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Full Length Research Paper

Screening of g.IVS5+1G to a mutation of *TG* gene and thyroid hormone level among Iraqi thyroid disorders

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***TG* gene mutations have been identified in some people who have a goiter but normal or boarder line thyroglobulin levels. These mutations are either caused by gene deletion or single nucleotide mutation as a result. This study aim to screen the frequency of g.IVS5+1G to A mutation of *TG* gene and thyroid hormone levels among Iraqi thyroid disorders. g.IVS5+1G to A mutation of *TG* gene and thyroid hormones levels were detected in Iraqi thyroid disorders including 16 metastatic follicular thyroid carcinoma, 31 toxic goiter, 32 non toxic goiter and 19 hypothyroidism in addition to 25 normal control. The g.IVS5+1G to A mutation of *TG* gene screened by LAN-PCR. The heterozygous point mutation g.IVS5+1G to A was detected at position +1 of the splice donor site of intron 5 in 57% of cancer patients, 55% of toxic goiter and in 44% non toxic goiter patients but not in patients with hypothyroidism. The results also showed no correlation between hormones levels and detected mutation. We conclude that the g IVS5+1G>A mutation which caused fusion of exons 4 and 6 was detected in high frequency in Iraqi patients with thyroid disorders and no correlation was found between mutation and the levels of the thyroid hormones.**

Key words: *TG* gene, thyroid cancer, mutation, goiter.

INTRODUCTION

Thyroglobulin (TG) is a large glycoprotein homodimer of 2749 residues and plays a major role in thyroid hormone synthesis, secretion and in the storage of iodine (Kopp, 2002). It is coded by a single copy gene, 270 kb long (GenBank accession number NT_008046), that maps on chromosome 8q24 and contains an 8.5 kb coding sequence (Gen Bank accession noNM_003235) divided into 48 exons (Malthie`ry and Lissitzky, 1987; Vono-Toniolo et al., 2005; Mendive et al., 1997; 2001; Targovnik et al., 2010). The integrity of the thyroglobulin (TG) structure is very important for synthesis and metabolic pathway of tri-iodothyronine (T3) and thyroxine (T4) (Targovnik et al., 2010). Many *TG* mutations such as missense mutations, nonsense mutations, splice site mutations and nucleotide deletions were detected among patients with thyroid disorders including congentil goiter (Hishinuma et al., 1999, 2005, 2006; Caron et al., 2003; Gutnisky et al., 2004; Mendive et al., 2005; Rivolta et al., 2005; Alzahrani et al., 2006; Kitanaka et al., 2006; Caputo et al., 2007; Niu et al., 2009; Rubio et al., 2009; Spitzweg et al., 2010; Targovnik et al., 2012), endemic (Perez-Centeno et al., 1996) and non endemic goiter (Corral et al., 1993; Gonza´lez-Sarmiento et al., 2001). Most of these mutations lead to overt or compensated hypothyroidism and are usually accompanied by large goiters due to chronic stimulation of thyroid gland by

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Table 1. LNA primer sequences and LNA base modification used for PCR amplification of *TG* gene- Exon 5/Intron..

Mutation	LNA Primer Forward (5'→3')	LNA Primer Reverse (5'→3')
	***TG* gene**	
	Exon 5/Intron	
g.IVS5+1 G>A	FW-*TG*-5 tctggtccacagctacaacagg FM-TG-6 tctggtccacagctacaacaga	RW-*TG*-7 gatgtagtaggcaccctagccg

Table 2. The LNA PCR amplification conditions.

Program step	Temperature (°C)	Time	No. of cycles
Preheat	95	10 min	1
Denaturation	95	30 s	
Annealing	56	30 s	30
Extension	72	30 s	
Termination	92	10 min	1
	30	3 min	

thyroid stimulating hormone (TSH).

Splice site mutations in the previously studies showed that the development of a widely metastatic follicular thyroid carcinoma correlated with the patients whom *TG* gene mutation was homozygous to the mutation g.IVS5+1G>A (Ieiri et al., 1991; Targonvnik et al., 1993; Gutnisky et al., 2004). These studies were also shown that the heterozygous g.IVS5+1G>A mutation has no significant clinical and biochemical abnormality of TG synthesis.

No molecular analysis of Iraqi patients with heredity long time with goitrous hypothyroidism and metastatic follicular thyroid carcinoma before. The current study aimed to detect TG mutation (g.IVS5+1G) in groups of thyroid disorder patients included cancer, toxic, non toxic and hypothyroidism.

MATERIALS AND METHODS

Patients

During July 2009 to October 2009 one hundred patient samples (28 male and 72 female) and twenty five control (12 male and 13 female) (ages ranged from 17 to 79 years) who attend the endocrinologist in Nuclear Medicine Department in Baghdad were selected. Clinical, ultrasonication and serum thyroid hormone were used for diagnosis. 5 ml of venous blood sample was collected by trained nurses from each individual of both thyroid cancer and normal. All samples were obtained after informed consent of the participants prior to their inclusion in the study. A structured questionnaire was used to elicit detailed information on age, affected side, residence, type of feeding and family history of thyroid disorders.

Thyroid hormones measurement

The levels of T3, T4 and TSH were measured with enzyme linked fluorescent assay (ELFA) by using VIDAS T3, T4 and TSH kits (Biomorieux, France). A positive control is included in each VIDAS T3, T4 and TSH kits and the measurement ranges of the VIDAS T3, T4 and TSH, is 0.9 to 2.3 nmol/ 60 to 120 nmol/l and 0.4 to 4.0µIU/L respectively. Serum thyroxine T4, T3, and TSH levels were measured in Nuclear Medical hospital laboratory. The laboratory results were evaluated by endocrinologist and collaborating general practitioner.

Genomic DNA

Genomic DNA from peripheral blood leukocytes was isolated following standard procedure of Wizard Genomic DNA purification kit (A1120) which supplied by Promega.

Polymerase chain reaction-locked nucleic acid (PCR-LNA)

For detection the g.IVS5+1G>A, three Locked Nucleic Acid (LNA) primers were designed using NCBI tools (Table 1) then used in PCR (Obika et al., 1998; Singh et al., 1998; Koshkin et al., 1998; Jarry et al., 1998). The optimum PCR conditions were listed in Table 2.

RESULTS

Among 53 *TG* mutations, 26(49.1%) were detected as heterozygous guanine to adenine transition g.IVS5+1G>A at position +1 of the donor splice acceptor site in exon-intron 5. Most of g.IVS5+1G>A mutations

Table 3. The mean and standard deviation of serum thyroid hormone levels and mutation screen among Iraqi thyroid disorders.

Patients group	n	g. IVS5 +1G<A	TSH (0.4-4.0 µIU/L)	T4 (60-120 nmol/l)	T3 (0.9-2.3 nmol/l)
cancer	3	Negative	0.17±0.115	120.3±34.79	1.03± 0.404
cancer	4	Positive	0.10 ± 0.000	117.3± 34.49	1.55 ±0.545
Toxic Goiter	5	Negative	0.23 ± 0.189	18.7± 16.36	2.24 ±1.250
Toxic Goiter	4	Positive	2.43± 2.265	25.8± 41.50	1.90 ±2.156
NonToxic Goiter	3	Negative	66.7± 33.68	1.2 ±0.33	129.6 ±197.71
NonToxic Goiter	7	Positive	106.0 ±54.56	2.7 ±1.45	11.6 ±6.01
Hypothyroidism	3	Negative	7.43 ±2.873	95.0 ±23.64	1.30 ±0.265
Hypothyroidism	0	Positive	-	-	-
control	3	Negative	1.0± 1.67	102.7 ±57.5	1.23± 0.643
control	0	Positive	-	-	-

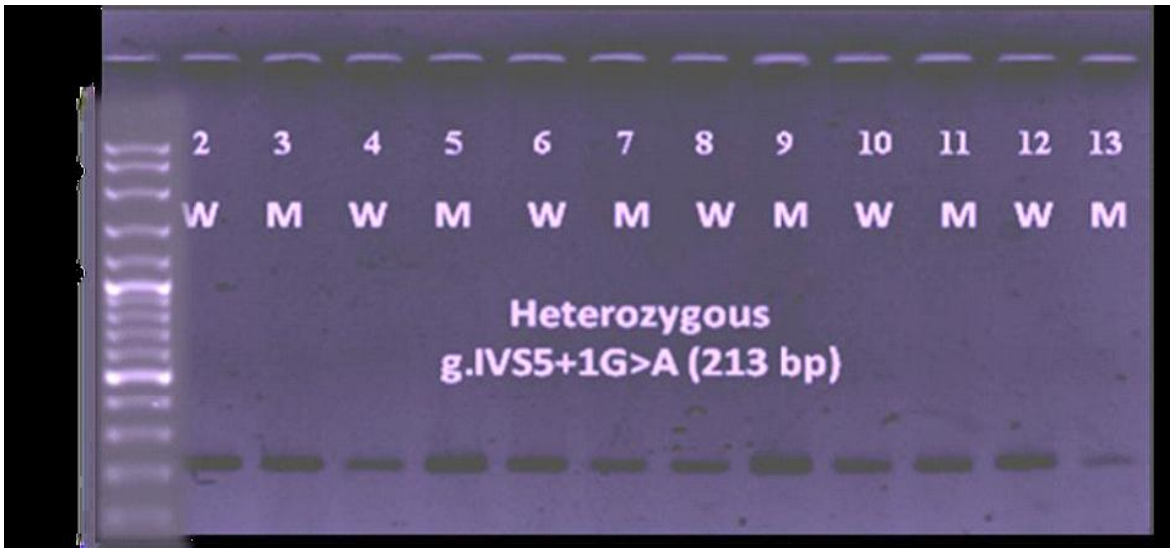

Figure 1. Ethedium bromide stained 1% agarose gel shows screening of DNA thyroid disorders samples for TG gene heterozygous g. IVS5+1G<A mutation by LNA-primer PCR, for 45 mins at 100 Volts. The wild type bands are present in all PCR products, lane mutant an amplified product (213bp) for mutant primer and the sequence of wild and mutant types.TG gene Wild type E5… .ggt cca cag cta E5+1I5 G ta agg gg ….. TG gene Mutant type E5 … ..ggt cca cag cta E5+1I5 A ta agg gg …….

were identified in thyroid toxic goiter and thyroid cancer (9 mutations each) and the rest *TG* gene mutations were detected as 4 mutations in hypothyroidism and 4 mutations in thyroid non toxic goiter (Table 3 and Figure 1). The thyroid hormones levels showed a complex pattern. TSH decreased level was detected in all thyroid cancer patients (including positive and negative to g.IVS5+1G>A mutation) with normal T3 and T4 levels. In patients with non toxic goiter TSH elevated level was detected along with T3 level and decreased T4 level in both mutation positive and negative groups. Elevated TSH was also detected in patients with hypothyroidism which was all negative to the mutation. Normal TSH and T3 levels were detected in all toxic goiter patients with low T4 level.

DISCUSSION

TG mutation g.IVS5+1G>A is a splice donor site mutation of intron 5 of the *TG* gene. The mutation caused skipping of exon 5. This resulted in the frame shift at codon position 141 and a premature stop codon at position 147, which result in a severely truncated TG (thyroglobulin) polypeptide chain (Alzahrani et al., 2006). The g.IVS5+1G>A mutation we identified could be cause a

shift of the reading frame and result in a severely truncated TG polypeptide chain. TG truncations were also detected in the g.IVS34−1G>C and g.IVS10−1G>A. g.IVS34−1G>C mutations include G to C transversion at position -1 in the acceptor site of intron 34(Carina et al., 2006; Hishinuma et al., 2006). This suggests that the splice site mutation might generate a total elimination of exon 35 generating frame reading mutation which produced a truncated protein. G to A transition was also detected at position -1 in the acceptor site of the intron 10 (g.IVS10−1G>A) causing skipping of exon 11 resulting substitution of (Asp-His) in the position 409. Such mutations were also detected by others (Carina et al., 2006; Hishinuma et al., 2006). Several other mutations in this gene have been reported. Many of these mutations occurred in the absence of exons from the gene transcript because of transversion at positions in the acceptor splice sites of many introns that replace the normal bases with abnormal ones (Yardena et al., 2007; Hector et al., 2010).

The results of thyroid hormones levels associated with g.IVS5+1G>A mutation showed no correlation between them where high levels of TSH,T3 and low level of T4 were detected in patients with non toxic in both positive and negative to g.IVS5+1G>A mutation and high level of TSH hormone was also detected in non mutant hypothyroidism patients. Moreover, decreased levels of TSH and T4 hormones were detected in cancer and toxic patients respectively in both positive and negative to the g.IVS5+1G>A mutation. This suggests that a normal copy of *TG* gene is sufficient to compensate the functional loss of the defective copy of the *TG* gene and other gene mutations could be involved. Gene mutations other than *TG* gene mutations were detected to be involved in thyroid disorders including TPO mutations (Ris-Stalpers and Bikker, 2010; Grasberger, 2010; Belforte et al., 2011), DUOX2 and DUOXA2 (De Marco et al., 2011; Neves et al., 2011), THOX2 (Pfarr et al., 2006), Foxe1/TTF2 (Castanet and Polak, 2010) and RET oncogene (Dvoraka et al., 2006; Moura et al., 2009; Hedayati et al., 2011). Normal levels of thyroid hormones were also detected in clinically unaffected persons with g.IVS5+1G>A heterozygous mutation (Alzahrani et al., 2006).

We conclude from these results that the g.IVS5+1G>A heterozygous mutations detected in this study have no effect on the thyroid hormones production since the levels appeared fluctuated in random pattern and other gene mutations could be involved.

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