Original Article

Determination of Frequency of Type 2 Deiodinase Thr92Ala Polymorphism (rs225014) in ^{131}I-treated Differentiated Thyroid Cancer Patients Undertaking L-thyroxine (L-T4) Suppression Therapy

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Abstract

Introduction: Type 2 deiodinase (DIO2) enzyme plays a vital role in peripheral T4 to T3 conversion and in the negative feedback regulation of pituitary thyroid-stimulating hormone (TSH) secretion. Thr92Ala polymorphism (rs225014) is a common single-nucleotide polymorphism (SNP) that lowers DIO2 activity and is associated with diverse physiological disorders. Differentiated thyroid cancer (DTC) patients are given L-T4 therapy after total thyroidectomy and ^{131}I treatment to suppress TSH levels. **Aim:** The aim of the study was to determine the frequency of rs225014 in DTC patients and to investigate its effect on the thyroid function tests (TFTs) and L-T4 dose required to suppress TSH levels. **Materials and Methods:** The study included a DTC patient group and a control group. TFTs were estimated by RIA/IRMA kits. Genomic DNA of all the subjects was screened for rs225014 SNP by polymerase chain reaction. **Results:** The frequency of Thr/Thr (wild type), Thr/Ala (heterozygous mutant), and Ala/Ala (homozygous mutant) genotypes in the DTC patients' group was 0.21, 0.52, and 0.27, respectively. T3 levels and T3/T4 ratio were significantly low in the Ala/Ala genotype in the DTC group indicating impaired DIO2 activity. L-T4 dose requirement to suppress TSH levels in the DTC patients harboring rs225014 SNP was not statistically different from the wild-type genotype. **Conclusion:** The SNP rs225014 was observed to be associated with T3 and T3/T4 ratio but not with the L-T4 dose in DTC harboring SNP suggesting the presence of a compensatory pathway to overcome DIO2 impairment. However, it is essential to study the genetic makeup of DTC patients showing reduced response to TSH suppression to enable quicker decision-making in the implementation of personalized L-T4 dose to prevent any adverse effects.

Keywords: *^{131}I treatment, differentiated thyroid cancer, L-T4 dose, thyroid function tests, type 2 deiodinase rs225014 single-nucleotide polymorphism*

Introduction

Thyroid hormones (TH) regulate overall growth, development, and metabolism. The thyroid gland secretes 80% of 3,5,3',5'-L-tetraiodothyronine (T4) and 20% of 3,5,3'-L-triiodothyronine (T3) hormones, respectively. T3 is the active form of TH, and the action of TH is mediated by binding of T3 to specific nuclear receptors present in almost all the tissues in the body. The major part of the circulatory and intracellular T3 is obtained by peripheral deiodination catalyzed by Type 1 (DIO1) and Type 2 deiodinase (DIO2) enzymes. Of these two enzymes, DIO2 plays a major role in the intracellular T3 availability. T3 levels in both the hypothalamus and pituitary glands are the major determinant of the inhibitory control of thyroid-stimulating hormone (TSH) secretion, and hence, DIO2 plays an important role in the T4-mediated negative feedback regulation of TSH and TRH levels at pituitary and hypothalamic levels, respectively.[1-3] The single-nucleotide polymorphism (SNP), i.e., Thr92Ala SNP (c.274A > G; T92A; rs225014) in the gene coding for DIO2, is associated with impaired peripheral T4 to T3 conversion.[4] The rs225014 is reported to be associated with diverse physiological disorders such as type 2 diabetes mellitus, gestational diabetes, insulin resistance, hypertension, osteoarthritis, Graves' disease, intelligence quotient alterations associated with iodine deficiency, psychological well-being, and response to T3 or T4 therapy, suggesting the possible involvement of this SNP in the disruption of the TH signaling.[1,5-14] Differentiated thyroid cancer (DTC) patients

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are administered L-T4 therapy after the thyroidectomy and ablative ^{131}I therapy to suppress the TSH levels to inhibit the recurrence of thyroid cancer. It is speculated that the ^{131}I-treated DTC patients harboring the rs225014 SNP may require a higher L-T4 dose for TSH suppression due to impaired DIO2 activity. There are conflicting results regarding the association of rs225014 SNP and the requirement of the suppressive L-T4 dose in DTC patients.[15-17] This study was carried out to determine the frequency of Thr92Ala SNP in the DTC patients at our center and to determine its association with thyroid function test (TFT) results and the L-T4 dose requirement.

Materials and Methods

The study included two groups, namely DTC and control groups, respectively. The DTC group consisted of 157 thyroidectomized DTC patients (113 females and 44 males) who had undergone ablative ^{131}I treatment at least 6 months before and were on L-T4 suppressive treatment. The inclusion criteria of the patients included age ranging between 10 and <70 years, undetectable thyroglobulin (Tg) levels with negative whole-body scan for thyroid malignancy, and absence of any other disease or medication affecting L-T4 absorption. L-T4 dose was taken under fasting condition. The control group consisted of 69 healthy volunteers. The age and gender of the healthy volunteers were matched with the patients. Informed consent was taken from all the subjects, and the study was approved by the Institutional Medical Ethics Committee (RMC-IMEC-P4/Feb/2018/OPA233616). The data were expressed as mean ± standard deviation and median (range) for the parameters having normal and skewed distribution, respectively. For statistical analysis, the deviation from Hardy–Weinberg equilibrium was analyzed using the Chi-square test, and the difference between the groups was carried out using ANOVA using SPSS statistic for Window Ver 21.0 (Armonk, NY, IBM Corp, USA) software. TFT levels, i.e., T4, T3, TSH, FT4, and antithyroid peroxidase antibody levels, in patients' serum samples were estimated by RIA and IRMA kits using an SR 300 STRATEC RIA autoanalyzer. Genomic DNA was extracted by the nonenzymatic salting out method. Tetra-primer amplification refractory mutation system polymerase chain reaction (PCR) was standardized based on a previously reported procedure to detect rs225014 polymorphism.[9] The primers for amplifying the A and G alleles of the DIO2 gene were as given below:

Primer (A allele):
- FW: ATTGCCACTGTTGTCACCTCCTTCGGT
- RV: CTATGTTGGCGTTATTGTCCATGCGGTC.

Primer (G allele):
- FW: AATTCCAGTGTGGTGCATGTCTCCATTG
- RV: TTTTGGGCCATTCTTTACATTACCTGCCA.

Each 30 µL PCR reaction mixture contained 2 µL DNA template, 2 µL primer mix (5 pM/µL), 20 µL master mix, and 6 µL nuclease-free water. PCR tests were done under the following program with initial denaturation of 3 min at 95°C followed by 30 cycles including 30 s denaturation (95°C), 30 s annealing (60°C), and 30 s extension (72°C) and an extra 15 min extension at the end of the 30 cycles. PCR amplicons were run on 1.5% agarose gel electrophoresis to analyze the pattern of bands to determine the type of genotypes. The product size for the "A" allele and "G" allele was 276 and 418 bp, respectively, whereas the product size of the two outer primers was 639 bp, as depicted in Figure 1.

Results

The frequency of homozygous (Ala/Ala) and heterozygous (Thr/Ala) rs225014 SNP in the DTC group was 0.27 and 0.52, respectively, which was comparable with the control group in the present study [Table 1]. The allele frequencies in the DTC and normal groups were consistent with Hardy–Weinberg equilibrium. Thr92Ala SNP was not found to be associated with DTC in this study (odds ratio = 1.1; 95% confidence interval = 0.6–1.9).

The results of TFTs in the three genotypes of rs225014 in the DTC patients are presented in Table 1. TFT levels were comparable among the three genotypes except for T3 and T3/T4 ratios. T3 levels in the heterozygous (Thr/Ala) and homozygous (Ala/Ala) genotypes were significantly lower than the wild-type Thr/Thr genotype. The T3/T4 ratio was significantly lower in the Ala/Ala genotype as compared to the other two genotypes. TSH levels were adequately suppressed in all three genotypes due to L-T4 suppressive therapy. The difference in the L-T4 suppressive dose requirement between wild-type and mutant genotypes, i.e., Ala/Ala and Ala/Thr genotypes, was not statistically significant [Table 2].

Discussion

Various factors including SNPs affect intraindividual TH set point and their homeostasis.[18-20] DIO2 is a major

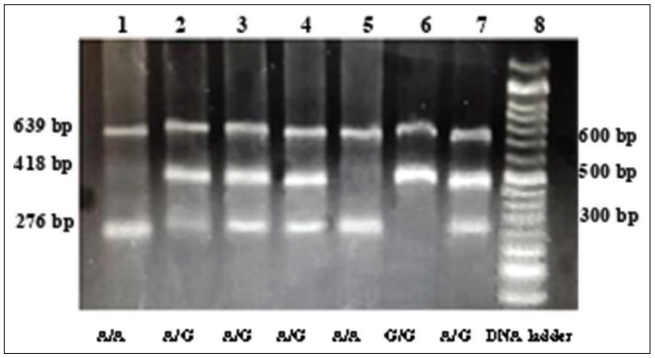

Figure 1: Lanes no. 1 and 5 show wild-type Thr/Thr genotype with band at 276 bp, Lanes no. 2, 3, 4, and 7 show heterozygous mutant Thr/Ala genotype with bands at 276 and 418 bp and Lane no. 6 shows homozygous mutant Ala/Ala genotype with band at 418 bp, the control band at 639 bp indicates product size of two outer primers, Lane no. 8 shows DNA ladder (product size for the Thr allele and Ala allele is 276 and 418 bp, respectively)

Table 1: Frequencies of Thr/Thr, Thr/Ala, and Ala/Ala genotypes of rs225014 in differentiated thyroid cancer and control groups along with the odds ratio evaluation to measure of strength of association

Subjects	Genotype frequencies			Allele frequencies	HWE (*P*)	OR
	Thr/Thr wild type, *n* (%)	Thr/Ala heterozygous mutant, *n* (%)	Ala/Ala homozygous mutant, *n* (%)			
DTC (*n*=157)	33 (0.21)	82 (0.52)	42 (0.27)	Thr (A)=0.47 Ala (G)=0.53	0.82	1.1 95% CI (0.6–1.9) *P*=0.7
Control (*n*=69)	17 (0.27)	34 (0.48)	18 (0.25)	Thr (A)=0.51 Ala (G)=0.49	0.93	

DTC: Differentiated thyroid cancer, HWE: Hardy–Weinberg equilibrium, OR: Odds ratio, CI: Confidence interval

Table 2: Thyroid function test parameters and L-T4 dosage comparison among the rs225014 DIO2 genotypes in the differentiated thyroid cancer group

Parameters (normal range)	DTC group genotypes (*n*)		
	Thr/Thr (26)	Thr/Ala (69)	Ala/Ala (39)
T4 (µg/dL): 4.2–13	13.3±3	13.1±3	13.4±3
T3 (µg/dL): 70–200	136±34	115±36*	113±28**
TSH (µIU/mL): 0.25–5.25)[#]	0.14 (0.01–3.9)	0.1 (0.01–5)	0.06 (0.01–2.1)
T3/T4 ratio	0.1±0.1	0.09±0.02	0.084±0.01**
FT4 (µg/dL): 0.65–1.8	1.9±0.8	1.82±0.4	1.9±0.4
Anti-TPO (U/mL): <12	4.5 (1.5–9.7)	4.8 (2–60)	5.6 (2–12)
Thyroxine dose (µg/day)	158±38	146±37	157±42

*P<0.01, **P<0.001, [#]Median and range given for TSH levels due to skewed distribution. DTC: Differentiated thyroid cancer, TSH: Thyroid-stimulating hormone, Anti-TPO: Antithyroid peroxidase

contributor to circulatory T3 as it has higher catalytic efficiency and lower Km than DIO1. It is also an important determinant of local T3 bioavailability and feedback regulation of pituitary TSH secretion.[3] Therefore, the SNPs associated with DIO2 are more likely to affect thyroid function parameters. The rs225014 SNP is an A/G polymorphism wherein a threonine (Thr) changes to alanine (Ala) at codon 92. This SNP is found to be more prevalent in the general population.[21-24] rs225014 has been reported to be associated with decreased DIO2 enzyme activity and linked with various disorders.[5-8,11-14,24,25] The frequency of homozygous and heterozygous rs225014 variants ranges from 0.12 to 0.34 and 0.39 to 0.60, respectively, in the patients' study population from different ethnicities.[5-7,9,14,16] In the present analysis, the frequency of homozygous and heterozygous rs225014 variants in the DTC patients' group and control group was on the higher side but was comparable to the frequency observed in the patients of Indian origin.[9] There are conflicting reports about the association of rs225014 SNP with serum TH levels. No association between the Thr92Ala SNP and TH levels was not detected in some of the reports.[10,16,23,26,27] However, Thr92Ala SNP was found to be associated with a TH level in some of the reports. Castagna *et al*. demonstrated that rs225024 reduced DIO2 activity and serum T3 levels in thyroid-deficient patients.[21] Butler *et al*. reported that the homozygous status for the rs225014 allele was associated with a decreased TSH-stimulated release of T3 from the thyroid in healthy subjects, indicative of a lower intrathyroidal conversion of T4 to T3.[4] In the present study, no changes in the serum T4, FT4, and TSH levels were observed among all three genotypes of rs225014 SNP. However, serum T3 levels and the T3/T4 ratio were significantly lower in the DTC patients with homozygous Ala/Ala allele indicating impaired T4 to T3 conversion. The reduction in the T3 and T3/T4 ratio was detected in the homozygous rs225014 carriers in the previous study carried out in type 2 diabetic patients as well.[9] No association between DIO2 SNP and thyroid cancer manifestation was observed in this study which is in accordance with previous studies.[15,16,28] Thyroidectomized DTC patients lack inherent T3 production and hence entirely depend on the DIO1 and DIO2 enzymes for catalytic conversion of L-T4 to T3. DTC patients are given L-T4 therapy to suppress TSH activity to keep the remnant malignant thyroid tissues dormant to inhibit the recurrence of thyroid cancer. However, DTC patients harboring rs225014 may require higher L-T4 to suppress TSH when compared to patients without the SNP due to differences in DIO2 gene makeup. Therefore, it would be important to identify the patients harboring rs225014 SNP to explore whether there is a requirement for redesigning the standard L-T4 suppressive therapy protocol. There are conflicting reports about the effect of rs225014 on the L-T4 dose requirement to suppress TSH in DTC patients. Torlontano *et al*. and AlRasheed *et al*. have speculated that DIO2 SNPs may have an important role in determining the L-T4 dose requirement in thyroidectomized and ^{131}I-treated DTC patients.[15,28] Torlontano *et al*. reported that the homozygous rs225014 variant was associated with approximately 20%

higher L-T4 dose requirement to suppress TSH levels in DTC patients.[15] However, Heemstra *et al*. and Santoro *et al*. did not find any association between rs225014 and higher L-T4 dose requirement.[16,17] In the present study, the observed difference in L-T4 dose requirement between the SNP carriers and the noncarriers was not statistically significant indicating no association between DIO2 rs225014 SNP and L-T4 dose. This probably indicated the presence of a compensatory mechanism to overcome DIO2 impairment. However, the nature of the present study was preliminary, and a large number of DTC patients need to be screened for confirmation regarding the possible variation in the L-T4 dose requirement in the patients harboring the rs225014 SNP. In addition, it would be important to analyze the synergistic effect of SNPs on the TSH suppressive L-T4 dose if more than one SNP are coexisting in DTC patients. Identification of the DTC patients' genetic susceptibility for differential response to L-T4 therapy would prevent delay in the evaluation of precise personalized TSH suppressive dose and hence would prevent the chances of recurrence or metastasis of the thyroid cancer. Nevertheless, it is essential to analyze the influence of genetic alterations in DTC patients for the precise implementation of the L-T4 therapy.

Conclusion

rs225014A was observed to be associated with T3 and T3/T4 ratio but not with the L-T4 dose in DTC harboring SNP suggesting the presence of a compensatory pathway to overcome DIO2 impairment. However, in the absence of any such salvage mechanism, DTC patients harboring rs225014 may require higher L-T4 doses to suppress TSH levels. Therefore, screening for rs225014 in the DTC patients showing reduced response to TSH suppression would enable quicker decision-making in the implementation of personalized L-T4 dose and save the patients from the development of any adverse effects. Nevertheless, it is essential to do a detailed study of the impact of the different SNPs associated with the DIO2 enzyme activity on the thyroid function and the treatment in the subjects from different ethnic origins as it is a key component in the TH signaling pathway.

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Conflicts of interest

There are no conflicts of interest.

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