PREDICTIVE VALUE OF SERUM THYROGLOBULIN IN TREATED PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA

D. Charles Stephen*, M.S. Seshadri, Regi Oommen®, Aravindan Nair[§], S.Swaminathan*, A.S.Kanagasabapathy***

Departments of Clinical Biochemistry*, Endocrinology**, Nuclear Medicine® and Surgery[§] Christian Medical College and Hospital, Vellore, 632 004.

ABSTRACT

An in-house radioimmuno assay for serum thyroglobulin was developed in our laboratory and compared its relative sensitivity with that of whole body scan in the detection of residual tumour or metastases and evaluated the predictive value of serum thyroglobulin in the clinical course of the disease. Ninety six patients after thyroidectomy were followed up for a maximum period of five years in this study.

The sensitivity and specificity of serum thyroglobulin were found to be close to that of whole body scan (85% and 94% respectively). According to this study, a serum thyroglobulin >40 ng/ml can differentiate between patients with metastases and those in remission. Serum thyroglobulin can replace whole body scan during the subsequent follow-up if the patient had concordant whole body scan and serum thyroglobulin during initial assessment.

KEYWORDS: Serum thyroglobulin, whole body scan and thyroid carcinoma.

INTRODUCTION

Diagnostic Iodine 131 (¹³¹I) whole body scan (WBS) and serum thyroglobulin (Tg) measurements are important tools to detect residual thyroid tumour and radioiodine therapy in patients with differentiated thyroid carcinoma (DTC)(1-3). However, some studies(2,4) indicated that there are some discrepancies between the serum Tg level and disease activity. Serum Tg can be occasionally detected in patients in the apparent absence of residual thyroid tissue (2,5-10). On the contrary undetectable serum Tg is

found in some patients with metastases(11-14). An inexpensive, reproducible, sensitive and specific Tg assay is not generally available in clinical laboratories in India. In spite of numerous studies on serum Tg in DTC patients abroad, till date no study has been reported on Indian patients. Further, there is no appropriate Tg reference range established for a patient who has been treated for DTC. The cut-off level for serum Tg reported in different studies from abroad range from 5 to 30 ng/ml(15,16).

The aim of this study was (a) to develop an in-house radioimmunoassay (RIA) for serum

Author for correspondence :
DR. A.S. Kanagasabapathy,
Prof. & Head at above address.

Tg; (b) to compare the relative sensitivity of serum Tg with that of WBS in the detection of residual tumour or metastases and (c) to evaluate the predictive value of serum Tg in the clinical course of the disease.

MATERIALS AND METHODS

a) Serum Tg Assay

The measurement of Tg was carried out by double antibody in-house RIA based on a previously described method(17). The method developed in our laboratory employed antibodies raised in rabbit against human Tg by repeated multiple injections. The antiserum was used in the assay at a dilution of 1 in 20,000 with 0.25 g% BSA-PBS, as diluent. The assay was carried out by overnight incubation (18 hr) of sample with diluted antiserum. ^{125}I -Tg was added and incubated for a further period of 4 hr. A purified goat anti-rabbit IgG was added and the resulting precipitates were separated by centrifugation. The radioactivity in the pellet was counted in Wallac 1470 WIZARD gamma counter. Tg was estimated from duplicate measurement by interpolation using standard curve drawn by log-logit data reduction. The antiserum was highly specific for Tg, with an extremely low crossreactivity to alpha-fetoprotein and undetectable crossreactivity to T4, T3, TSH and LH. The normal range of serum Tg (up to 21 ng/ml) was determined from Tg values measured in sera from 86 normal adults, having a mean $\pm$ SD value of 7.8 ± 6.7 ng/ml. The calculated analytical sensitivity of the assay was 2.5 ng/ml. The inter-assay CV of the controls containing two different levels of Tg (10 ng/ml & 60 ng/ml) were 5.2% and 8.4%, respectively (n=30).

Recovery studies resulted in a mean recovery of 102% ranging from 93% to 108%. Serum Tg values of 60 patients samples obtained by the in-house RIA correlated very well with the values obtained using DPC RIA kit - $r=0.98$ (Diagnostic Products Corporation, Cat. No. KTGD1).

b) Patients and Clinical Methods

We evaluated serum Tg levels (off-T4 therapy) in 96 patients (62 females and 34 males) during a period of 5 years from 1993 to 1998 who were treated in the Christian Medical College and Hospital, Vellore, India. At the time of diagnosis, age of the patients ranged between 18 and 72 years, with a mean $\pm$ SD of 45.5 ± 11.3 years. Histological examination showed papillary cancer in 64 patients, follicular cancer in 28 patients and mixed in 4 patients. Patients were treated by a standard protocol. All patients were treated with total thyroidectomy and modified neck dissection to clear the neck of lymphnode metastases. When there was local infiltration of soft tissue by tumour at the time of surgery, patients received external radiation therapy to the neck. All patients underwent a whole body ^{131}I uptake and therapeutic dose of ^{131}I was also given to most of the patients. At follow-up, if ^{131}I uptake showed only minimal uptake in the thyroid bed or a decreasing uptake compared to previous scans, patients were followed up without intervention. The mean period of follow-up of patients was 42 months ranging from 18 to 60 months. The follow-up scheme included clinical examination of the patient, a Chest X ray, and WBS. A sample of blood was collected each time a patient was examined. The blood sample collected during the first visit of the follow-up was

considered as the initial sample and the latest was the final sample. The sera were frozen at -20°C until analysis of Tg.

The patients were divided into four groups. Group 1 are those patients whose serum Tg was normal in the initial stage of measurement and remained so throughout the follow-up. Group 2 patients had an elevated serum Tg level in the beginning and normalized on subsequent follow-up / treatment. Group 3 patients had elevated serum Tg level in the beginning and during all follow-up periods. Patients in Group 4 had normal serum Tg at the initial stage and at one stage of follow-up, but began to rise subsequently. (recurrence).

RESULTS

The clinical and pathological details of the patients are provided in Table 1. A patient was considered to be in remission (disease negative) if clinical examination, X rays and WBS failed to reveal evidence of active disease. Table 2 shows serum Tg levels in all 4 groups of patients obtained at the initial and the final assessment stages in this study. Table 3 compares serum Tg levels with WBS in initial and final assessment of the patients studied.

In this study, serum Tg values < 10 ng/ml and > 10 ng/ml were considered as 'Tg negative' and 'Tg positive', respectively. Standard whole body scans were performed at 48 and 72 hr. after the administration of 2 mCi of ¹³¹I.

As shown in Table 3 all 40 patients in Group 1 had the initial serum Tg level <10 ng/ml, (negative), but 19 WBS were positive and 21 WBS were negative. All 19 patients with

positive WBS showed uptake only in the thyroid region and not elsewhere, indicating minimal residual disease. Out of these 19 patients only 8 needed an additional ¹³¹I ablation therapy since, in the remaining 11 patients, the scans showed only minimal uptake and there was no extrathyroidal disease. However, in the final follow-up visits, only 2 were WBS positive (only in the thyroid region) and 17 of the 19 became WBS negative. These two patients were not subjected to any further treatment. All of them were clinically normal and all remained Tg negative (final).

In Group 2, where initially 29 patients were serum Tg positive, 24 had concordant WBS (WBS positive), and 5 had discordant WBS (WBS negative) results. Out of 24 patients only 3 had a repeat ¹³¹I ablation therapy (one patient had 5 times ¹³¹I therapy). In the final assessment all the 29 patients had become serum Tg negative. However, 2 patients remained WBS positive of whom 1 patient had only uptake in the thyroid region and the other patient was found to have lymphnode metastases.

In Group 3, all 24 patients had initial serum Tg and WBS positive (100% concordant results). However, while all these patients remained Tg positive in the final assessment only 20 patients had WBS positive and 4 became WBS negative. In this group 16 patients had repeated ablation, the frequency of ¹³¹I therapy dose ranged from 2 to 7 times and all of them had external radiation. The serum Tg level in the four patients who became WBS negative ranged from 33 to 490 ng/ml with a mean of 164 ng/ml.

All the 3 patients in Group 4 were serum Tg negative, with 2 patients having negative WBS

Table 1. Sex and histological findings of the patients studied in different groups.

Groups	Numbers (n)	Sex		Histology		
		Female	Male	FOL	PAP	MIX
1	40	28	12	11	29	0
2	29	20	9	6	21	2
3	24	12	12	10	12	2
4	3	2	1	1	2	0

Table 2. Serum Tg Levels (initial & final) in all groups of patients.

Group	Initial Tg ng/ml		Final Tg ng/ml	
	Mean	SD	Mean	SD
1	7.1	6.3	3.3	1.6
2	34.2	30.3	4.3	2.7
3	194.4	153.7	130.8	121.9
4	6.2	3.4	17.3	3.5

Table 3. Serum Tg versus WBS in initial and final assessment in all groups.

Group	Initial Tg		Initial WBS		Final Tg		Final WBS	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
1	0	40	19	21	0	40	2	38
2	29	0	24	5	0	29	2	27
3	24	0	24	0	24	0	20	4
4	0	3	1	2	3	0	3	0

Table 4. The relative sensitivity and specificity of Tg versus WBS

	Initial Assessment	Final Assessment
Sensitivity	71%	85%
Specificity	82%	94%
Pos. Predictive	91%	85%
Neg. Predictive	53%	94%

and 1 patient having positive WBS at the initial assessment. However, later on all 3 of them had both serum Tg and WBS positive.

Based on the above data the overall clinical sensitivity and specificity as well positive and negative predictive values of serum Tg when compared to WBS in the assessment of all patients were calculated according to Watson(18) and presented in Table 4.

DISCUSSION

Most authors (8,9,13,14,19,20) consider a Tg value of 5 or 10 ng/ml as the upper limit of normal in patients who have undergone total thyroidectomy. Authors(9,19) whose assays had detection limit of 1 ng/ml used 5 ng/ml as the upper limit of normal, while those who used assays with higher detection limits recommended a cut-off of 10 ng/ml. In our assay the analytical sensitivity was 2.5 ng/ml, hence we used a cut-off of 10 ng/ml.

Discordant results between serum Tg and WBS are quite possible because it is well known that metastatic deposits from DTC are not always capable of taking up iodine but still can secrete Tg(5-10). On the contrary very rarely we see the opposite situation where tumour still takes up iodine with undetectable serum Tg(11-14).

There is a statistically significant difference between the mean initial serum Tg level in Group 1 with that of Group 2 and 3 ($p < 0.001$). If Tg values were not available for these patients then, going by only WBS it would have been necessary to give ^{131}I ablation therapy for all 19 patients who were WBS positive. In fact, in this study out of the 19, 11 were subjected to ablation

therapy as their uptake level was higher compared to the remaining 8 patients. This could have been avoided if one goes by only Tg levels.

In Group 2 it is to be noted that the mean Tg is only 34.2 ng/ml when compared to 190 ng/ml of Group 3. Out of the only 2 patients in Group 2 who had discordant results (WBS positive and Tg negative) during the subsequent follow-up one patient was found to have lymphnode metastases where the tumour probably cannot synthesize and secrete Tg and WBS played a role in the diagnosis over serum Tg. The other patient had minimal uptake over the thyroid region and remained clinically normal.

An elevated serum Tg > 40 ng/ml needs careful consideration. In our study it was noted that most of them had metastases,(92% (22/24) in Group 3). Hence a serum Tg value could differentiate between patients with metastases and patients in remission. Because of the repeated ablation therapy in 4 patients, the ability of the tumour to take up radioiodine was lost, but they remained disease active which was shown by raised serum Tg. In these patients serum Tg level was a better marker than WBS. Interestingly, out of the three patients in Group 4 who had serum Tg values which increased during their follow-up period, 2 had not received ^{131}I ablation therapy and they developed the recurrence only after an average period of 3 years.

Conclusion

Our study clearly proves that serum Tg level can be used to follow the patients with DTC either during treatment or once they have become apparently free of disease. Its use is

rather simple in patients totally ablated where the presence or the rise of circulating Tg is always suspicious of recurrent or persistent tumour. Serum Tg measurement can replace WBS during the subsequent follow-up if the patient had

concordant WBS and serum Tg results during initial follow-up. In conclusion we can say that Tg assay is a useful and cost-effective method for the management of patients with treated DTC.

REFERENCES

1. Pacini, F., Pinchera, A., Giani, C., Grosso, L. and Baschieri, L. (1980) Serum thyroglobulin concentrations and ^{131}I whole body scans in the diagnosis of metastases from differentiated thyroid carcinoma (after thyroidectomy). Clin. Endocrinol. (Oxf). 13, 107-110.
2. Refetoff, S. and Lever, E.G. (1983) The value of serum thyroglobulin measurement in clinical practice. J.Amer. Med. Assoc. 250, 2352-2357.
3. Bladh, W.H., Drickman, M.V., Porter, C.W., Hill, V.A. and Baumgartner, W.A. (1984) Serum thyroglobulin, a monitor of differentiated thyroid carcinoma in patients receiving thyroid hormone suppression therapy. J Nucleic. Med. 25, 673-676.
4. Sheppard, M.C. (1986) Serum thyroglobulin and thyroid cancer. Q J Med. 59, 429-433.
5. Tang Fui, S.C., Hoffenberg, R., Maisey, M.N. and Black, E.G. (1979) Serum thyroglobulin concentrations and whole body radioiodine scan in follow up of differentiated thyroid cancer after thyroid ablation. Brit. Med. J. 2, 298-300.
6. Schlossberg, A.H., Jaccobson, J.C. and Ibbertson, H.K. (1979) Serum thyroglobulin in the diagnosis and management of thyroid carcinoma. Clin. Endocrinol. (Oxf).10, 17-27.
7. Schlumberger, M., Fragu, P., Parmentier, C. and Tubiana, M. (1981) Thyroglobulin assay in the follow-up of patients with differentiated thyroid carcinomas: comparison of its value in patients with or without normal residual tissue. Acta Endocrinol. (Copenh). 98, 215-221.
8. Barsno, C.P., Skosey, C., DeGroot, L.J. and Refetoff, S. (1982) Serum thyroglobulin in the management of patients with thyroid cancer. Arch. Intern. Med. 142, 763-767.
9. Black, E.G., Cassoni, A., Gimmette, T.M.D., Harmer, C.L., Maisey, M.N. and Oates, G.D. (1981) Serum thyroglobulin in thyroid cancer. Lancet. 2, 443-445.
10. Pacini, F., Lari, R., Mazzeo, S., Graso, L., Tadei, D. and Pinchera, A. (1985) Diagnostic value of single serum thyroglobulin determination on and off thyroid suppressive therapy in the follow-up of patients with differentiated thyroid cancer. Clin. Endocrinol. (Oxf). 23, 405-411.
11. Grant, S., Luttrell, B., Reeve, T., Weisman, J., Wilmshurt, E., Steiel, J., Donohoe, D., Cooper, R. and Bridgeman, M. (1984) Thyroglobulin may be undetectable in the serum of patients with metastatic disease secondary to differentiated thyroid carcinoma. Follow-up of differentiated

- carcinoma. *Cancer*. 54, 1625-1628.
12. Muller-Gartner, H.W. and Schneider, C. (1988) Clinical evaluation of tumour characteristics predisposing serum thyroglobulin to be undetectable in patients with differentiated thyroid cancer. *Cancer*. 61, 976-981.
 13. Schneider, A.B., Line, B.R., Goldman, J.M. and Robbins, J. (1981) Sequential serum thyroglobulin determination, ^{131}I scans, and ^{131}I uptake after triiodothyronine withdrawal in patients with thyroid cancer. *J. Clin. Endocrinol. Metab.* 53, 1199-1206.
 14. Ramanna, L., Waxman, A.D., Brachman, M.B., Sensel, N., Tanasescu, E.E., Berman, D., Calltz, B. and Braunstein, G.D. (1985) Correlation of thyroglobulin measurement and radioiodine scans in the follow-up of patients with thyroid cancer. *Cancer*. 55, 1525-1529.
 15. Kastrup, J., Feldt-Rasmussen, U., Bartram, H.R., Witten, J.S. and Hassen, H. (1985) An enzyme linked immunosorbent assay for measurement of human serum thyroglobulin. Evaluation of the influence of thyroglobulin autoantibodies. *Scand. J. Clin. Lab. Invest.* 45, 471-476.
 16. Kato, R., Noguchi, S. and Noguchi, A. (1987) Human serum thyroglobulin determination with monoclonal antibody one-step assay: minimum interference of autoantibody. *Endocrinol. Jpn.* 34, 171-178.
 17. Ozata, M., Suzuki, S., Miyamoto, T., Liu, R.T., Renoy, F.F. and Degroot, L.J. (1994) Serum thyroglobulin in the follow-up of patients with treated differentiated thyroid cancer. *J. Clin. Endocrinol. Metab.* 79, 98-105.
 18. Watson, R.A. and Tank, D.B. (1980) The predictive value of prostatic acid phosphatase as a screening test for prostatic cancer. *N. Engl. J. Med.* 303, 497-499.
 19. Black, E.G., Sheppard, M.C. and Hoffenberg, R. (1987) Serial serum thyroglobulin measurement in the management of differentiated thyroid carcinoma. *Clin. Endocrinol. (Oxf)*. 27, 115-120.
 20. Erichson, U.B., Tegler, L., Lennquist, S., Christensen, B., Stahl, E. and Thorel, J. (1984) Serum thyroglobulin in differentiated thyroid carcinoma. *Acta Chir. Scand.* 150, 367-375.