Evaluation of Serum Total Sialic Acid (TSA),Total Protein(TP) Levels in Patients with Thyroid Cancer

Manal Kamal Rasheed AL-Ani.
M.Sc.

-Background: It has been repeatedly observed increased concentration of sialoglycoproteins on tumor cells. Serum TSA and TSA/TP may serve as a valuable tumor marker for a variety of malignant neoplasms.

Objective: The present study was applied to determined potential clinical application of TSA and TSA/TP in the evaluation of patients with thyroid cancer in terms of severity of disease and the response of patients to the chemotherapy after thyroidectomy and to compare it with normal subjects.

Subjects & methods: A total of 100 subjects were enrolled in this study, they were divided into two groups: 50 healthy individuals (control group) and 50 patients with thyroid cancer (patients group). These patients were treated by surgery and multimodality therapy, including debulking operation, polychemotherapy and radiation therapy. Treated thyroid cancer patients were subdivided into three groups according to the remnants tissues after thyroidectomy.

Results: There was a significant elevation in serum total sialic acid and TSA/TP in patients with thyroid cancer when compared to healthy individual’s (control). While a significant decrease in level of serum TSA and TSA/TP after thyroidectomy was observed. Patients underwent thyroidectomy revealed variation in serum TSA and in TSA/TP levels depending on remnant tissues after surgery.

Conclusion: This simple available methods might aid in detecting the activity of thyroid cancer. They may be useful as marker of thyroid activity during follow-up of patients under treatments.

Key word: Thyroid cancer, TSA, TP.

Introduction:
Thyroid cancer is more common in female, with an increased incidence in patients who received radiation therapy applied to the head and neck[1]. Primary thyroid malignancy is rare occurring for less than 1% of all carcinomas. Thyroid carcinomas are histologically divided into: Well differentiated papillary or follicular and poorly differentiated anaplastic types[1].

* Papillary Carcinoma is the most common type of thyroid carcinoma and accounts for 60% of all thyroid cancers[2] and 90% of irradiation induced thyroid cancer. It is associated with local invasion and lymph node spread. Poor prognosis is associated with thyroid capsule invasion, a size more than 2.5 cm, an age of onset more than 45 year and lymph node involvement[2]. Many papillary carcinoma secrete thyroglobulin, which can be used as a marker for recurrence or metastases of the cancer[2].

* Follicular Carcinoma is slightly more aggressive than papillary carcinoma and it accounts of 20% of all thyroid malignancies[4,5].

* Anaplastic Carcinoma occurs in older individuals and is very aggressive leading to rapid enlargement of thyroid gland over 2-3 months, presenting with pain, dysphagia and hoarseness[4]. Sialic acid may be present in human body in three forms: free Sialic acid (FSA), presents in trace amount in serum, protein associated Sialic acid (PASA) as a terminal sugar of glycoprotein in the cell membrane and other cell component, and lipid associated Sialic acid (LASA), which represents lipid bound Sialic acid present in glycolipids[8]. Serum or plasma Total Sialic Acid (TSA) and Lipid Bound Sialic Acid (LSA) are useful markers for human cancer[9]. Sialic acid levels have been demonstrated in various types of human cancer including brain, thyroid, and Hodgkin’s[8]. The results show that Sialic acid can be used as a beneficial marker for detecting malignancies, but it cannot be used as criteria for identifying tumor types[8].

SA’s are universal component of membrane glycoprotein occupying terminal site, and SA residue is the most abundant terminal carbohydrate residue of mammalian cell[9]. The unique structural features of this molecule, which include a negative charge owing to a carboxyl group, enable it to play an important role in cellular functions, such as cell–cell repulsion, transportation of positively charged compounds and tumor cell metastasis[10].

These Sialic acids apparently enter the circulation by either shedding or cell lyses are of considerable interest because of their potential diagnostic value[10,11]. SA’s level in serum is found to be closely related to the stage of some disease, such as cancer, collagen disease, inflammatory disease[12,13,14].

TSA/TP: When TSA is normalized to total serum protein (TP), many cancer patients exhibit significant increased TSA/TP values[15]. Snyder and Ashwell[16] reported significant difference in
carbohydrate/protein ratio for cancer patients as compared with normal subjects and patients with non malignant disease (renal, rheumatic fever or infectious diseases). The concentration of both protein-bound carbohydrate and serum TP are increased, whereas, in the cancer patients protein-bound carbohydrate was increased, but values for serum TP were normal or lower\cite{16}.

**Patients & methods:**

**Patients:**

In this work 100 subjects were included. Their age ranged between 16-75 years, with a mean age was \((33 \pm 9.77)\) years; 50 healthy individuals were considered as control group, with a mean age was \((28 \pm 9.93)\) years. The 50 patients (17male, 35female) with histologically confirmed thyroid cancer were selected for single serum sample study. These patients who were treated by surgery and received radioactive iodine 131 (after withdrawal of thyroxin one month ago) in the Institute 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 under went in vitro and in vivo thyroid function tests \((T_3, T_4 \text{ and TSH})\) 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:**

1- **TSA Determination:**

   The principle of this adopted method depends on the formation of chromogen on addition of resorcinol reagent into the test tube\cite{17}.
   1- Resorcinol stock (2% w/v).
   2- \(\text{CuSO}_4\) (0.1 M): prepared by dissolving 4 gm of the salt in 250 ml distilled water.
   3- \((\text{Resorcinol – HCL})\) reagent: 10 ml of the stock + 9.75 distilled water + 0.25 ml 0.1 M \(\text{CuSO}_4\), the volume was completed to 100 ml with concentrated HCL (prepared daily).
   4- Butyl acetate /Methanol (85:15) reagent:
   5- Standard Sialic Acid: The standard Sialic acid different concentrations \((0 – 38)\ \mu g / ml\) were prepared by serial dilution with distilled water.

**Procedure:**

1- 20 \(\mu l\) of sample or standared Sialic acid solution and 980 \(\mu l\) of distilled water placed in test tubes, vortexes and placed on ice.
2- To each assay tube, 1 ml of resorcinol reagent was added, and placed in a 100 C°. Boiling water bath for exactly 15 minutes flowed by 10 minutes on ice bath.
3- 2 ml of butyl acetate / methanol (85:15 v/v) was added to each tube, then vortexes and centrifuged for ten minutes at 3000 rpm .The extracted chromophore was read at 580 nm.
4- The standard curve was prepared and used for determination of Sialic acid \cite{18} fig 1.

![Graph](image.png)

**Figure 1:** Standard curve for determination of Sialic acid concentration in human sera
2. Total protein Determination:
Peptide bond of the protein reacts with cupric ion (Cu\(^{2+}\)) in alkaline medium to form colored product whose absorbency is measured at 540 nm \(^{[18]}\).

Results:
Table 1 shows that serum TSA levels in patients with thyroid cancer (Mean 80.48 mg/dl ± SD 10) as compared to healthy controls (Mean 56.06 mg/dl ± SD 6.5), the difference was significance (P<0.0005).

| Table 1: Serum TSA levels (mg/dl) in patients with thyroid cancer and in healthy individuals control. Serum total Sialic acid (mg/dl). |
|---|---|---|
| | Patients | Controls |
| Sample size | 50 | 50 |
| Mean ±SD | 80.48 ±10 | 56.06± 6.5 |
| t.test | 15.26 |
| Probability | P<0.0005 |

Figure 2: The mean levels of TSA (mg/dl) in patients with thyroid cancer as compared to healthy individuals.
Serum TSA/TP level was measured to evaluate its usefulness in diagnosis and follow of patients with thyroid cancer Table 2, Figure 3. Table 2 Figure 3 shows that serum TSA/TP levels in patients with thyroid cancer (Mean 10.58 mg/gm ± SD 0.4) as compared to healthy controls (Mean 8.01 mg/gm ± SD 0.54), the difference was significance (P<0.0005).

Table 2: Serum TSA/TP levels in patients with thyroid cancer. Serum TSA/TP level: mg/gm.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>10.18±0.4</td>
<td>7.5 ±0.54</td>
</tr>
<tr>
<td>t.test</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Probability</td>
<td>p&lt;0.0005</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: The mean levels of TSA/TP in patients with thyroid cancer.

Table 3 Figure 4 shows levels of TSA in the patients with thyroid cancer treated by surgery and with or without radioactive iodine 131 are divided into three group according to remnant tissue after surgery Figure 5 were:

1. Intact thyroid gland after surgery (two lobe)
   Serum TSA levels were reduced (Mean 30.3 mg/dl ± SD4.118) were compared to healthy individuals control (Mean 56.06 mg/dl ± SD6.5), the difference was significance(p<0.0001).
2- With lobectomy (one lobe):
Serum TSA levels were reduced (Mean 23.3 mg/dl ± SD2.7) were compared to healthy individuals control (Mean 56.06 mg/dl ± SD6.5), the difference was significance(p<0.0001). the serum TSA levels of 18 patients with treated thyroid cancer (tow lobes) when compared with 16 patients treated thyroid cancer (one lobe) , the difference was significance(p<0.000001).

3- Total thyroidectomy (no thyroid tissue):
Serum TSA levels were reduced (Mean 20.11 mg/dl ±SD1.61) were compared to healthy individuals control (Mean 56.06 mg/dl ±SD6.5), the difference was significance(p=0.0049). The serum TSA levels of 18 patients with treated thyroid cancer (no thyroid tissue) when compared with 16 patients treated thyroid cancer (one lobe) , the difference was significance(p<0.0001).

Table 3 : The levels of serum TSA (mg/dl) in patients with thyroidectomy.

<table>
<thead>
<tr>
<th></th>
<th>Intact thyroid gland(Tow lobe)</th>
<th>With lobectomy (one lobe)</th>
<th>Total thyroid (no thyroid tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>18</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Mean± SD</td>
<td>30.3± 4.118</td>
<td>23± 2.76</td>
<td>20.11± 1.61</td>
</tr>
<tr>
<td>t-test</td>
<td>*35.88</td>
<td>***20</td>
<td>***9.78</td>
</tr>
<tr>
<td></td>
<td>**9.7</td>
<td></td>
<td>*****12.7</td>
</tr>
<tr>
<td>Probability</td>
<td>* P&lt; 0.000001</td>
<td>***P&lt;0.0001</td>
<td>****P&lt;0.0049</td>
</tr>
<tr>
<td></td>
<td>**P&lt;0.0001</td>
<td></td>
<td>*****P&lt;0.0001</td>
</tr>
</tbody>
</table>

*Intact thyroid gland x with lobectomy.  
*** Lobectomy x healthy control  
**** Total thyroid x healthy  
***** Total thyroid x Intact thyroid gland.

Figure 4: The mean levels of TSA in patients with treated thyroid cancer as compared to healthy individuals control.
Thyroid cancer, TSA, TP.

Manal Kamal Rasheed AL-Ani


A: Intact thyroid gland after surgery "two lobe"

B: With lobectomy "one lobe"

C: With total thyroidectomy "no thyroid tissue"

Figure 5 (A,B,C): Iodine $^{131}$ thyroid scan in treated cancer patients

Table 4 Figure 6 demonstrate the mean ±SD of serum TSA/TP.

Levels in patients with thyroidectomy which treated by surgery and with or without radioactive iodine 131 are divided into three group according to remnant tissue after surgery were: (Figure5)

1- Intact thyroid gland after surgery (two lobe):
   Serum TSA/TP levels were reduced (Mean 4.3 mg/gm ± SD1.5) were compared to healthy individuals control (Mean 8.04 mg/gm ± SD0.54), the difference was significance (p<0.005).

2- With lobectomy (one lobe):
   Serum TSA/TP levels were reduced (Mean 3.0mg/gm ± SD0.8) were compared to healthy individuals control (Mean 8.04 mg/gm ± SD0.54), the difference was significance (p<0.0005). The serum TSA /TP levels of 18 patients with treated thyroid cancer (tow lobes) when compared with 16 patients treated thyroid cancer (one lobe), the difference was not significant (p>0.05).

3- Total thyroidectomy (no thyroid tissue):
   Serum TSA/TP levels were reduced (Mean 2.5 mg/gm ± SD0.6) were compared to healthy individuals control (Mean 8.04 mg/gm ±SD0.54), the difference was significance (p=0.0049). The serum TSA/TP levels of 18 patients with treated thyroid cancer (no thyroid tissue) when compared with 16 patients treated thyroid cancer (one lobe), the difference was not significant (p=0.04).
Table 4: The levels of serum TSA/TP (mg/gm) in patients with thyroidectomy:

<table>
<thead>
<tr>
<th></th>
<th>Intact thyroid gland (Tow lobe)</th>
<th>With lobectomy (one lobe)</th>
<th>Total thyroid (no thyroid tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>18</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Mea± SD</td>
<td>4.3±1.5</td>
<td>3.0± 0.8</td>
<td>2.5± 0.6</td>
</tr>
<tr>
<td>t-test</td>
<td>*3.2</td>
<td>***11</td>
<td>****4.73</td>
</tr>
<tr>
<td>Probability</td>
<td>*NS (p=0.08)</td>
<td>***P&lt;0.005</td>
<td>*****NS (p=0.04)</td>
</tr>
</tbody>
</table>

*Intact thyroid gland x with lobectomy.  **Intact thyroid gland x healthy control.  ***lobectomy x healthy control.  ****Total thyroid x lobectomy.

Figure 6: The mean levels of TSA/TP (mg/gm) in patients with thyroidectomy as compared to healthy individual’s control.

Discussion:
The documentation of alterations in the metabolism of cell surface glycoproteins and sialoglycolipids has encouraged the study of Sialic acid as a possible biochemical marker in blood of patients with thyroid diseases [19]. In more general studies, serum TSA level has been found to be elevated in malignant diseases when compared to normal healthy individual controls [20], and that it’s level correlated with clinical course of malignant disease [21]. A serious limitation of procedures was the findings that Sialic acid levels were also elevated in non malignant conditions such as inflammatory or chronic diseases resulting in false positive findings [22].

The results presented in this study revealed highly significant elevation in serum TSA leveling thyroid cancer patients when compared to healthy individual’s control, these findings were in agreement with findings reported by Kokoglu,E,1992 [23]. Two possible sources of an increased serum TSA concentration in malignant tumors were currently being considered: first an intensified output of tumors cells with high content of Sialic acid caused disintegration higher cell metabolism- [24] are related to increase binding of SA residues with glycoprotein of the cell surface and membrane[24] and secondly, the high turn over tumor cells and the consequently increased release supposedly cause high SA concentration in serum [23]. In patients with treated thyroid cancer serum TSA levels revealed a decrease [table 4 figure 6] which depended upon presence or absence of cells after thyroid surgery, and number of radioactive iodine. Increase metabolism of remnant cells lead to TSA level decrease due to increase depolymerisations of glycoprotein of cell membrane and accelerated secretion of glycoprotein with urine this different from hypothyroidism (medical treatment), and euthyroid [23]. These results were in agreement with the findings reported by Uniyal,1998 [25].

Plucinsky,1986 [26] suggested that TSA/TP was a good marker, although it is quite sensitive , its lack specificity since its a significant increase in TSA and TSA/ TP as disease progress, suggesting that this marker may be useful in monitoring therapeutic intervention and disease progression.
Thyroid cancer, TSA, TP.

The present results c/w (concentration /weight) with this findings, TSA/TP increased significantly in cancer patients as compared to healthy individual's control.

These data revealed that serum TSA/TP levels are significantly higher in patients with thyroid cancer. The high levels of TSA/TP at the onset of disease, although decreasing during clinical remission, this result is in agreement with those reported by Feijoo [26].

In conclusion:

a- assist in the diagnosis and classification of thyroid cancer after surgery according to remnant tissues (two lobe, one lobe, no thyroid tissue)

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

References:
1-Christopher R.W. Edwards , IANA.D.Bowchier ; David Son’s principles and practice of medicine; 1993,P:537.
2- Anderoli , Bennett, Charpenter ,Plum ; Simith ;
9-Schumacher — U; Mukhtar –D ; Stehling-P; Reutter- W. Is the lectin binding pattern of human breast and colon cancer cells influenced by modulators of sialic acid metabolism? Histochem –cell –Biol;1996, 106 (6).
12-Yoko Sugawara , Masuo I wamori, Jaceewes Portoukul and Yoshitaka Nagai : Determination of N-acetyl neuraminic acid by gas chromatography . Mass spectrometry with as

22- Mark, C., Plucinsky, W.; Micheal Riley; Joseph. J.Prorok;: Total and lipid associated serum sialic levels in cancer patients with different primary sits and different degree of metastatic in volument. Cancer 1986,58 P: 2580-2685 ..
23- Kokoglu E, H Snmez, Euslu and Iuslu , Cancer Biochem . Biophys. : Sialic acid levels in various types of cancer, 1992, 13 (1) P: 57-64,