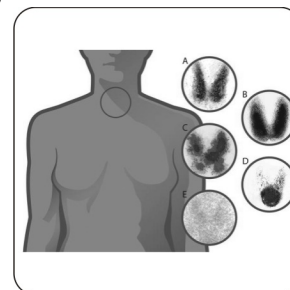


ORIGINAL

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# SERUM AND TISSUE MUCOPROTEINS AND SIALIC ACID IN THYROID DISEASES

**DR. ZARFAR IQBAL**

Associate Professor,  
Department of Biochemistry  
Kabeer Medical College, Peshawar.

**DR. TASNEEM ZAFAR**

Associate Professor,  
Department of Biochemistry  
Wah Medical College, Wah Cantt.

**ABSTRACT... Objective:** To find the correlation coefficient of serum mucoproteins and sialic acid to thyroid tissue in thyroid diseases. **Study Design:** The study was carried out at J.P.M.C, Karachi. **Materials and Methods:** A total of twenty four thyroid patients and twenty normal subjects were studied. The cases were divided into non toxic goiter fourteen, and toxic goiter ten on the basis of clinical and physical examination, thyroid scanning and hormone analysis. Thyroid hormones were estimated in serum. Mucoproteins and sialic acid were estimated in serum as well as in thyroid tissue. **Results:** Estimations of thyroid hormones and thyroxine binding globulin (T.B.G.) are useful clinically to evaluate the functions status and diagnosis of different thyroid disorders. Thyroid tissue is mainly composed of glycoprotein, thyroglobulin, and different studies showed that serum glycoprotein fractions varied in different thyroid diseases. Significantly increased levels of  $T_4$  were observed in toxic goiter group. The levels of TBG was significantly increased in non toxic goiter, toxic goiter patients while  $T_4$ /TBG ratio was significantly decreased in non toxic goiter. Coefficient correlation ( $r$ ) of serum to tissue mucoproteins shows an inverse relationship in non toxic goiter and toxic goiter patients. In non toxic goiter and toxic goiter patients the serum sialic acid levels to tissue sialic acid levels also shows an inverse relationship. **Conclusion:** Correlation coefficient analysis showed an inverse relationship of serum mucoproteins and sialic acid to thyroid tissue. It signifies that carbohydrate moieties are increased in the blood when the tissue are hyperactive and in the pathological state. It further concludes that thyrotoxic state might be converted into nodular form even into cancerous form later on.

## INTRODUCTION

Thyroid glands is indispensable for the maintenance of the normal growth, development and well being of the organism. The major constituent of thyroid glands is colloid which is a large glycoprotein. Thyroglobulin which comprises 70-80% of protein<sup>1</sup> Spiro (1965) studied the thyroglobulin of sheep, calf, pig, and human and showed

that thyroglobulin is a high molecular weight glycoprotein<sup>2</sup>. The association of serum glycoproteins and mucoproteins with cancer was observed by Winzler (1967)<sup>3</sup>.

Total protein bound carbohydrate in normal serum and its variation in different diseases like tuberculosis,

subacute bacterial endo-carditis, advanced cancer and parenchymatous liver disease was investigated<sup>4,5</sup>. There is evidence that oncogenic transformation is associated with increased size cell surface carbohydrate<sup>6</sup>. Immunoelectrophoresis of serum of rat having inflammation induced by terpineol showed a considerable increase in mucoprotein, mucopolysaccharide and glycoprotein<sup>7,8,9</sup>.

Extensive variation in thyroglobulin structure is frequently associated with goiter and may be involved in the pathogenesis<sup>10</sup>. The level of serum thyroglobulin rises in autoimmune thyroid diseases, thyroid injury or inflammation and thyroid cancer.

Thyroglobulin provides a useful marker in thyroid toxicity to indicate recurrence of disease and for further studies and therapy<sup>11,12</sup>. Thyroxine and triiodothyronine are the only physiologically active hormones. Ratio of  $T_4/T_3$  would be proportional to thyroid function. Estimation of  $T_4$  and  $T_3$  would be considered as better index of thyroid function as these are the basis for the grouping of the patients as non toxic to toxic form. Ophthalmopathy is clinically apparent in 20-40% of patients<sup>13</sup>. Thyroid stimulating hormone (TSH) increases the synthesis of mucopolysaccharide in the thyroid gland<sup>14</sup>. TSH induces transfer of newly synthesized protein from the follicle cells to the follicle lumen by exocytosis i.e. emptying of specific apical vesicle<sup>15,16</sup>.

It was also observed that a significant thyroglobulin release in man usually occurs only after intense and prolonged TSH stimulation of the thyroid gland<sup>17,18</sup>. Degree of glycosylation depends upon the concentration of plasma protein as well as amounts of sugar present in blood. Increased glycosylation of plasma proteins was noted in diabetes mellitus and this increase is directly proportional to the increase in blood glucose level<sup>19</sup>.

Thyroidectomy in man is followed by accumulation of extracellular fluid rich in hyaluronic acid containing mucoprotein and forms the basis of clinical myxoedema<sup>5</sup>.

Sialic acid N-Acetyl Neuraminic Acid, (NANA) is a component of thyroglobulin and is present at the terminal portion in the oligosaccharide chain of thyroglobulin and it is suggested that sialic acid incorporation may be used as an index for the secretion of glycoprotein<sup>20</sup>. Bland et al (1984)<sup>21</sup> also observed that serum thyroglobulin levels equal to or below 20 ng/ml indicated the absence of thyroid carcinoma and values exceeding 60 ng/ml were indicative of active thyroid cancer but might include some patients without clinical evidence of disease. No study has been conducted till now both in blood and tissue level simultaneously. The purpose of this study was to estimate the levels of T<sub>4</sub>, TBG, T<sub>4</sub>/TBG variations in blood to assess the basis of toxicity. It was also planned to estimate the levels of mucoproteins and sialic acid in serum and thyroid tissue simultaneously in patients suffering from thyroid disorders which could be helpful to understand the pathogenesis of thyroid gland and its conversion into nodular and cancerous form later on.

## MATERIALS & METHODS

### Patients

Patients suffering from thyroid disorders admitted in Surgical Units of Jinnah Postgraduate Medical Centre Karachi, Surgical Units and ENT Wards of Civil Hospital Karachi were selected. Diagnosis of the patients was carried out on the basis of history of patients physical examination and radio-isotopic studies such as thyroid scanning and Isotopic iodine uptake.

### Control Subjects

Age matched subjects having no history of thyroid disease were selected from normal population. Most of the normal subjects were selected from amongst the students and staff members of Basic Medical Institute, J.P.M.C Karachi.

### Blood Collection

Fifteen to twenty ml of blood was collected from each patient by venepuncture. About 5 ml of blood was transferred into a clean centrifuge tube containing a mixture of sodium fluoride and potassium oxalate in a

ratio 1:3 as an anticoagulant. Remaining blood was transferred to a dry centrifuge tube. Plasma was separated by centrifugation after 30 minutes of collection of blood and serum after complete coagulation of blood, approximately within one hour of blood collection. Plasma and serum after separation were transferred into dry glass bottles with plastic stoppers. 0.5 ml of serum was stored into a plastic container for hormone analysis. All the samples were stored in the freezer at  $-20^{\circ}\text{C}$ . Before analysis, samples were allowed to attain room temperature. Sialic acid and mucoproteins were estimated by Natelson<sup>23,24</sup> methods respectively.

### Thyroid Tissue Collection

Fresh human thyroid tissue was obtained at the time of operation and preserved in ice cold tris-hydrochloric acid buffer at pH 7.5. The tissue was dried on filter paper. Adherent fascia and fibrous tissue was removed. The tissue was weighed, minced and chopped. Homogenate 20% was prepared in 0.2M Tris-hydrochloric acid buffer (hydroxymethyl-aminomethane 99.0-99.5% of Sigma Chemical Company, USA) at pH 7.5 with Aloe homogenizer (Aloe Scientific company, USA). The homogenate was centrifuged at  $1500^{\circ}\text{g}$  for 15 minutes at  $4^{\circ}\text{C}$  in a refrigerating centrifuge. It was labeled and stored at  $4^{\circ}\text{C}$  in a refrigerator.

### RESULTS

The results are summarized in table-IV and  $\pm$  s.e.m. of each is shown. Twenty apparently healthy normal subjects and 24 patients suffering from thyroid diseases were studied. The cases were divided into two groups namely non toxic goiter (14) and toxic goiter (10). Mean values of physical findings ie. body temperature, blood pressure (systolic and diastolic) and pulse rate in different groups are shown in table-I. The mean levels of body temperature expressed in clinically euthyroid subjects i.e. controls (36.87) and in toxic goiter (36.90) were remarkably similar. In non toxic goiter patients, the mean level of body temperature (36.75,  $p<0.001$ ) was significantly decreased as compared with control group.

The mean levels of systolic blood pressure in non toxic goiter (122.21,  $p<0.001$ ) and in toxic goiter (157.00,  $p<0.001$ ) were significantly increased as compared with control group. The diastolic blood pressure was only significantly increased in toxic goiter (87.50,  $p<0.001$ ) as compared with control group (73.95). In this study, pulse rate was significantly increased in toxic goiter group (108.00,  $p<0.001$ ) as compared with control group. The mean values of pulse rate observed in control subjects (74.50) and in non toxic goiter (74.42) were remarkably similar.

**Table-I. Physical findings of patients with non toxic goiter and control subjects**  
The number of patients or units are given in parenthesis.  
The values are mean  $\pm$  s.e.m.

Group	Temperature $\text{C}_0$	Blood Pressure		Pulse (Rate/min)
		Systolic (mmHg)	Diastolic (mmHg)	
Control (20)	36.87 $\pm$ 0.02	109.90 $\pm$ 1.73	73.95 $\pm$ 1.05	74.50 $\pm$ 0.41
Non toxic goiter (14)	36.75** $\pm$ 0.02	122.21** $\pm$ 1.95	76.78 $\pm$ 1.19	75.42 $\pm$ 1.01
Toxic goiter (10)	39.90 $\pm$ 0.01	157.00** $\pm$ 6.59	87.50** $\pm$ 2.00	108.00 $\pm$ 2.70
* $P<0.01$ as compared with normal control subjects.				
** $P<0.001$ as compared with normal control subjects.				

Mean values of serum T<sub>4</sub>, TBG and T<sub>4</sub>/TBG quotient are shown in table-II. The mean levels of T<sub>4</sub> expressed in micro g/dl observed in control (8.17) and in non toxic goiter (7.57) were similar. The mean levels of T<sub>4</sub> in toxic goiter (17.79, p<0.001) was significantly higher as compared with control group. The mean levels of TBG

expressed as micro g/ml in control subjects were (13.16). The significant increase was observed in non toxic goiter (34.88, p<0.001) but in toxic goiter patients (19.13, p<0.001). In toxic goiter patients the mean levels were (10.57±2.55).

**Table-II. Variations of thyroxine, thyroglobulin, and T<sub>4</sub>/TBG quotient control subjects and in patients**  
The number of patients or units are given in parenthesis.  
The values are mean ± s.e.m.

Group	T <sub>4</sub> (µg/dl)	TBG (µg/ml)	T <sub>4</sub> /TBG Quotient
Control (20)	8.17 ± 0.41	13.16 ± 0.72	6.29 ± 0.48
Non toxic goiter (14)	7.57 ± 1.17	34.88 ± 1.16***	2.16 ± 0.32***
Toxic goiter ( )10	17.79 ± 2.84**	19.13 ± 1.19*	10.57 ± 2.55
*P<0.05, 0.02 as compared with normal control subjects.			
**P<0.01 as compared with normal control subjects.			
***P<0.001 as compared with control subjects.			

Table-III shows the serum mucoprotein levels in controls and serum and tissue mucoprotein levels in non toxic goiter and toxic goiter patients with their correlation coefficient ( r ) ration. The mean levels of serum mucoproteins expressed in mg/dl in non toxic goiter is 88.05 ± 8.91 and mean levels of tissue mucoproteins expressed in mg/100mg of tissue in this group is 294.41± 34.06. The correlation coefficient ( r ) of serum

mucoproteins to tissue mucoproteins in this group is - 0.05. An inverse relationship was observed i.e. if the levels of mucoproteins is increased in blood then decreased levels were observed in the tissue. In toxic goiter patients mean serum mucoproteins levels and tissue levels, expressed in mg/100 gm of tissue are 58.21±5.61, 270.17 ± 43.91 respectively. These findings also show an inverse relationship.

**Table-III. Correlation coefficient of blood mucoproteins to the tissue in thyroid patients.**  
The number of patients or units, are given in parenthesis  
The values are mean ± s.e.m.

Group	Mucoprotein		
	Blood (mg%)	Tissue (mg/100gm)	Correlation coefficient ( r )
Control (20)	67.87 ± 47.33	-	-
Non toxic goiter (14)	88.05 ± 8.91	294.41 ± 34.06	-0.05
Toxic goiter ( )10	58.21 ± 5.61	270.17 ± 34.91	-0.22
*P<0.05, 0.02 as compared with normal control subjects.			
**P<0.01 as compared with normal control subjects.			

Mean levels of serum sialic acid (mg/dl) in control subjects and in non toxic and toxic goiter patients along with their tissue levels expressed in mg/100gm of tissue and correlation coefficient ( r ) are shown in table-IV.

Serum sialic acid level in control group is  $40.83 \pm 2.03$ . The mean values of serum sialic acid expressed in mg/dl

in non toxic goiter and in toxic goiter group are  $55.90 \pm 3.19$  and  $55.68 \pm 2.91$  respectively. Tissue sialic acid levels in non toxic goiter is  $273.69 \pm 13.91$  and values in toxic goiter are  $159.26 \pm 17.57$ . Correlation coefficient ( r ) i.e; serum sialic acid to tissue sialic acid in non toxic goiter and toxic goiter patients are - 0.07, -0.68 respectively. These values shown an inverse relationship.

**Table-IV. Correlation Coefficient ( r ) of blood Sialic Acid to Tissue Sialic Acid in Thyroid Patients**  
The number of patients or units, are given in parenthesis  
The values are mean  $\pm$  s.e.m.

Group	Sialic Acid		
	Blood (mg%)	Tissue (mg/100gm)	Correlation Coefficient of Tissue ( r )
Controls (20)	$40.83 \pm 2.03$	-	-
Non Toxic Goiter (14)	$55.90^{**} \pm 3.19$	$273.69 \pm 13.91$	-0.07
Toxic Goiter (10)	$55.68^{**} \pm 2.91$	$159.26 \pm 17.57$	-0.68*
<i>*P&lt;0.05, 0.02 as compared with normal control subjects.</i>			
<i>**P&lt;0.01 as compared with normal control subjects.</i>			

## DISCUSSION

Thyroid tissue mainly composed of colloid material, the major constituents of the colloid is a large glycoprotein, the thyroglobulin (70-80%). Extensive variation in thyroglobulin are frequently found in patients with goiter which may be involved in pathogenesis and its conversion into cancerous form later on. Elevation of serum glycoprotein may arise as a result of the depolymerization of the ground substance of the connective tissue with the release of solubilized components into the circulation either in the tissue destructive processes e.g. inflammation, destructive disease, carcinoma etc. or in tissue proliferative processes e.g. prostatic hyperplasia, pregnancy, blood loss etc.<sup>24, 25, 26</sup>. Winzlar (1967)<sup>3</sup> observed significant increase in hexosamine, sialic acid, fucose and mucoproteins in advance cancer, acute tuberculosis, sub bacterial endo-carditis and parenchymatous liver

diseases.

It was also found that various fractions of glycoproteins were increased in serum in different thyroid disorders, urinary stone formers and diabetics<sup>27, 28, 29</sup>. It has also been observed by Wade that in cancer diagnosis if serum thyroglobulin level is below 50 ng/ml residual tumor deposits are probably present and if level is above 50 ng/ml secondary deposits are invariably present<sup>21</sup>. Thyroid hormones increases metabolism in the tissues and causes more rapid utilization of oxygen, cardiac output and heart rate<sup>30</sup>. Thyroid hormone probably has direct effect on the excitability of heart which in turn increases heart rate<sup>31</sup>.

These effects were also observed by Buccino, that thyroid hormone augments the contractile state of isolated cardiac preparations by mechanism thought not

to involve catecholamines<sup>32</sup>. Pulse rate was significantly increased in toxic goiter (108.00,  $p < 0.001$ ) as compared with control group. The results are in accordance with Klin,<sup>31</sup>. These results are also in agreement with those of Perveen and also observed by Sterling that increase may be due to super sensitivity of heart to catecholamine in hyperthyroidism and several reports indicate that the number of myocardial  $\beta$ -adrenergic receptors is increased<sup>26, 33</sup>.

Normally, mucoprotein serves as a cementing substance between the individual fibers of the gland tissue. The mean levels of tissue mucoproteins expressed as mg/100g of tissue in non toxic goiter (294.41), and toxic goiter (270.17) were observed. Coefficient correlation of serum mucoprotein and tissue mucoprotein was calculated. The non toxic goiter group ( $r = -0.05$ , P N. S) and toxic goiter patients ( $r = -0.22$ , P N. S) showed an inverse relationship i.e. if the level of mucoproteins in blood increased there was a decrease in the tissue mucoproteins levels.

The mean levels of serum sialic acid observed in controls (40.83),. In non toxic goiter (55.90,  $P < 0.001$ ), in toxic goiter (55.68,  $P < 0.001$ ), the mean values were significantly higher than the controls (40.83). Sialic acid is present at terminal position in oligosaccharide chain of thyroglobulin and it is suggested that sialic acid incorporation may be used as an index for the secretion of glycoprotein<sup>21</sup>. It has also been observed that incorporation of sialic acid and iodine takes place at the same time in a newly synthesized molecules of thyroglobulin<sup>20, 34</sup>.

In this study the mean values of sialic acid in toxic goiter group is significantly higher. This may be possible that in this group the thyroid hormone formation is greater, the incorporation of sialic acid expressed as mg/100 gm of tissue in non toxic goiter (273.69) and in toxic goiter (159.26) were observed. Monaco reported the low contents of sialic acid in thyroglobulin of congenital goiter<sup>21, 35</sup>. Correlation coefficient ( $r$ ) between blood sialic

acid to tissue sialic acid contents were determined. It was found a negative  $r$  value ( $-r$ ) in non toxic goiter ( $r = -0.07$ , P N. S) and in toxic goiter ( $r = -0.68$ ,  $P < 0.02$ ).

## CONCLUSION



It was concluded from correlation coefficient analysis (blood to tissue levels) that in non toxic goiter and toxic goiter patients mucoproteins and sialic acid showed an inverse relationship. It is suggested that research may be progressed to explore the more biochemical basis for the conversion of euthyroid goiter into nodular (simple or multi nodular) goiter and even its conversion into cancerous into cancerous form.

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