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## Serum Ribonuclease Level (RNase) as Tumor Marker in Patients with Thyroid Cancer

Manal Kamal Al-Ani  
MSc

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**Back ground:** Evidence is presented to show that most patients with thyroid cancer have an increased level of serum RNase, assayed with the synthetic compound, (yeast RNA). In addition, the results suggest that the enzyme level may be useful indicator of response to chemotherapy with  $I^{131}$ . Preliminary results of enzyme levels in the serum of patients with thyroid cancer that was high are due to excessive entry of RNase into the serum.

**Objective:** 1- measure RNase levels in serum of patients with thyroid cancer, and to point out any relationship with these disease by comparing its level with that of healthy individuals (control group).

2-Study the possibility of using RNase as a tumor marker for thyroid cancer.

**Subjects and Methods:** A total of 100 subjects were enrolled in this study, they were 50 healthy individuals (control group) and 50 patients with thyroid cancer (patients group). The thyroid cancer patients treated by surgery and with or without radioactive iodine  $I^{131}$ . The latter subdivided into three other subject groups categorize according to the remnant tissue after thyroidectomy.

**The method** it is possible to measure the RNase reaction by observing in absorbance of UV light of wavelengths at 260 nm in reaction solution, the rate of hydrolysis of yeast RNA at PH 7.6 is determined by measuring the amount of acid soluble oligonucleotide liberated under defined condition.

**Results:** There was a significant elevation in serum RNase leveling patients with thyroid cancer as compared to healthy individual's control, while the level of serum RNase in patients after thyroidectomy decrease deepened on the remnant tissue after surgery and who exhibited clinical response to radioactive iodine therapy.

**Conclusion:** This simple available method might aid to use it as a biochemical marker in detection and follow up during treated of thyroid cancer.

**Key word:** Thyroid cancer, RNase.

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### Introduction:

Many literatures have been published concerning serum RNase by using of RNA substrates in the enzyme assays. The results represent summation of enzymes with varying specificities acting on yeast RNA at an average optimal pH. Spectrophotometric determination of acid soluble reaction products has been made at 260 nm<sup>[1]</sup>.

RNases, are probably the oldest enzymes, they are logical regulatory molecules<sup>[2]</sup>. RNase may be defined as any of group of nuclease enzymes that cleave phosphodiester bonds in chain of RNA<sup>[3]</sup> RNase is a very important enzyme for RNA metabolism in almost all organisms. They include those that hydrolyze only single – stranded RNA, double -stranded RNA, and RNA hybridized with DNA<sup>[4,5]</sup>, which catalyses the hydrolysis of polyribonucleic acid to their component nucleotides<sup>[6]</sup>. Thyroid hormones, bind it self to specific high – affinity receptors in the target cell nucleus<sup>[7]</sup>.

A major effect of  $T_3$  and  $T_4$  is to induce or repress proteins by increasing or decreasing gene transcription, by binding to specific

intranuclear receptors. This ligand –receptor complex, binds to thyroid hormone, response element in target genes to regulate the rate of synthesis of specific mRNA<sup>[8]</sup>. This result's in a change in amount and activity of conjugate protein, which in turn alters the rate of a metabolic process<sup>[9]</sup>. In hyperthyroidism there is an increase in the metabolism then the rate of protein degradation in liver and skeletal muscle. This is decreased in the hypothyroid state<sup>[10]</sup>, due to increase the levels of the RNase in hyperthyroidism, and decrease in hypothyroidism. Alteration in nucleic acid metabolism have been demonstrated in a variety of malignant conditions, and it was reasonable to suppose that increased synthesis and turnover of nucleic acid RNA might be associated with the enhanced protein synthesis in the abnormal proliferating plasma cells in multiple myeloma<sup>[11]</sup>.

### Patients & Methods:

#### Patients:

In this work 100 subjects were concluded. The age range was between 16 – 75 years, with a mean age was (33±9.77) years; 50 healthy

individuals were considered as control group, with a mean age was (28±9.93) years. The 50 patients (15 male, 35female) with histologically confirmed thyroid cancer were selected for single serum sample study. These patients who treated by surgery and received radioactive iodine 131 (after withdrawal of thyroxin one month ago) in Instituted of Radiology and Nuclear Medicine either one therapy dose or multiple doses . All cases were selected after clinical diagnosis with history, and physical examination of thyroid gland by Nuclear Medicine Specialists " which is reported in special formal (Questionnaire)" .All cases underwent in vitro and in vivo thyroid function tests (T<sub>3</sub>, T<sub>4</sub> and TSH) utilizing radioimmunoassay method and using a commercial kit (competitive double antibody RIA technique) and Nuclear Enterprises NE-1600 gamma counter . Thyroid patients were excluded if they had significant complication such as hypertension, diabetes mellitus and were taking medication specifically known to affect the results of this study.

#### Methods:

The RNase assays were perfumed with yeast RNA as a substrate utilizing an adaptation of the method reported by Umeda in 1969<sup>[12]</sup>. For estimation of RNase . Dilutions of serum (1 in 5 DW) were made in polyethylene test tubes with 0.1 ml of ( Tris buffer PH=7.6,0.2 M) and 0.2ml of (1 gm RNA substrate 1%) . The mixture was incubated for 20 min. at 37 °C , cooled to 0 °C, and mixed with an equal volume of solution (1ml HCL in 70% ethanol ).After 1 hour, the specimens were centrifuged and the supernatant filtered. A solution was read at 260 nm. Blank was treated as above except water (0.2 ml) was added instead of enzyme.

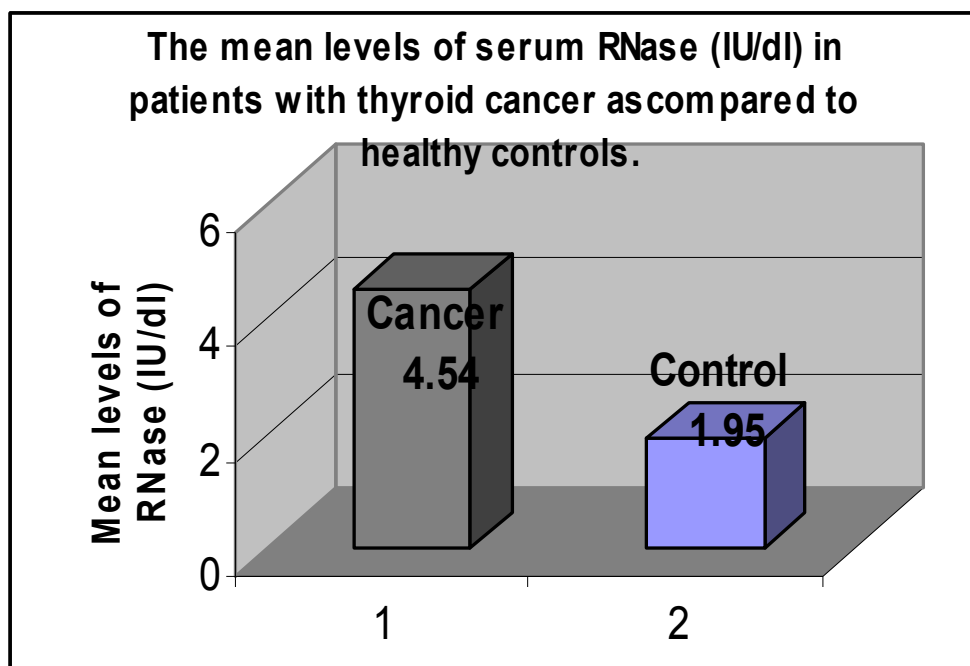
#### Results:

RNase in normal and in thyroid cancer serum . Results of serum RNase determinations in normal adults and patients with untreated thyroid cancer are given in table I fig.1.

The mean level observed in the thyroid cancer series was more than two and half times that of the normal. The difference between the means is highly significant statistically (p<0.0001).

**Table I: Serum RNase levels (IU/dl) in patients with thyroid cancer and in healthy individual's control.**

|                    | Patients  | Control    |
|--------------------|-----------|------------|
| <b>Sample size</b> | 50        | 50         |
| <b>Mean ±SD</b>    | 4.54 ±2.4 | 1.95± 0.14 |
| <b>t-test</b>      | 8.93      |            |
| <b>Probability</b> | P<0.0001  |            |



**Figure 1: The serum RNase levels in patients with thyroid cancer as compared to healthy individual's control.**

The serum RNase levels in patients with thyroid cancer who treated by thyroidectomy and with or without radioactive iodine<sup>131</sup> were instituted significant decrease table II fig. 2. Treated thyroid cancer subdivided into three groups depended on the remnant tissue after surgery fig.3.

1- Data obtained in a patients who intact thyroid gland after surgery (two lobes).

Table II Fig.3 shows that mean of serum RNase levels in the (18 patients) were (Mean 2.9 IU/dl  $\pm$  SD 0.5). The difference between the means to normal is highly significant statistically ( $p < 0.0001$ ).

2- With lobectomy (one lobe):

Table II Fig.3 shows that mean of serum RNase levels in the (16 patients) were (Mean

2.27 IU/dl  $\pm$  SD 0.29). The difference between the means to normal is highly significant statistically ( $p < 0.0001$ ), and the difference between the means of one lobe to two lobes is significance ( $p < 0.001$ ).

3- Data obtained to a patients who exhibited a good clinical response to total thyroidectomy and iodine 131 therapy are shown in table II Fig 3 shown that the mean serum RNase levels in the (16 patients) were (Mean 2.01 IU/dl  $\pm$  SD 0.29). The difference between the means to normal is non significance statistical ( $p > 0.05$ ). But the difference between the means of no thyroid tissue to two lobes is highly significance ( $p < 0.001$ ).

Table II : The levels of serum RNase in patients with thyroidectomy.

|             | Intact thyroid gland(Tow lobe) | With lobectomy(one lobe) | Total thyroid (no thyroid tissue) |
|-------------|--------------------------------|--------------------------|-----------------------------------|
| Sample size | 18                             | 16                       | 16                                |
| Mea± SD     | 2.9± 0.5                       | 2.27± 0.29               | 2.01± 0.29                        |
| t-test      | *18                            | **20.3<br>***6.02        | ****8.9<br>*****3                 |
| Probability | * P< 0.0001                    | **P<0.0001<br>***P<0.001 | ****P<0.001<br>*****P>0.05        |

\*Intact thyroid gland x with healthy control

\*\* Lobectomy x healthy control.

\*\*\* Intact thyroid gland x with lobectomy

\*\*\*\* Total thyroid x intact thyroid gland.

\*\*\*\*\* Total thyroid x healthy control

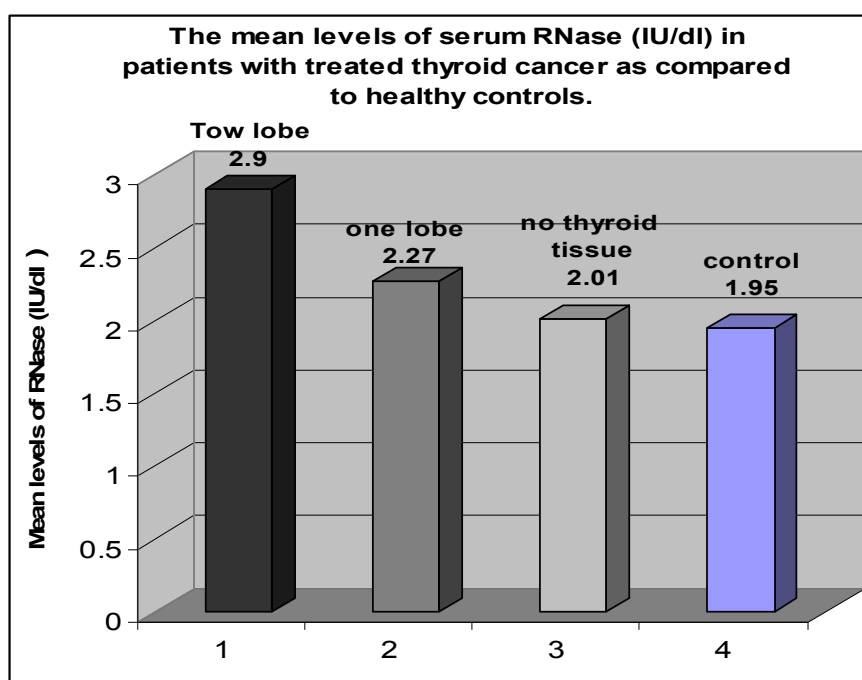
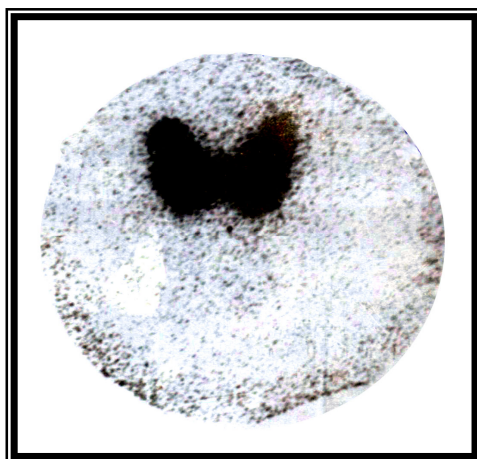
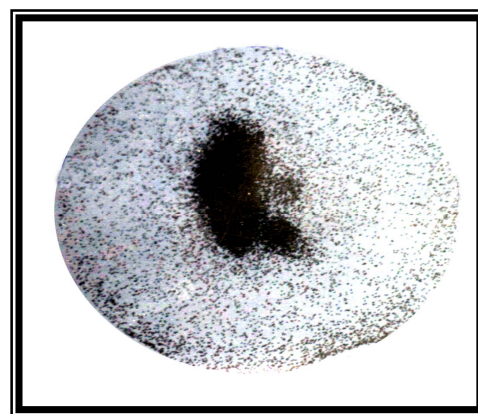
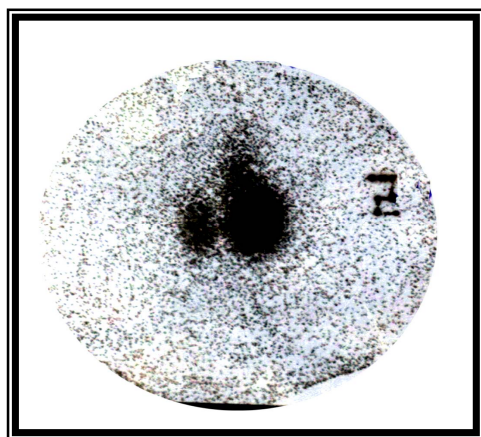


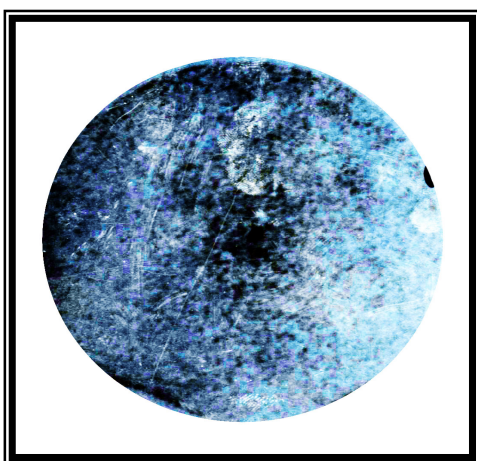
Figure 2: The levels of RNase (IU/dl) in patients with thyroidectomy



**A: Intact thyroid gland after surgery "two lobe"**



**B: With lobectomy "one lobe"**



**C: With total thyroidectomy "no thyroid tissue"**

**Figure 3(A,B,C): Iodine I<sup>131</sup> thyroid scan in treated cancer patients**

**Discussion:**

The increase of RNase level in patients with thyroid cancer is probably due to the increase of metabolism caused by increase production of mRNA resulting from augmented gene transcription, proliferation of ribosomal constituents involved in protein synthesis, and increase in translation efficiency. These finding in our study are in agreement with published literature by Oppenheimer., 1985<sup>[13]</sup>.

Elevated levels of serum RNase have been related to a bewildering variety of diseases, with several mutually exclusive claims that RNase is highly specific biochemical parameter. There are many potential reasons for this conclusion, among them the stability and easily detection of the enzyme. Increased level of RNase in cancer patients were noted by several investigations about four decades ago.<sup>[14,15]</sup>, nevertheless, only few systematic attempts to determine the usefulness of the assay in clinical oncology had appeared. This was because serum RNase elevation was not found in all studies of cancer patients, the source and biological activity of enzymes have not been clarified<sup>[16]</sup>.

**The increase in the level of serum RNase may be due to one of the following reasons<sup>[17]</sup>:**

- 1- General increase in all type of RNases.
- 2- Malignant de novo synthesis of one or several RNase, not present in a healthy body.
- 3-Increased expression of few physiologically present RNase.

Our result supported the first and second reasons.

In patients treated thyroid cancer, the increase serum RNase activity was due to the increased metabolism which depended on remnant of cells after thyroid surgery which killed or stop their activity by different doses of I<sup>131</sup> radioactive.

**Two factor effect:**

Remnant tissue after surgery, and number of iodine 131doses delivered to the patients as treatment. We can decide the optimum results from thyroid surgery and I<sup>131</sup> treatment according to RNase levels. Moreover it even had good surgery and number of iodine 131doses we noted different of RNase and we think this finding due to thyroxin medication.

**In Conclusion:**

RNase is much more simple, less expensive and they can:

- a- assist in the diagnosis and classification of thyroid cancer after surgery according to

remnant tissues b- They may be useful as a marker of thyroid cancer activity during follow-up of patients under treatment.

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