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 border 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 congenital 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; Gonzalez-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
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.

<table>
<thead>
<tr>
<th>Program step</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>No. of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preheat</td>
<td>95</td>
<td>10 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>30 s</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>56</td>
<td>30 s</td>
<td>30</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>30 s</td>
<td></td>
</tr>
<tr>
<td>Termination</td>
<td>92, 30</td>
<td>10 min</td>
<td>1</td>
</tr>
</tbody>
</table>

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 (Biomerieux, 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.

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

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
n 11 resulting
ital study have no
, Targovnik HM
. Endocrinol. Metab., 88: 3546
, Dvoraka
ated with
exon from the gene
Dvoraka
would like to thank Suha Azize and Mrs. Faeza Ahmed in
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REFERENCES
Follicular Thyroid Carcinoma A rising from Congenital Goiter as a
result of a Novel Splice Donor Site Mutation in the Thyroglobulin
Carina M, Rivolta CM, Targovnik HM (2006). Molecular advances in
Novak, BB (2006). New multiple somatic mutations in the RET proto-
oncogene associated with a sporadic medullary thyroid carcinoma.
Thyroid, 16(3): 311-316.
Gonzalez-Sarmiento R, Corral J, Morries MT, Corrales JJ, Miguel-
region of the thyroglobulin gene as a cause of sporadicvnonendemic
simple goiter. Thyroid, 11: 789-793.
Grasberger H (2010). Defects of thyroidal hydrogen peroxide generation
Gutnisky VJ, Moya CM, Rivolta CM (2004). Two distinct compound
heterozygous constellations (R277X/IVS34-1GNC and R277X/R1511X)
in the thyroglobulin (TG) gene affected in individuals of a Brazilian kindred with congenital goiter and defective
substitutions(C1263R and C1995S) of Thyroglobulin cause a defect
in intracellular transport of thyroglobulin In patients with congenital
goiter and the variant type of adenomatous goiter.
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 Bikkor, 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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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).

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