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The association study between deiodinase2 gene polymorphism with thyroid nodule

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ABSTRACT

Introduction: A thyroid nodule is a palpable swelling in a thyroid gland with an otherwise normal appearance. Palpable thyroid nodules occur in 4 to 7 percent of the population. Nodular thyroid diseases are more frequent in women than in men. Most actions of thyroid hormone are mediated by the active form of thyroid hormone. D2 is essential for formation of active hormone of T3 through deiodination of triiodothyroxine. The single-nucleotide polymorphism D2 Thr92Ala is associated with decreased enzyme activity. The purpose of this study was to investigate an association of Rs225014 deiodinase2 gene polymorphism with thyroid nodule.

Methods: A case-control study comprising 110 patients with thyroid nodule and 110 healthy controls was performed. Thyroid nodule was established by ultrasonography. We analyzed D2 Thr92Ala polymorphism by Tetra-Arms PCR method. The distribution of alleles and genotypes of this polymorphism in two groups were determined and the data were analyzed by chi square and logistic regression analysis.

Results: In the 110 of case group, the frequency of TT, TC and CC genotypes of Rs225014 polymorphism were 25(80.6%), 81(44%) and 4(80%), respectively that indicated a significant difference compared with 110 of control group: TT 6(19.4%), TC 103(56%), CC 1(20%) (P 0.0001)

Conclusion: Rs225014 polymorphism in D2 gene is associated with thyroid nodule. However, further multicenteric and functional studies with more participants and evaluation for other polymorphisms in D2 gene are necessary to determine why this association is exist and determine its mechanism.

Key words: Thyroid nodule, Deiodinase2 Gene Polymorphism, Association

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Introduction:

A thyroid nodule is a palpable swelling in a thyroid gland with an otherwise normal appearance. Palpable thyroid nodules occur in 4 to 7 percent of the population (10 to 18 million persons), but nodules found incidentally on ultrasonography suggest a prevalence of 19 to 67 percent (1). The greatest prevalence of thyroid nodule is in pre-menopausal women, and the ratio of women to men is at least 4:1 (2). The clinical presentation of thyroid cancer is usually as a solitary thyroid nodule or increasing goitre size. Although thyroid nodules are common, thyroid cancers are rare (3). The prevalence of thyroid nodule is 5.9% (3% male, 8.3% female) in Iran. Thyroid nodules are 2.5 times more common in areas with endemic goiter (4).

Thyroid hormones play an essential role in organism development and in the maintenance of metabolic homeostasis (5). Most actions of thyroid hormone are mediated by the active form of thyroid hormone, T_3 (6). Deiodinase D2 is essential for the local production of T_3 through deiodination of triiodothyroxine (T4) (6). Several polymorphisms in D2 have been described (6-8). The single-nucleotide polymorphism (SNP) in D2 Thr92Ala is associated with decreased enzyme activity and greater insulin resistance in nondiabetic and in type 2 diabetes mellits (6-8). Also, Torlontano *et al*. reported in thyroidectomized differentiated thyroid carcinoma (DTC) patients that homozygous carriers of the D2-Ala92 allele needed higher dosages of T_4 (9). In this work, the association of thyroid nodule with SNP of Rs225014 deiodinase2 gene was evaluated.

Methods:

In this case-control study, 110 patients with thyroid nodule and 110 healthy control without thyroid nodule, with mean age 42 were consecutively recruited from the endocrinology clinic of Arak University of Medical sciences. Thyroid was assessed by ultrasonography and people based on the presence of thyroid nodule were classified in case and control groups. A written consent was obtained from all cases.

Genetic evaluation

Peripheral blood samples about 5cc in tubes containing EDTA (ethylene demine tetra acetic) were taken, was frozen and stored at -20 ℃ until the time of DNA extraction. Genomic DNA was extracted based on instructions of manufacturer by a genomic DNA purification kit (DNG plus DNA Extraction Kit, Sinaclon Co., Iran) and was held at 4℃ until performing PCR. Alleles and genotypes were assessed by Tetra-Arms PCR (Tetra-primer amplification refractory mutation system-polymerase chain). Primers were designed by Primer Blast tool.

PCR reactions were carried out in a volume of 25µL containing 12.5 µL of 2x PCR master mix (Sinaclon Co, Iran), 0.5 µL (10 pmol/µL) of internal primers , 0.7 µL (10 pmol/µL) of outer primers, 0.5µL (10Mm) $MgCl_2$, 1µL (~100 ng/µL) template DNA and 8.5 7µL deionized distilled water. Amplification was carried out in a Peqlab thermal cycler. PCR tests were done under the following program with Initial denaturation (4 min at 94℃) followed by 35 cycles (45 sec at 94℃, 45 sec at 55 ℃, then 45 sec at 72℃) and final extension 10 min at 72℃.

The PCR products were electrophoresed through 8% acrylamide gel, and visualized in the silver nitrate stained gels. Products of Tetra-ARMS-PCR reaction for Rs225014 polymorphism are including 500 bp (base pairs) (from activity of OF and OR primers as control) are present in all genotypes. T allele with 196 bp is result of activity of OF and IR primers and C allele with 324 bp is result of activity of OR and IF primers). Genotypes were defined as TT (500, 196), TC (500, 196 and 324) and CC (500,324). Primers sequences for detecting alleles and genotypes of Rs225014 (T>C) polymorphisms deiodinase2 gene are shown in Table 1.

Table 1. Primers used for detecting of alleles and genotypes of Rs225014 of D2.

	Name	5'.....3'		TM
1	Of	GAACTCTTCCACCAGTTTGCG		59.74
2	OR	GTTAACTCCAGTGCCTTCAGTG	CACTGAAGGCACTGGAGTTAAC	59.19
3	IF	CCACTGTTGTCACCTCCTTCTGT		63.32
4	IR	TGTGGTGCATGTCTCCAGTg	CACTGGAGACATGCACCACA	60.25

F1: Forward Internal, RI: Reverse Internal, FO: Forward Outer, RO:Reverse Outer

Table 2. Frequency of genotypes of polymorphism Rs225014

	Control N(%)	CASE N(%)	OR	95% CI Bunder	Upper	p-value	test Fisher exact	p-value
CC	4(80)	1(20)	1/04	11/09	0/09	0/9	16/34	0/0001
CT	81(44)	103(56)	5/29	13/52	2/07	0/0001		
TT	25(80/6)	6(19/4)	-	-	-			

Table 3. Allele frequency of polymorphism Rs225014.

	Control N(%)	CASE N(%)	OR	95% Confidence Interval Bunder	Upper	test X^2	p-value
C	89(45/9)	105(54/1)	0/744	1/08	0/51	2/3	0/12
T	131(53/3)	115(46/7)					

Statistical analysis:

Statistical analysis was performed by SPSS 16 (Chicago, USA). The allele and genotype distribution in the control group versus the case group was compared by Chi-squared or Fisher's exact tests. Mean and standard deviation Quantitative variables in the control group versus the case group was compared using t- student Logistic regression was used to evaluate. The odds ratio (OR) and 95% confidence interval (95% CI) provide a measure of the strength of association.

Results:

In this study, 4.1% of patients were men and 95.9% were women. The results were analyzed after detection of genotypes for target point in 220 samples.

In the 110 of case group, the frequency of TT, TC and CC genotypes of Rs225014 polymorphism were 25(80.6%), 81(44%) and 4(80%), respectively that indicated a significant difference compared with 110 of control group: TT 6(19.4%), TC 103(56%), CC 1(20%) (P 0.0001). Also, the frequency of C allele was more in case group than in control group and inversely T allele was more frequent in control group than in case group. The patients with thyroid nodule showed statistically significant lower frequency of TT and CC genotype compared to patients without thyroid nodule. The frequency of genotypes and alleles in case and control groups are shown in Table 2 and 3. The chance of thyroid nodule in persons who had CT genotype compared to TT genotype is significant (OR=5.29).

Conclusion:

In this study, we have performed a case-control study of genetic association between thyroid nodule and Thr92Ala polymorphism, which demonstrate that heterozygosis for this polymorphism have increased risk for thyroid nodule. The Thr92Ala polymorphism is characterized by the first amino acid substitution 114 located in the 115 instability loop of D2, a site of importance for D2 turnover rate (10,11). The association of polymorphism of Rs225014 with multiple disorder including insulin resistant and DM2 due to decrease of expression level of D2 (deiodinase2) was determined in many studies (12,13). Similar results with our study were reported by Peeters et al. (14) which found significantly lower plasma TSH levels and TSH/T4 ratios in heterozygous subjects, but no association with circulating iodothyronine levels.

Alina et al. (15) reported that the carriers of Ala92Ala genotype have high frequency of remission in Graves' disease patients because they had lower activity of the enzyme and less active education of T3 in tissues. Also, Gumieniak et al. (16) reported that Ala92 carriers of Type 2 iodothyronine deiodinase Thr92Ala significantly more frequent in hypertensive subjects compared to normotensive subjects (64.8% versus 47.1%; *P*_0.011).

Dora et al. (12) reported that homozygosis for the Ala allele of the single-nucleotide polymorphism Thr/Ala in codon 92 of the D2 is asssociated with increased risk for DM2.

Totally, this polymorphism is significantly associated with thyroid nodular disease. To explain of this association, there is maybe a role based on other studies for the effect of this polymorphism on

T_3 expression. This subject is needed to be more researched in aspect of induction isoform in thyroid tissue and also in aspect of the effect of allele in site.

We found a significant association of CC and TT genotypes of Rs225014 polymorphism of D2 and the presence of thyroid nodule. Although, we suggest further studies with larger sample sizes to confirm these findings.

Conflict

Authors declare no conflict of interest.

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