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Ultrasound features and molecular diagnosis testing of thyroid mass

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Abstract:

Thyroid gland is one of the most important glands in human body due to the necessary hormones it produces. It can be affeced by different types of infections and diseases, one of the most risky abnormalities of this gland is thyroid masses. The aim of this study is to find how does thyroid masses start from the molecular stages and is there any relation with it and genetics, for this purpose we scanned more than 273 patients by ultrasound from different ages groups, and genders, thyroid mass detected in 17 of them, 3 with cystic fluid filled mass, and 14 with solid mass . Blood samples from thes patients were analysed and molecular study were performed by taking exon 11 and 15 from Braf gene mution was detected in all patients included frame shift mutaion a deletion of adenine in same position , while no sequence variation was found in the exon 11 Braf gene. In conclusion According to DNA sequence analysis, genomic variation was not found in exon 11 of the BRAF gene in all patients, an exonic alteration was detected in exon 15 in 7 patients was deletion of (A) in coding nucleotide , which the BRAF mutational hot spot region are found. The result of the deletion mutation is conversion of all of codons after deletion.

Key Words: *Thyroid mass, Braf gene, DNA sequencinq*

Introduction:

One of the largest endocrine glands in human body is Thyroid gland. The shape of healthy normal thyroid lobe is pear-shaped it looks like a butterfly in the transverse view. Its location is in front of the trachea just inferior to the thyroid cartilage. Thyroid gland is located in front of the larynx and trachea in the neck at the level 5th, 6th 7th cervical spine and 1st thoracic vertebrae. It weighs about 25 gm, it is a highly vascular gland and a fibrous capsule surrounding it (Khatawkar et al 2015). It consists of two lobes, one on either side of the upper cartilaginous rings of the trachea. A narrow isthmus joins the lobes like a bridge, lying in front of the trachea. The lobes are about 3 cm wide and 5 cm long. The posterior surface of each lobe The Two parathyroid glands lie against and are sometimes embedded in thyroid tissue. Figure 1 shows the anatomy, and the position of thyroid gland as well as right and left lobe for a human being. Measurement of the thyroid involves three measurements, which are the width, depth and length (Kollorz et al 2008). The normal thyroid gland is 2cm or less in width and depth and 4.5 – 5.5 cm in length.

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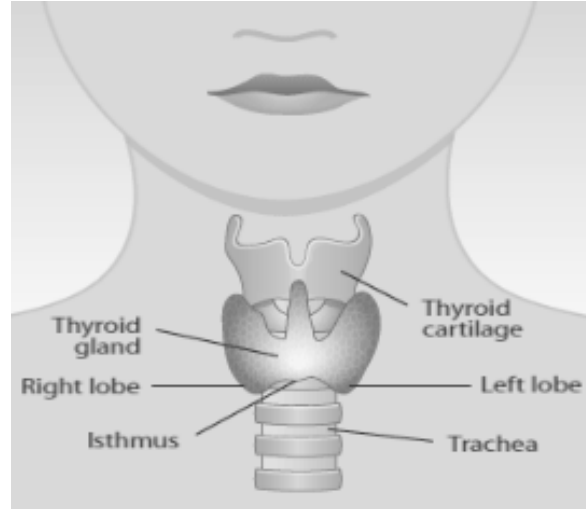


Figure 1 anatomy and position of thyroid G(http://underactivethyroid.net/ (date access, 8 August 2011)

Thyroid nodular disease is to commom clinical finding. The prevelence of palpable nodules ranges from 3-7% in the general population (Gharib et al., 2008). Thyroid nod-

ule an abnormal growth of thyroid cells that forms a lump within the thyroid while most thyroid nodules are non-cancerous(benign), 5-10% are canceous (malignant) (Jeong, 2013). The etiology of thyroid tumors is multifactorial, including environmental, genetic and endogen hormonal factors. Iodine deficiency is the major environmental factor, contributing the development of both endemic and sporadic nontoxic nodular goiter (Knauf et. al. 2005). The evidence of genetic predisposition is best established for medullary carcinoma and approximately 25% of these tumors. BRAF gene located on chromosome 7q34 encode a cytoplasmic serine kinase (Cantwell-Dorris et al. 2011). One of the most studied somatic point mutation in thyroid cancer is BRAF gene mutation that leads to the contitutive activation of BRAF kinase and stimulation of MARK pathway that is tumorigenic for thyroid cells (Knauf et al., 2005). Asymptomic thyroid nodules are detected up to 65% on ultrasound and 50% in pathologic examination of autopsy studies(Knauf et. al. 2005). For small, soild nodules, experienced cytopathologists can accurately distinguish benign nodules and papillary cancers. However, cytological features do not distinguish benign from malignant follicular neoplasms, and cystic papillary thyroid cancers are a common cause of false nagative results(Farid, 2005). The recent use of molecular markers has been helpfule in sorting out the presence of cancer, espacially the presence of the BRAF gene mutation(Jeong, 2013). The aim of this study was to determine wheather several features (ultrasound appearance of the thyroid mass and the presence of BRAF mutation) are helpful to predict cancer in thyroid nodule.

Materials and Method:

Patients

In the present study (273) of cystic/ solid mass and normal samples of thyroid (273) were obtained by ultrasonography at harer hospital in Erbil- Iraq. Genomic DNA was extracted from peripheral blood of (17) patients and normal samples and placed in EDTA tubes and kept at -20 C°until DNA extraction.

Table (1) The number of patients samples, according to gender and type of mass

mass types	Male/	Female	Total
Cystic	-	3	3
Soild	3	11	14

Imaging studies Ultrasonography:

Thyroid masses is one of the most risky types of the thyroid diseases, in this study more than 25000 patients scanned by ultrasound for different purposes as general ultrasound in Harer general hospital, 273 of them scanned for thyroid. 17 patients among them have thyroid mass, the scanning procedure started with lying supine position with full neck flextion on the scanning patient's bed, fine small pillow positioned beneith the neck, after that translucent gel applied on the frontal neck (Adam's appl), then by using linear probe (transdducer), with (6.5-8.2 MHz) frequency by 2D ultrasound machine. The best view of thyroid gland is transvese (coronal) section with caudally angulation and from right to left directions to get the best view of the both lobes with isthimus, parameters that should been measured and are echotexture of the gland's lobes, its size, detecting the mass, differentiating between it whether it is cystic or solid mass echogenecity (hyper or hypoechoic), then measuring it. After scanning thyroid gland and detecting the abnormality, blood were collected from the patients for molecular study as shows in figures (2,3,A,B).

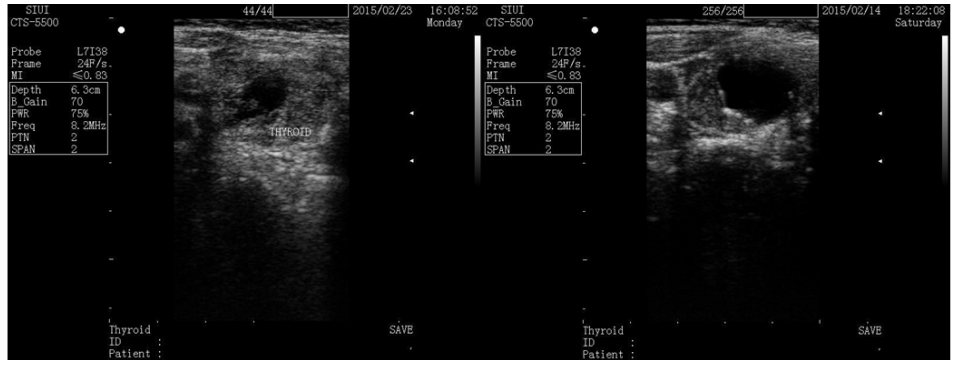


Figure . 2. Shows Lt. lobe of thyroid gland with a large fluid filled cystic mass which may be related to infections or hormonal disorders, the image shows Lt. lobe completely in transverse section and the fluid collections insde thyroid nodule clearly defined with an increasing of the Lt. lobe's size due to the internal cyst.

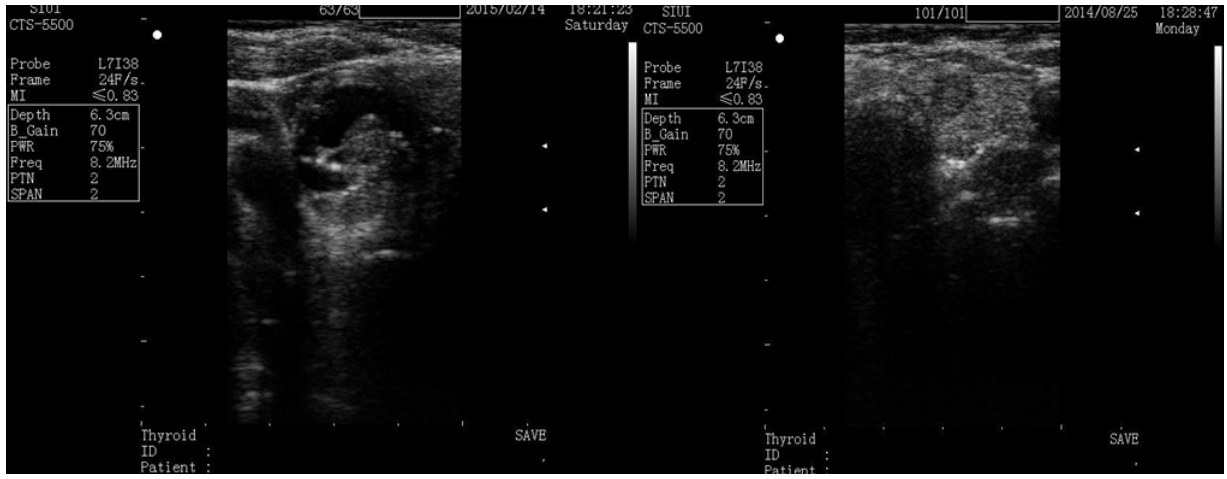


Figure. 3 . A. Shows Lt. lobe of thyroid gland with mixed cytic/solid mass. B. show a hypoechoic solid mass in the upper part of the Lt. lobe of the thyroid gland

Molecular study:

DNA samples from the blood of patients with thyroid masses were extracted using a commercial extraction kit (geneaid, USA) according to manufacture's instruction. Quantification and qualification of DNA concentration was performed using Nanodrop (Thermo Scientific. UK) . The PCR was carried out using specific pair of primers for exons 15, and 11 of BRAF gene Forward 5'-TCCTATGTTCATATTGCTACCTCAA- 3' , Revers 5'-TCCCTGTGGAAAAAGTGAGAAACAA-3' for exon 15 and Forward 5'-TCCTATGTTCATATTGCTACCTCAA- 3' ,Reverse 5'ACTGTGGAAAAAGTGAGAAACA AA-3' for exon 11 respectivly. Using the following PCR thermal program initial denaturation at 95C° for 5 minutes, followed by 30 cycles for denaturation at 30 seconds, annealing at 60.9 C° for exone 15 and 60.1 C° for exon 11for 30 seconds and elongation at 72 C° for 30 seconds. After the last cycle a final extention at 72 C° for 5minites was performed. The result ant PCR products were ; separeted and visualized, a 2% agarose gel stained with safe stain (fig 3). DNA sequencing of PCR- amplified product was carried out bidirectionally.

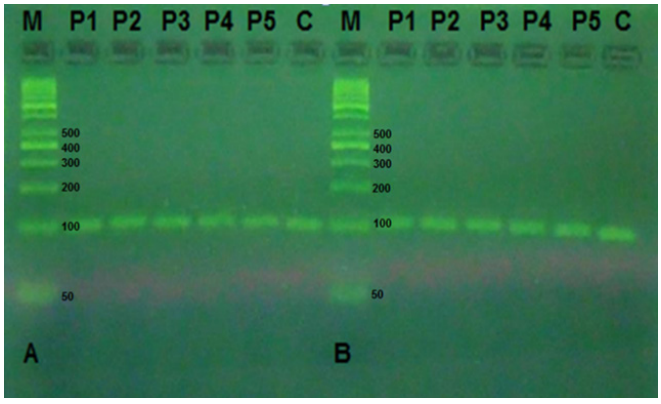
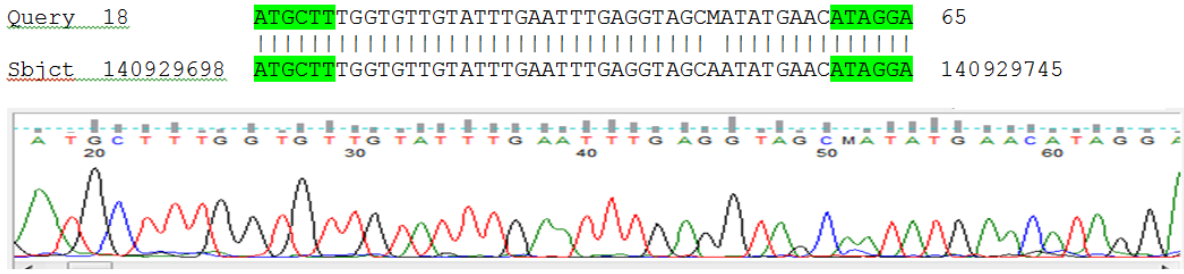


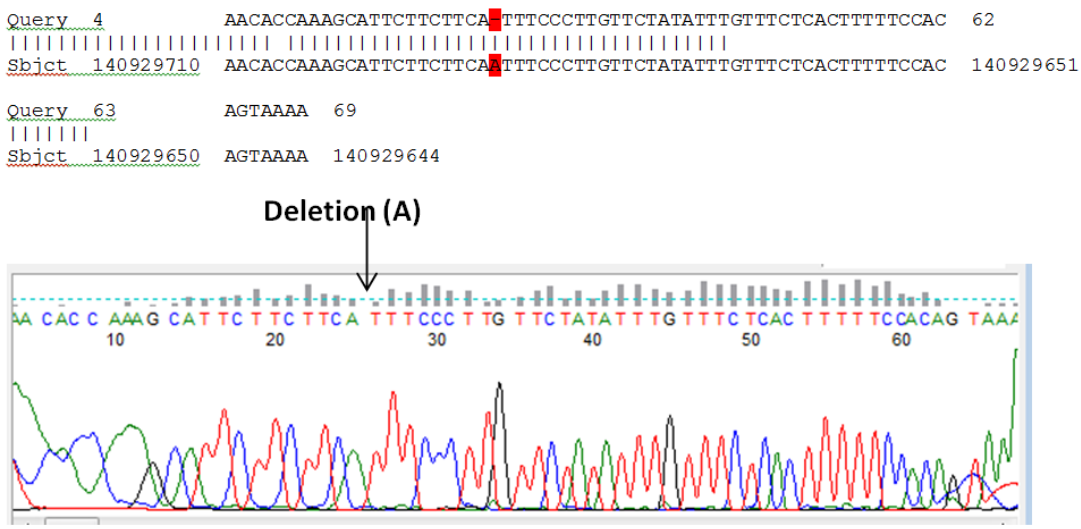
Figure (3) Electrophoregram of the safe stain stained 2% Agarose Gel showing Amplified PCR product of Exon (A=11, B=15,) the (M) refers marker, (P) refers to patient and (C) refers to control involved in present study. And length product exon (11=100, 15=98).

Result and Discussion :

To evaluate the pattern of somatic mutation in BRAF, we screened the coding sequence in exon 11, and 15 for mutations in genomic DNA. Using the PCR product , and sequencing. The DNA sequence of BRAF gene exone 15 and 11 was obtained from NCBI website. According to DNA sequence analysis, genomic variation was not found in exon 11 of the BRAF gene in all patients(Fig 4)., an exonic alteration was detected in exone 15 in 7 patients was deletion of (A) in coding nucleotide , which the BRAF mutational hot spot region are found (Fig 5). The result of the deletion mutation is conversion of all of codons after deletion.



Figure(4) DNA sequencing showing there is no any sequence variation in exone 11 Braf gene in all patients with thyroid mass.



Figure(5) DNA sequencing showing a fram shift mution as a result of deletion of adinine at position (140929688) in exone 15 Braf gene in all patients with thyroid mass.

Ultrasound scanning is one of the best imaging modalities to diagnose thyroid masses or abnormalities, furthermore it provides full information about the gland whether it is normal or not, what is the abnormality as there are different types of thyroid abnormality, one of the most risky abnormalities is thyroid mass. Ultrasound helps to define the mass as cystic (fluid filled) or solid (hyperecoic, or hypoechioc) mass, measuring its size, and detecting microcalcification, all of these features can be detected by conventional 2D ultrasound scanning. Thyroid nodules are common, and are palpable in approximately 5% of normal adults (Vander et al. 1968) and visualized by sonography in one-third or more of normal adults (Brander et al. 1991). 5–8% of palpable thyroid nodules are cancerous, a major task of the initial evaluation of thyroid nodules is to rule out malignancy (Werk et al. 1984, Belfiore et al. 1989).

Numerous studies have consistently shown a high prevalence of BRAF mutation in thyroid cancer, ranging from 29 to 83% (Namba et al. 2003, Kim et al. 2004)

Recently, occurrence of activation mutations with high frequencies is discovered in BRAF gene. About 43 somatic mutations in BRAF gene were discovered which are associated with human cancers and located in exon 11 and 15 (Davies et al., 2002; Pakneshan et al., 2013).BRAF mutations probably were involved in tumor initiation. Most of the PTCs are removed by surgery in early stages regardless to genotype. Many studies indicate that PTC carrying BRAF mutations are predominant among cases representing during

advanced stage. Two mutation hot spots were discovered in BRAF gene exon 11 and 15, the most common point mutation is T1796A transversion which occurs in exon 15 while lead to glutamine for valine substitution (V600E). V600E somatic mutation codes constitutively active B-RAF kinase (Ranjbari et al. 2013). Guan et al. (2009) reported that there is an association between BRAF mutation and iodine intake. According to their report BRAF mutation was found in 69% of PTCs in high iodine content in natural drinking water but it was found onlyin 53% of PTCs in normal iodie content natural drinking water in china.

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خصائص أورام الغدة الدرقية بواسطة الموجات فوق الصوتية وتشخيص التحليل الخلوي(الجزيئي)

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الخلاصه:

الغدة الدرقية هي واحدة من أهم الغدد في جسم الإنسان وذلك بسبب ضرورة الهرمونات التي تنتجها. ويمكن ان تتأثر بأنواع مختلفة من الالتهابات والحالات المرضية ، وأحد هذه من الحالات الغيرطبيعية والأكثر خطورة هي أورام الغدة الدرقية . الهدف من هذه الدراسة هو معرفة كيف تبدأ هذه الاورام من المراحل الخلوية وهل يوجد بينها وبين الجينات او المورثات اية علاقة ام لا في علم الوراثة، لهذا الغرض تم فحص وتشخيص 273 مريضا بواسطة الموجات فوق الصوتية لمجموعات مختلفة من الأعمار والأجناس، تم تشخيص أورام الغدة الدرقية في 17مريضا ، 3 منهم كانوا ذو أورام كيسية مليئة بمادة سائلة ، و 14 حالات ذو أورام صلبة. وقد تم تحليل عينات الدم من هؤلاء المرضى و ثم أجراء الدراسة الجزيئية عن طريق أخذ اكسون 11 و 15 من البراف جينgene BRAF, تم تشخيص الطفرة من نوع الحذف للقاعدة النيتروجينية الأدنين في الموقع نفسه من كل المرضى ، في حين لم يظهر اختلاف في التسلسل في اكسون 11 من البراف جينBRAF gene .