Detection of homozygous g.IVS5+1G>A, g.IVS34-1G>C and c.886C>T mutations among Toxic Goiter and Thyroid Cancer patients in Iraq.

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Introduction

1. Thyroglobulin (TG) gene is a key element in thyroid hormones synthesis and production.
2. It codes thyroglobulin protein, a large homodimeric protein with a molecular weight of 330 KDa per monomer.
3. TG protein iodinated by thyroid peroxidase (TPO) via forming thyroxine (T4) and triiodothyronine (T3) in process done by thyroid follicular cells.
4. The failure of this process led to a variant type of thyroid disorders.

5. Most of this disorders due to mutations occurred either in TG genes or TPO gene.
6. The most frequent mutations detected were heterozygous mutations in TG gene.
7. A rare homozygous mutations of the TG gene were recorded among thyroid disorders until now.
8. These including mutations : g.IVS5+1G>A, c.886C>T, g.IVS30+1G>T , p.Q2142X , p.C1245R, p.C1977S and p.C1058R.
9. On the other hand, no homozygous g.IVS34-1G>C mutation is detected in thyroid disorders yet.

Aim of study:

The present work aimed to detect the frequency of TG homozygous mutations among thyroid disorder patients.

Material and Methods

48 Patients .Ages 30 to 70 years

.18 male

.30 female

31 with Toxic goiter

.17 with Thyroid Cancer

The patients were attended the endocrinologist in Nuclear Medicine Hospital and Al Yarmok Nuclear Medicine Department in Baghdad-Iraq during a period from July 2009 to October 2009.

Isolation of genomic DNA

Three ml venous blood was collected in EDTA tubes and genomic DNA was extracted according to DNA extraction Kit protocol.

Locked Nucleic Acid (LNA)-primers-PCR

LNA-primer PCR was carried out using designed mutations primers including g.IVS5+1G>A, g.IVS34-1G>C and c.886C>T located in exons 5, 34 and 10 of *TG*, respectively.

Table 1: LNA- primer sequences and LNA base modification used for PCR amplification of *TG* gene.

Mutation	LNA Primer Foreward (5'→3')	LNA Primer Reverse (5'→3')
TG gene Exon 5/Intron g.IVS5+1G>A		
	FW-*TG*- tctggtccacagctacaacagg	RW-*TG*- gatgtagtaggcaccctagccg
	FM-TG- tctggtccacagctacaacaga	
Exon 7 c.886 C >T		
	FW-TG- caatcagtcatctctggcagattcc	RW-TG- ggcggcagcttggaacaa
	FM-TG- caatcagtcatctctggcagattct	
Exon 34/Intron IVS34+1 G>C		
	FW-TG- ccttcggatggtaccagaagcccag	RW-TG- atcatggcacactgaagaagttg
	FM-TG- ccttcggatggtaccagaagcccac	

PCR Program Steps + Conditions:

	Temperature	Time	No. of Cycle
Preheat	95 ºC	10 mins	1 cycle
Denaturation	95 ºC	30 sec	30 cycles
Annealing	56 ºC	30 sec	
Extension	72 ºC	30 sec	
Termination	92 ºC	10 mins	1 cycle
	30 ºC	3 mins	

Results

- 26 *TG* mutations were detected, 13(50%) in thyroid cancer and 13(50%) in toxic goiter.
- Among these mutations, 23 mutations were detected as heterozygous mutations(Data not seen) and 3 mutations as homozygous.
- Two of these homozygous mutations were detected in two toxic goiter patients as guanine to adenine transition g.IVS5+1G>A at position +1 of the donor splice acceptor site in exon-intron 5 (Figure 1) and transversion that replace guanine by cysteine (g.IVS34-1G>C) in the exon 34 (Figure 1).
- The third homozygous mutation was detected among one thyroid cancer patient as transition that replace cysteine by thymine c.886C>T in the exon 7 (Figure 2).

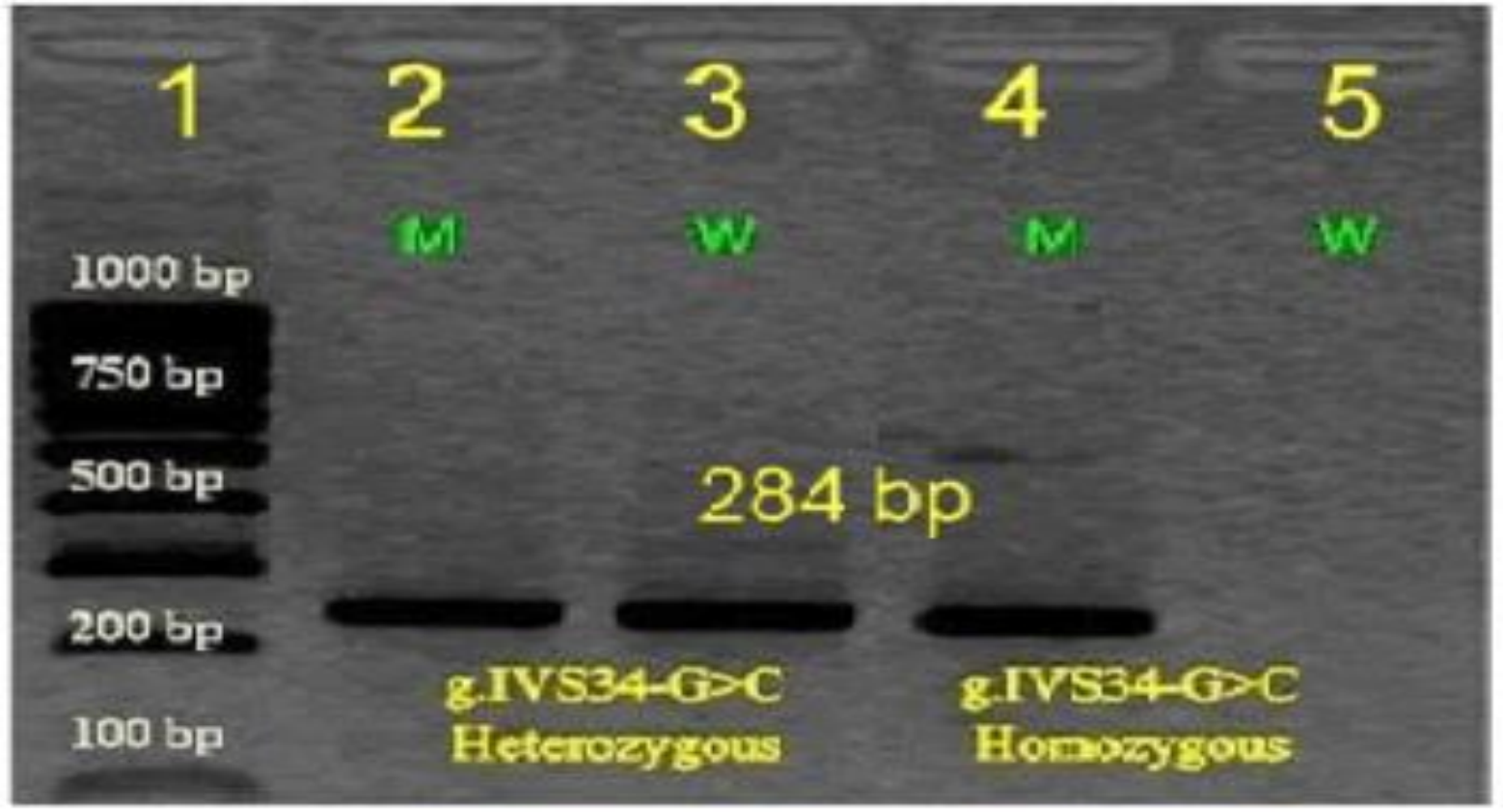

Figure 1: Ethidium bromide stained 1% agarose gel, showed TG gene heterozygous and homozygous g.IVS34 G>C mutations among toxic goiter patients. Lane: 1 Marker, Lanes 2 and 4: mutant (M), Lanes 3 and 5: wild types (W)samples.

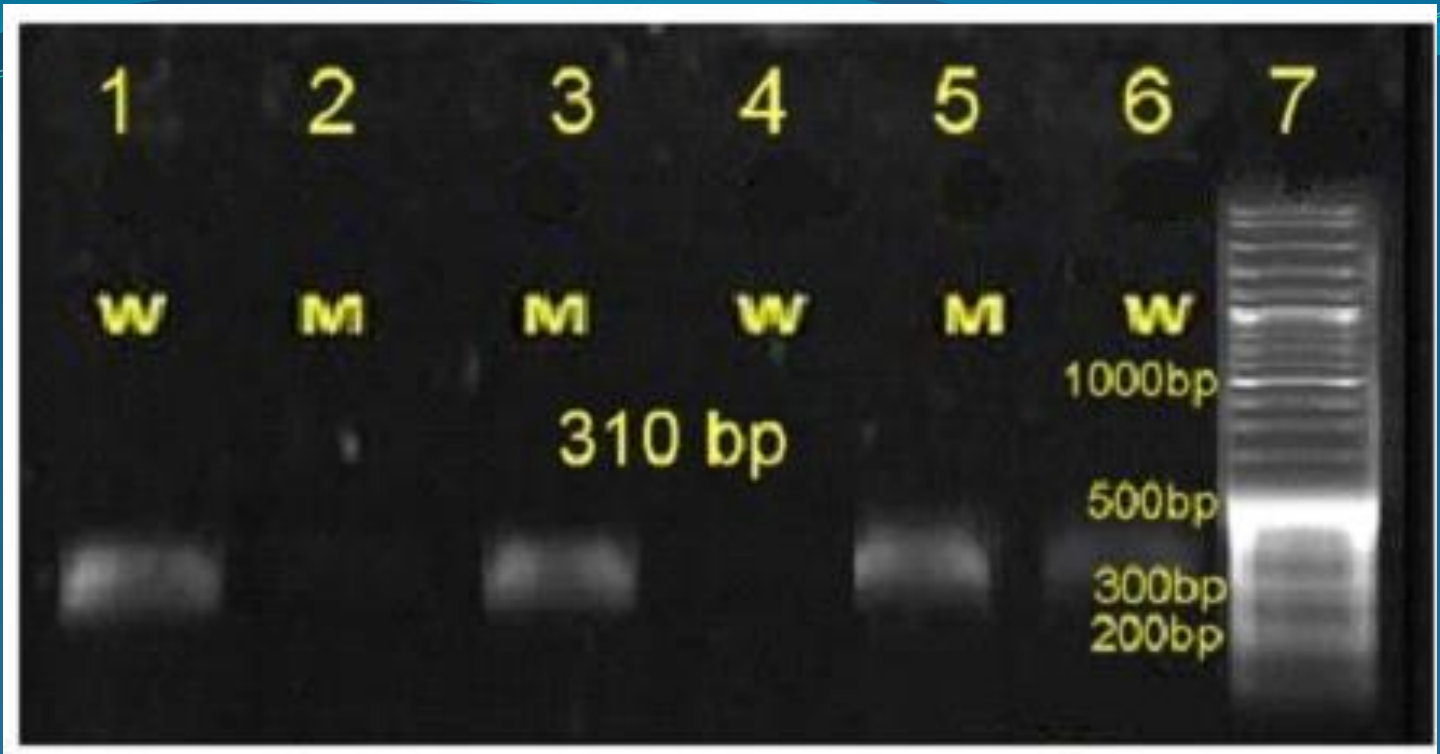

Figure 2: Ethidium bromide stained 1% agarose gel, showed TG gene heterozygous and homozygous c.886 C>T mutations among thyroid cancer patients. Lanes 1,4 and 6: wild types samples(W) , Lanes 2, 3 and 5: cancer samples(M) (Lane 2 no mutation, Lane 3 homozygous mutation and Lane 5 heterozygous mutation) and Lane 7: marker.

Conclusions

1. g.IVS5 +1 G>A mutation was found to be the most frequent mutation among toxic goiter and thyroid cancer groups.
2. Three homozygous mutations were also detected among thyroid toxic goiter and thyroid cancer groups which reflect high DNA instability among thyroid toxic goiter and thyroid cancer groups.

Recomendations:

Further studies need to carry out to understand the role of these mutations in thyroid disorders.

خلاصة

حللت 48 عينة DNA تعود الى 31 مريض بتضخم غدة الدرقية السام و 17 مريض بسرطان الغدة الدرقية من أجل تعقب الطفرات الوراثية g.IVS5+1G>A, g.IVS34-1G>C, c.886C>T لمورث الثايروجلوبيولين Thyroglobulin (TG) . وجد من خلال التحليل ان هناك ثلاثة طفرات وراثية متجانسة الزيجة Homozygous . أثنان منها تم تعقبها في مريضين بتضخم غدة الدرقية السام و كانت الطفرات عبارة عن استبدال مكافئ للجوانين بالادنين (g.IVS5+1G>A) في الموقع +1 لفاصل المحور 5 و استبدال غير مكافئ للجوانين بالسايتوسين (g.IVS34-1G>C) في المحور 34 . أما الطفرة الثالثة متجانسة الزيجة المسجلة فقد تم تعقبها في مريض بسرطان الغدة الدرقية و كانت عبارة عن استبدال مكافئ للسايتوسين بالثايمين في المحور 7 (c.886C>T). لم تسجل سابقا الطفرة الوراثية (g.IVS34-1G>C) كطفرة متجانسة الزيجة عند مرضى الغدة الدرقية و سجلت هنا لاول مرة عند مريض واحد مصاب بتضخم الغدة الدرقية السام.

THANK YOU SO MUCH FOR LESTINING