Evaluation thyroglobulin level during suppressive therapy measured by ultrasensitive technique in the prediction of excellent response in patients with differentiated thyroid cancer

Original Article

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ABSTRACT

Introduction: When ultrasensitive thyroglobulin (uTg) is measured serially in the first 12 months of treatment, it can probably predict recurrence of thyroid cancer without discontinuation of T4 or administration of rhTSH.

Methods: Measurement of TSH, Tg, Anti-Tg Ab, and uTg was performed in all consecutive patients with low-intermediate risk DTC 2, 6, and 12 months after initial therapy. One year after surgery, response to therapy was evaluated. Tg and uTg levels in different time points and the trend of changes were used to predict response to therapy.

Results: Overall, 37 patients with a mean age of 40.3 years were studied. The mean initial uTg was significantly lower than fTg (P=0.01). Overall, the correlation coefficient was 0.45 and increased to 0.71 in ftg<10 ng/ml (P=0.005). The majority of patients (91.9%) received a mean dose of 3.3± 2.2GBq of I-131. One year later, there was no significant difference in mean Tg and mean uTg between the two techniques (P=0.62). The mean offT4uTg is not significantly different between patients with incomplete response compared to other groups at the end of the follow-up (P=0.1). The slope of onT4uTg was 0.04±0.18 versus 0.24±0.82 in patients with and without incomplete response respectively (P=0.45). Using ROC analysis, onT4uTg slope of 0.0005 was the best cutoff to differentiate incomplete response from other responses, however, the sensitivity was only 54.5% and specificity was 75%.

Conclusion: The trend of thyroglobulin level during suppressive therapy measured by the ultrasensitive technique cannot accurately predict excellent response in DTC patients.

Key words: Ultrasensitive thyroglobulin assay; Thyroid cancer; Response to therapy

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INTRODUCTION

Differentiated thyroid cancer (DTC) is the most common endocrine cancer and its incidence has been increasing in the last decades [1]. Treatment and follow up of patients with DTC varies from a lobectomy and replacement therapy in microcarcinomas to extensive surgery plus radio-iodine therapy and external radiotherapy in more aggressive metastatic lesions [2]. After initial treatment, a dynamic risk stratification is highly important to tailor further treatments [3]. As TSH stimulated thyroglobulin is a very sensitive marker of recurrence, American Thyroid Association (ATA) recommends measurement of serum thyroglobulin after discontinuation of thyroid hormones or after administration of rhTSH, 6-12 months after initial therapy. However discontinuation of thyroid hormones is associated with severe hypothyroid symptoms and decreased quality of life [4]. Administration of rhTSH could be used instead of thyroid hormone discontinuation and will result in better quality of life with the same diagnostic and therapeutic results [5, 6]. However considering high cost of rhTSH, it is not cost effective in most of the countries especially in developing or under developed countries [7]. On the other hand, as onT4 Tg level is strongly correlated with stimulated Tg level, it is reported that if thyroglobulin level during suppression (onT4-Tg) was <0.1 ng/ml, only 2.5% of patients would have stimulated Tg (sTg) level of >2ng/ml [8, 9].

Some authors suggest using Tg slope instead of single Tg measurement, in prediction of recurrence of thyroid cancer [10]. An upward Tg trend was associated with tumor recurrence while downward trend was more in favor of remission [11, 12]. However, those studies did not use ultrasensitive Tg assays.

It is hypothesized that if we measure onT4-Tg serially using an ultrasensitive assay in the first 12 months, and we look at the trend of Tg, we could probably predict recurrence or remission without discontinuation of T4 or administration of rhTSH. However not enough studies tested this hypothesis and it is not clear that if this approach is optimal in all or in a special group of patients. We tried to test this hypothesis using logistic regression analysis for prediction of recurrence of thyroid cancer and including Tg trend in the model.

METHODS

All consecutive patients with low and intermediate risk DTC (up to T3, any N, M0), who referred for radio-iodine therapy after initial surgery recruited in the study. Upon presentation, levothyroxine was replaced with liothyronin for 2 weeks followed by 2 weeks of no thyroid hormone and measurement of TSH (Padyab Teb, Iran), Tg (IZOTOP, Hungry) and Anti-Tg Ab (Medizym® Anti-Tg, MEDIPAN GMBH, Berlin, Germany) was done in all patients. Analytical sensitivity of Anti-Tg Ab was 17 IU/ml with intra-assay CV of 6% and inter-assay CV of 7%. Radio-iodine therapy was performed in intermediate and high risk patients according to the ATA risk stratification [2]. Suppressive therapy was began with levothyroxine in all patients after initial treatment. Ultrasensitive serum Thyroglobulin measurement was done using high sensitive ELISA technique (Medizym® Tg Rem, MEDIPAN GMBH, Berlin, Germany) 2, 6 and 12 months after initial therapy. The functional sensitivity of uTg was 0.03ng/ml using this high sensitive ELISA technique. Measurement of Tg was done in the laboratory of Nuclear Medicine Research Center (NMRC) according to the instruction of the manufacturing company. Ultrasonography of the thyroid and neck was done 2, 6 and 12 months after initial therapy using a Mediso ultrasound system (ACCUVIX 10) equipped with a 12MHz linear probe. If there was any evidence of disease during the follow up (defined as presence of metastasis, neck mass in physical examination / hypoecho mass>10 mm in U/S or onT4-Tg>10ng/ml), appropriate therapeutic work up was followed according to the ATA recommendations [2]. In case of no evidence of disease, the patients were studied one year later after discontinuation of levothyroxine and measurement of Tg in off T4 state. Also, physical examination and U/S and if indicated diagnostic whole body iodine scan was performed.

Patients were categorized as “excellent response, acceptable response and incomplete response” according to the response categorization suggested by Tuttle et al. [3].

The trend of Tg was calculated using three points (2,6 and 12 months) or two points(6 and 12 months) and patients were categorized into three groups: Slope<0 , 0<Slope<0.1 and slope>0.1

Statistical analysis

Descriptive analysis was done using univariate analysis. The results of treatment were evaluated in three different response groups. A cross table was produced and difference in three groups was tested using Chi-Square test. Also logistic regression analysis was done for prediction of incomplete response using multiple variables including Tg slope. P<0.05 was considered significant in all comparisons.

RESULTS

Forty-one patients with DTC entered into the study and initial evaluation revealed distant metastasis in 4 patients which were excluded from the study. Overall, 37 patients (35 female and 2 male) with mean age of 40.3±11.2 years were studied. Pathology report

showed papillary thyroid carcinoma in 32 patients (86.5%), follicular carcinoma in 3 patients (8.1%) and Hurtle cell carcinoma in 2 patients (5.4%). All patients with FTC had widely invasive histology. Classic subtype of pathology was reported in 65.7% of patients. Table 1 shows general characteristics and TNM staging of the studied group.

Table 1: General characteristics and TNM stage of our patients (Version 8).

Variable		
Mean age ± SD (years)	40.3 ±11.2	
Sex	Female	35 (94.6%)
	Male	2 (5.6%)
Pathology	PTC*	86.5%
	FTC*	8.1%
	Hurtle cell Ca	5.4%
Pathology subtype	Classic	65.7%
	Other	34.3%
T stage	T1a	13.5
	T1b	27%
	T2	37.8%
	T3a	13.5%
	T3b	8.1%
N stage	N0	45.9%
	N1a	8.1%
	N1b	43.2%

*PTC: Papillary thyroid carcinoma; FTC: Follicular thyroid carcinoma

The first laboratory examinations was done around 6 weeks after surgery and about 4 weeks after discontinuation of thyroid hormones and the mean TSH level was 122.0 ± 53.1 IU/ml , mean first Tg (fTg) was 10.4 ± 18.7 ng/ml and corresponding uTg level was 3.36± 4.7 while first Anti-Tg Ab level (fAnti-Tg Ab) was 398.6±815.8 mIU/ml . Using paired t-test, mean initial uTg was significantly lower than fTg (P=0.01). Overall, correlation coefficient was 0.45 that was increased to 0.71 in ftg levels of less than 10 ng/ml (P=0.005). Furthermore there was no significant difference between mean fTg and uTg in patients with fTg<10ng/ml.

Overall, 34 patients (91.9%) treated with radio-iodine and received a mean dose of 3.3±2.2 GBq (90.2±60.7 mCi). Six months later, the mean Tg on suppression (2.78± 9.4 ng/ml) was not significantly different with the mean uTg (1.02±2.7ng/ml, P=0.32). The mean uTg on suppression, one year after therapy was 0.86±2.19 ng/ml. We already measured Tg level on suppression in 5 patients at this time to get a correlation with uTg and found that there was no significant difference in mean Tg and mean uTg between two techniques (P=0.62). Table 2 shows comparison of Tg and uTg levels in different time points.

Response to treatment was assessed one year after therapy, in off T4 state and we performed ultrasonography and measurements of anti-Tg Ab, Tg and uTg. Excellent, indeterminate and incomplete response were noted in 14 (38.9%), 10(27%) and 12 (32%) patients respectively. One patient had missing first year follow up. From 12 patients with incomplete response, 10 patients received another dose of radio-iodine because of persistent disease. The mean last follow up time was 2.01±0.81 years and in the last follow up, 22 (66.1%) patients had excellent response, 5 patients (13.9%) had indeterminate response and 9 (25%) patients had incomplete response.

We looked at the Tg level during T4 suppression (onT4Tg) to find out that if it can predict Tg level after discontinuation of the drugs (offT4Tg) 1 year after therapy. All patient with onT4Tg level of less than 0.15 had offT4Tg<0.1 and all patients with onT4Tg levels of >1.5 had higher offT4Tg levels. However it was not true for uTg levels, as 23% of patients with onT4uTg levels of <0.1 ng/ml, had offT4uTg levels of >0.1 and one patient had offT4uTg levels of >10 ng/ml. Conversely, 50% of patients with onT4uTg levels of >0.1 ng/ml, had lower offT4uTg levels and in one patient it was undetectable.

Table 2: Comparison of Tg and uTg levels in different time intervals with (on) or without (off) consumption of levothyroxin.

Variable	Mean Tg (ng/ml)	Mean uTg (ng/ml)	P value
First Tg (off)	10.4±18.7	3.36±4.7	0.01
2 months (on)	0.33±0.45	0.13±0.31	0.22
6 months (on)	2.78±9.4	1.02±2.7	0.32
11months (on)	0.06±0.07	0.03±0.03	0.62
12 months (off)	0.07±0.07	0.86±2.19	0.21

Table 3: Comparison of offT4uTg, onT4uTg slope and offT4Tg levels between two groups of incomplete response and complete/acceptable response in all patients as well as in patents with negative Anti-Tg Ab.

	AntiTg Ab< 100 IU/ml			All patients		
	Complete /Acceptable response	Incomplete response	P value	Complete /Acceptable response	Incomplete response	P value
OffT4uTg	0.97±2.8	4.22±5.24	0.24	0.68±2.2	6.1±5.20	0.08
OnT4uTgslope	0.025±0.099	0.44±1.1	0.39	0.13±0.48	0.01±0.47	0.53
OffT4Tg	1.37±1.92	23.9±25.5	0.058	1.94±3.5	18.6±24.2	0.07

Mean offT4uTg was 4.4±5.1 ng/ml in patients with incomplete response while it was 0.73±2.3 ng/ml in patients with complete or acceptable response one year after therapy (P=0.1). The same comparison was done for offT4Tg level and it was significantly higher in patients with incomplete response (15.8±21.5 Vs 1.2±1.7 ng.ml, P=0.03). We compared the slope of onT4uTg in these groups and noted that it was 0.04±0.18 Vs 0.24±0.82 in patients with and without incomplete response (P=0.45). Using logistic regression, incomplete response could be predicted using different variables by a marginal statistically significant model (P=0.06). Anyhow, the variables with highest beta in the model was radio-iodine therapy and uTg slope. Using ROC analysis, we noted that onT4uTg slope of 0.5×10^{-3} was the best cutoff to differentiate incomplete response from other responses, however the sensitivity was only 54.5% and specificity was 75% at this point (Figure 1).

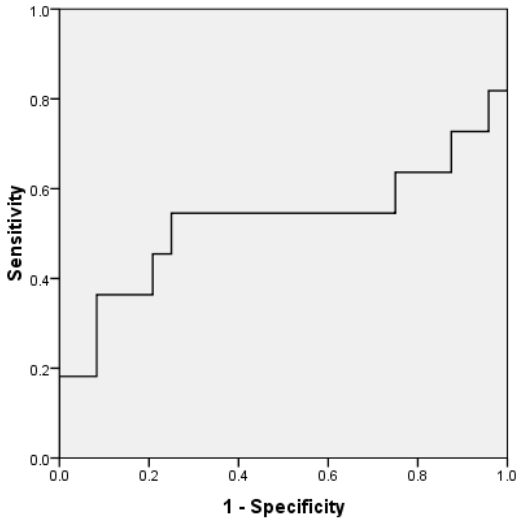

Fig 1. Receiver Operating Curve of onT4-uTg-slope for prediction of incomplete response (AUC=0.527).

Twenty-one patients had anti-Tg Ab of less than 100 IU/ml at the first presentation and a separate analysis was done in this group. Again there was not statistically significant difference between patients with incomplete response with other responses in terms of offT4uTg level, onT4uTg slope and offT4Tg level (Table 3, P>0.05).

DISCUSSION

Stimulated serum thyroglobulin level is a very important factor in evaluation of DTC patients either for initial staging or during follow up [13, 14]. However levothyroxin withdrawal is associated with lower quality of life and unpleasant symptoms that may be improved by administration of recombinant TSH (rhTSH) instead of thyroid hormone withdrawal [15, 16]. On the other hand, rhTSH is very expensive and seems to be not cost effective in most of the developing countries [7].

Thyroglobulin is measured using different techniques with a functional sensitivity of about 0.1ng/ml that must be calibrated against international CRM-457 standard [2]. Strong direct correlation of Tg level during suppression with Tg level after thyroid hormone withdrawal or rhTSH injection was reported [9]. Also, it is suggested that Tg measurement with ultrasensitive methods even during thyroid hormone consumption may be used for early detection of recurrence/persistent disease in patients with DTC [17]. However, not all of the studies were confirmatory [17, 18]. We used a new ultrasensitive kit (Medipan, Germany) to test the hypothesis that if nonstimulated ultrasensitive Tg measurements or its trend during follow up could predict incomplete response.

Our study showed that uTg is not correlated with stimulated Tg and cannot accurately predict the off-T4 Tg level.

Giovanella et al. in a study of 195 patients with DTC measured Tg using an ultrasensitive Tg assay 6 months after initial therapy. If the Tg level was undetectable and neck U/S was negative, the patients were considered without disease. In case of detectable Tg, patients underwent specific diagnostic and therapeutic work up. They noted that sensitivity of the uTg test was 100%, but 3 false positive test was noted and in all of these patients Tg level decreased without

any intervention during follow up, which means that specificity of the test was also perfect [12].

In another study, Lervasi et al looked at performance of ultrasensitive Tg assays and found that 0.16 ugr/l is the 99 percentile cut off level for detection of recurrence in stage I patients. In that study, if onT4-Tg level was <0.1 ng/ml, stimulated Tg level would be <1 ng/ml in all patients [19]. This is similar to our findings that all patients with onT4Tg level of less than 0.15ng/ml had offT4Tg<0.1ng/ml. However it was not correct for uTg levels, as 23% of patients with offT4uTg levels of <0.1 ng/ml, had offT4uTg levels of >0.1ng/ml. This may be due to large number of patients with elevated Anti-Tg Ab in our patients.

Shlumberger et al. tested 7 different Tg assays in patients with DTC and measured Tg level 3 and 9-12 months after initial therapy. Using a ROC analysis, they noted that the best cutoff for Tg for detection of remnant disease would be 0.22-0.27 ng/ml. They suggested that using ultrasensitive Tg and looking into the Tg trend may replace stimulated Tg measurement [20]. However, in contrast to those predictions, it is more than 2 decades that ultrasensitive Tg was introduced and it is not replaced stimulated Tg measurements in clinical practice.

Our study showed that onT4-uTg does not correlate with stimulated Tg and cannot accurately predict the off-T4Tg level. This may be explained by elevated Anti-Tg Ab in about half of our patients that may adversely affect measurements of uTg levels [9]. Although we analyzed separately the patients with negative Anti-Tg Ab, the number of patients was not large enough for a reliable conclusion. Others, also reported a discrepancy between uTg and Tg levels when the Anti-Tg Ab was elevated [21]. It seems that other technical factors may also affect optimal measurement of uTg [22]. This may explain the reason behind unpopularity of uTg measurements in clinical practice, despite its introduction for more than two decades.

It has been shown that the Tg doubling time and trend of Tg during follow up is a better predictor of recurrence/persistent disease than single Tg level in DTC patients [9]. We calculated uTg slope, using uTg values in different time points during patient follow up. In patients with negative anti-Tg Ab, we observed higher slopes for incomplete response compared to acceptable/excellent response; however, it did not reach statistical significance.

Another interesting finding in our study was higher uTg and Tg correlation in Tg levels of less than 10ng/ml. This is particularly important as stimulated Tg level of less than 10ng/ml categorizes patient in acceptable versus excellent response and uTg may play more important role in differentiation of these two groups. This however needs further work to invistigate its applicability in this particular group.

Limitations

Our study however suffered from some limitations including limited number of patients who agreed to participate in this study and high number of patients with positive Anti-Tg Ab that might adversely affected the results.

CONCLUSION

Our study showed that Tg and ultrasensitive Tg has a high correlation in Tg levels of less than 10ng/ml, but it cannot replace measurements of stimulated Tg in DTC patients and it needs more technical optimization before its wide clinical applications.

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