaline RNase activity and specific activity in saliva of control and patient groups.

| GROUP     | Sample Number | Age (year)<br>Mean±SD | Mean±SD                         |                           |
|-----------|---------------|-----------------------|---------------------------------|---------------------------|
|           |               |                       | Activity×10 <sup>3</sup><br>U/L | Specific<br>Activity U/mg |
| Control   | 32            | 35.66±11.64           | 15.60±3.89                      | 7.67±2.23                 |
| Benign    | 14            | 35.78±15.63           | 15.51±9.20                      | 6.62±5.23                 |
| Malignant | 19            | 46.57±15.21           | 15.84±7.27                      | 4.27±2.61*                |

\*Significant difference in comparison to control at (p ≤ 0.01) .

### **Acid RNase activity and specific activity**

The results of sera acid RNase activity (U/L) and specific activity (U/mg) were presented in Table (5) and reflect a non significant difference (P> 0.05) in both activity and specific activity of benign and malignant groups in comparison to that of the control group. The results in Table ( 6) showed the activity and specific activity of acid RNase in saliva of the three studied groups, and reveal a non significant difference ( P>0.05 ) in both activity and specific activity of benign and malignant groups in comparison to that of control.

Table 5: Mean value of acid RNase activity and specific activity in sera of control and patient groups.

| GROUP     | Sample Number | Age (year)<br>Mean±SD | Mean±SD                         |                           |
|-----------|---------------|-----------------------|---------------------------------|---------------------------|
|           |               |                       | Activity×10 <sup>3</sup><br>U/L | Specific Activity<br>U/mg |
| Control   | 32            | 35.66±11.64           | 17.42±4.27                      | 0.34±0.22                 |
| Benign    | 14            | 35.78±15.63           | 24.96±14.89                     | 0.30±0.19                 |
| Malignant | 19            | 46.57±15.21           | 24.65±17.65                     | 0.30±0.20                 |

Table 6: Mean value of acid RNase activity and specific activity in saliva of control and patient groups.

| GROUP     | Sample Number | Age (year)<br>Mean±SD | Mean±SD                         |                           |
|-----------|---------------|-----------------------|---------------------------------|---------------------------|
|           |               |                       | Activity×10 <sup>3</sup><br>U/L | Specific Activity<br>U/mg |
| Control   | 32            | 35.66±11.64           | 5.98±2.20                       | 4.17±1.47                 |
| Benign    | 14            | 35.78±15.63           | 8.30±6.65                       | 4.01±3.20                 |
| Malignant | 14            | 46.57±15.21           | 6.60±5.59                       | 2.64±3.21                 |

### Alkaline RNase isoenzymes

Figure (3) and Figure (4) showed the electrophoretic patterns of the protein and RNase activity respectively of sera and saliva samples among the studied groups. Figure (4) showed that the enzyme present in different forms in sera samples of the studied groups. Reflect the different sources of this enzyme in the sera. These forms were different in their relative mobilities, most of them were with relatively low mobility on the gel. Also the electrozymogram showed that there were RNase's bands with different intensities that moved faster than the others in all sera samples which reflected changes in their molecular weight and charges. When enzyme activity was localized on the gel for the saliva samples, Figure (4) showed that the enzyme presented in one form in saliva samples of each group with different mobility in the gel.

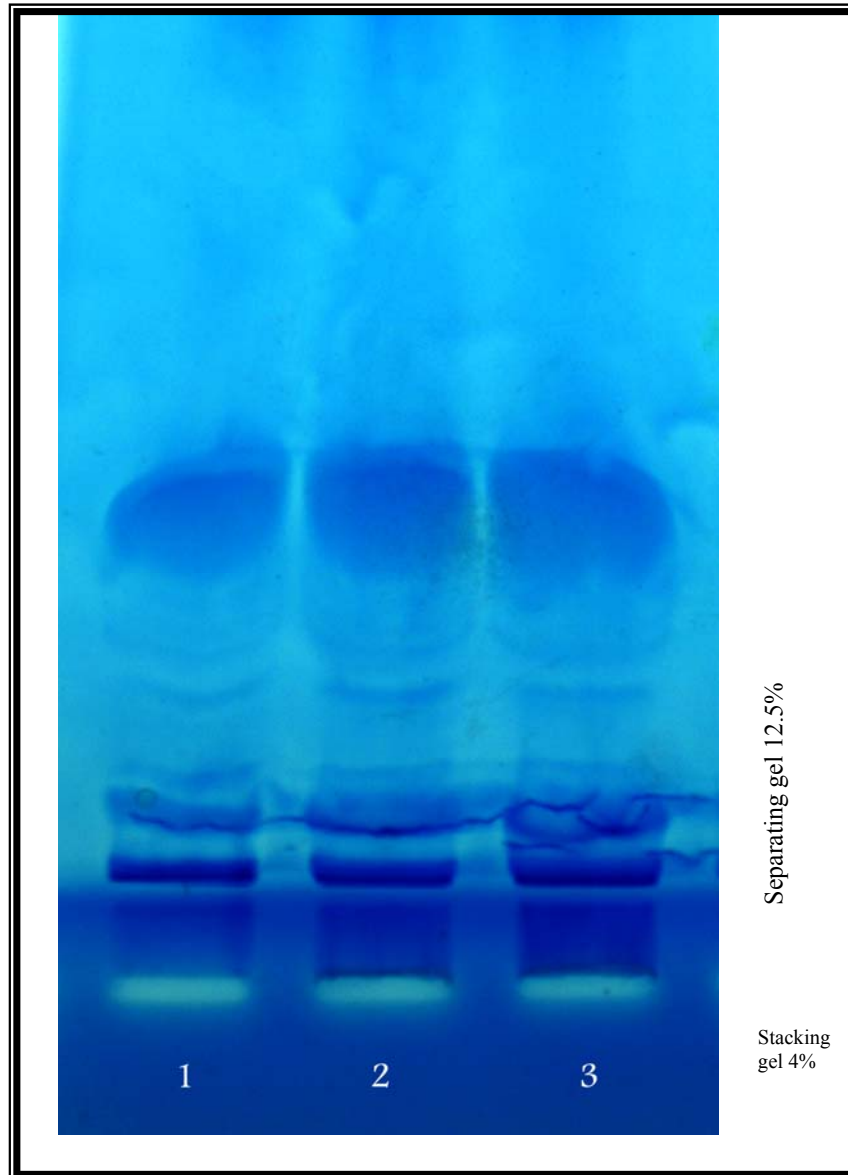
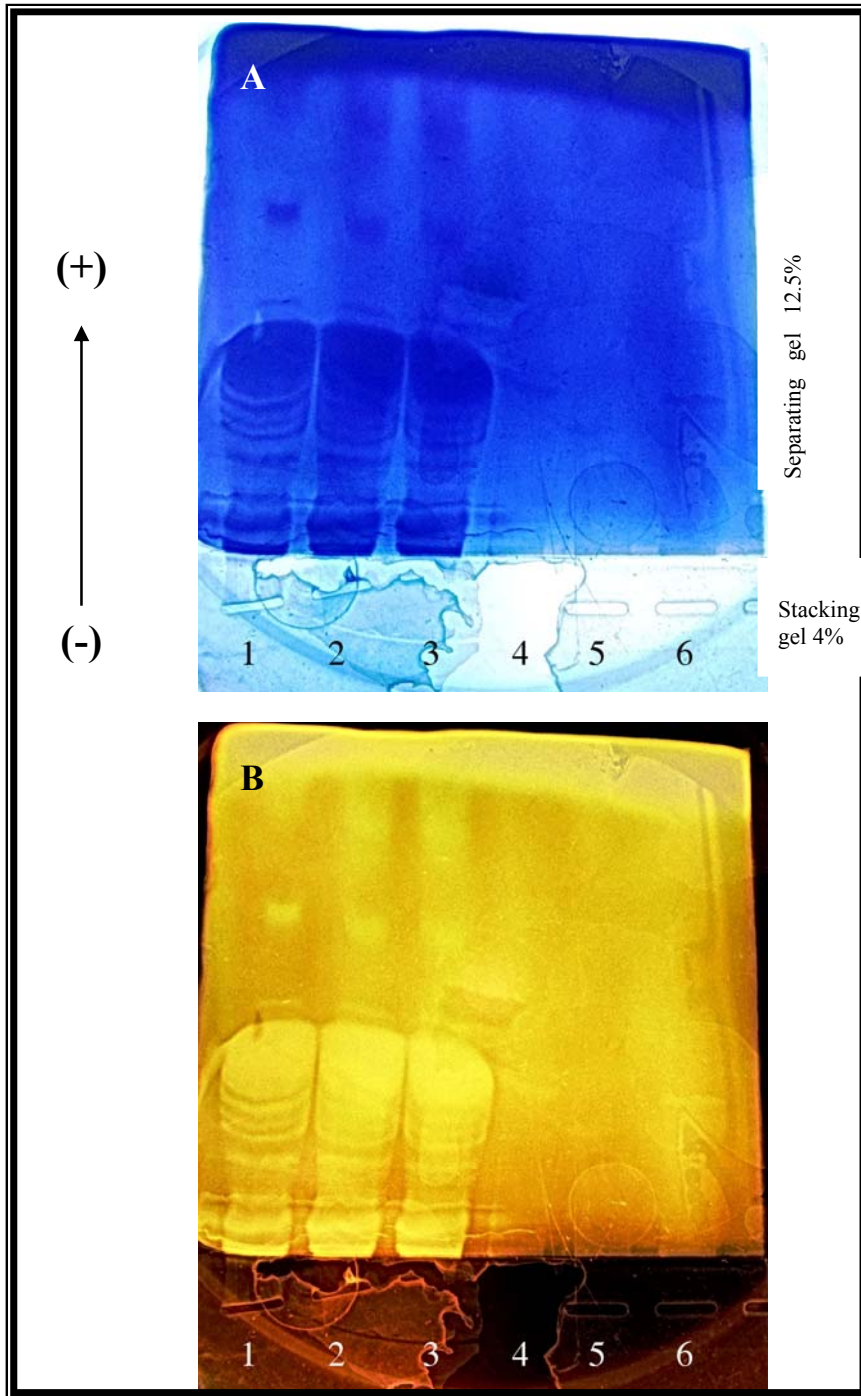


Figure 3: Conventional poly acrylamide gel electrophoresis 12.5%, using tri-glycin pH 8.2 as electrode buffer. The gel was stained for protein. The samples used were as follows:

- 1- pooled crude serum (control)
- 2- pooled crude serum (benign)
- 3- pooled crude serum (malignant)



A- Positive

B- Negative

Fig. 4: Conventional poly acrylamide gel electrophoresis 12.5%, using tri-glycin pH 8.2 as electrode buffer. The gel was stained for alkaline RNase activity. The samples used were as follows:

- |                                   |                                    |
|-----------------------------------|------------------------------------|
| 1- pooled crude serum (malignant) | 4- pooled crude saliva (control)   |
| 2- pooled crude serum (benign)    | 5- pooled crude saliva (benign)    |
| 3- pooled crude serum (control)   | 6- pooled crude saliva (malignant) |

## DISCUSSION

Hyperamylasemia has been reported to occur in many malignancies such as lung, pancreas, and to a lesser extent ovary (Yokoyama *et al.*, 1977, Warshaw *et al.*, 1977) and also with multiple myeloma (Pinlli *et al.*, 2006). The alpha amylase present in sera samples actually is the total amylase i.e. consists of both P-type and S-type. Therefore the elevation of sera alpha amylase (total amylase) may be attributed to the S-type that is present within the sera alpha amylase, which secreted from variety of tissues. Therefore the elevation of sera amylase (total amylase) may not only occur in pancreatic disorders (referred to elevated P-type), but also in mumps, intestinal obstruction and other intestinal disorders and also in cases of malignancies. In addition to its well-known function as a digestive enzyme, salivary alpha amylase has been reported to act as an antimicrobial enzyme (Tenovuo, 1998) and interacts specifically with certain oral bacteria and may play a role in modulating the adhesion of those species to teeth (Scannapieco *et al.*, 1993). In the current study, salivary amylase activity showed a significant decrease in oral tumor patient groups in comparison to that of the control group. This result is in agreement with other (Shpitzer *et al.*, 2007) on their study on Oral Squamous Cell Carcinoma (OSCC) patients and disagrees with Bssalyk (Bassalyke *et al.*, 1992) where they observed a significant increase in salivary alpha amylase in patient with malignant tumors of the oral mucosa. . It has been suggested that amylase make up about 1/3 of the total protein content in parotid saliva (Pederson *et al.*, 2002) so amylase is thought to be an indicator of acinar cell function in parotid glands (Rantoneu, 2003). It has been found earlier that amylase increases with saliva flow rate, and it is generally considered to be a reliable marker of serous cell function (Almstahl, 2001). The observed reduced amylase activity in this study may be due to the low salivary flow rate noticed in almost all the patients under the present study. The altered concentration and activity of various enzymes, electrolytes, and ions in saliva may compromise various salivary functions, so the reduced salivary amylase activity may impair salivary digesting ability (Shpitzer *et al.*, 2009) and indicate dysfunction in parotid glands.

The separation of amylase isoenzymes by gel electrophoresis has been shown to be reliable and reproducible (Rantoneu, 2003). The differences in sera and saliva amylase activity was confirmed by the electrophoretic profile (Figure 1) and (Figure 2) respectively, where it showed that the sera amylase consist of P-type and S-type which they differ in their mobility toward the anode. The increased isozymes referred to the secretion of S-type from variety of tissues including malignant one. Salivary amylase zymogram showed a decreased in bands number and intensity in benign and malignant groups when compared with the control group, and this may due to the dysfunction of parotid gland which is the major source of salivary alpha amylase in the saliva.

Throughout this study, acid and alkaline RNases activities were measured in sera and saliva samples of control and oral tumor patient groups. The results reveal that oral tumor patient groups have highly significant increased RNase activity in sera samples. The observed increased in sera alkaline RNase is in agreement with many other studies in different kinds of cancer such as ovarian cancer (Al-Shaikhly, 2004), pancreatic cancer (Ester, 2000), , and uterine cancer (Al-Shamarre, 2002). It have been suggested by a number of authors that the levels of

RNase activity in human body fluids, such as serum, urine, and saliva may be an indicative of disease states (Park *et al.*, 2006). Generally it is known that cancer cells are characterized by uncontrolled increase in the number and size (Sano *et al.*, 2007), which mean that there will be an increase in the synthesis of different proteins and this explain the need for high RNase activity. Although many human tissues express ribonucleases, the origin of the serum RNase has not been established (Tran *et al.*, 2007). The increase in RNase activity may be attributed to many reasons such as the lack of host defense mechanisms, or it may be produced by malignant cells, a secondary destructive process in other cell or tissue, and this is confirmed by the appearance of different bands on the gel which have different mobilities in the acrylamide gel.

RNase activity in saliva during this study was also investigated, and the results revealed a non significant increase in RNase activity. There are some reports on human RNase which indicate the presence of an elevation in the activity of this enzyme in saliva of patients with different diseases such as cystic fibrosis (CF) (Bardon, 1980), influenza, ovarian and breast tumors (Al-Shaikhly, 2004). Actually little is known about human salivary RNases and its molecular nature and properties (Li *et al.*, 2004). Saliva is known to contain ribonucleases from various sources (Westra *et al.*, 2004). It is not clear how RNA and RNase can coexist in the saliva, RNA can enter the oral cavity through various routes, including saliva secretions from the three major salivary glands and minor glands, like many micro and macro molecules, RNA in salivary glands secretions could originate from acinar cells (Aps *et al.*, 2005), or from Gingival Crevice Fluid (GCF). Many blood cells and their cellular components are released into the oral cavity from GCF (Delima *et al.*, 2000), or from desquamated oral epithelial cells, where turnover of epithelial cells in the oral cavity could be another source of RNA (Hu, 2006). Also micro wounds inside the oral cavity could release the RNA directly from the blood (Park *et al.*, 2006). The cause of such elevation in activity may be attributed to the alteration in cell permeability of tumor cell membranes where a number of changes in the biochemical characteristic of malignant cell surface have been observed. Such changes lead to transport of enzymes from the blood to the saliva via salivary glands, and one of these enzymes is RNases. Furthermore, some divalent cations or other parameters that activated the RNase may be transported from the blood to the saliva with the disease such as cystic fibrosis (Bardon, 1980), and this increase in their level in saliva, may lead to the observed elevation in salivary RNase. Salivary RNA is protected from degradation by the association with macromolecules (Park *et al.*, 2006). Saliva contains mucin (proteins that forms oligomers which are highly glycosylated) (Lamblin *et al.*, 2001), and it is possible that in saliva some RNA is protecting from degradation by their association with these oligomers. This may be the reason for the reduction of salivary RNase specific activity. The electrophoretic profile of RNase in sera and saliva samples Figure (4) reflect the increase in RNase activity in the oral tumor patient groups, where it is obvious the presence of increased isoenzymes in benign and malignant groups and also these isoenzymes (forms) are different in their mobility in the gel, that mean different in their molecular weight.

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