THE ROLE OF GENETIC MUTATIONS OF BRAF AND RET IN PAPILLARY THYROID CARCINOMA IN IRAQI PATIENTS: CROSS SECTIONAL STUDY

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ABSTRACT

Background: Thyroid cancer is one of the most frequent malignant lesions seen in daily surgical practice and women are far more frequently affected than men. The diagnosis of thyroid abnormality in general and thyroid cancer in particular is primarily based on histopathological diagnosis and microscopical features that are often characterized by conventional H and E stain and in certain situations immunohistochemical stain may be helpful. The subtle intermingling among various histopathological classes of thyroid cancer suggested the revaluation of the diagnosis classification of thyroid cancer based on the existence of certain genetic mutations such as BRAF and RET genes mutations.

Aim of the study: The current study was assess the prevalence rate and types of BRAF and RET gene mutations in Iraqi patients with papillary thyroid cancer and their possible diagnostic and prognostic role.

Patients and methods: This cross sectional study was done on 45 patients with papillary thyroid carcinoma. The age range was from 26 to 50 years. 4 male and 41 female were enrolled in the study. The patients have been diagnosed as papillary thyroid cancer by two pathologists depending on histopathological criteria of the disease. FISH CytoCell RET Breakapart (10q11.21) from (Oxford Gene Technology-USA), and BRAF gene mutation detection kit were used. The procedure is according to user guide provided by the manufacturer.

Results:

Conclusion: High rate of BRAF and RET mutations in men with thyroid papillary cancer in comparison with women is suggestive of prognostic role for these mutations beside their diagnostic properties.

Key words: BRAF, RET mutation, papillary thyroid carcinoma, Iraqi

I. INTRODUCTION:

Thyroid carcinoma is one of the increased incidence carcinoma in the last decades. It is the most common type of endocrine cancers. Of which, many types have been diagnosed depending on the histopathological criteria (Curado et al., 2007). The papillary thyroid carcinomas (PTC) occupy the leads in the incidence of thyroid neoplasms. Many variants of PTC have been diagnosed (Morris et al., 2008).

Till, the cause of the elevated count of the cases with thyroid carcinoma as awhile, or especially the PTC, is attributed to the development of diagnostic facilities in one hand and the increase the risk of carcinogenic factors that is the people subjected to, in the other hands. Yet, genetic events play an important role in diagnostic and prognostic aspect of the patient with PTC (Mulligan, 2014). RET oncogene is one of the genetic diagnostic features used in the diagnosis of PTC. The thyroid cancers are associated with rearrangement or deletion of this gene. The RET gene is encoded on the long arm of gene 10q11.2 and is composed of 21 exons (Suet et al., 2016). The frequency of RET rearrangements is noticed in approximately one fourth of cases of PTC. The RET breaking and the partner genes and their fusion are thought to be the result from incorrect repair of DNA double-strand breaks. Because the RET and its most common fusion partners (CCDC6 and NCOA4) are thought to
besusceptible to breakage as they map in a fragile sites of DNA (Yakushina, Lerner and Lavrov, 2018). These fragile sites are non-random DNA loci that are, under normal conditions, are stable, but they become a site of chromosomal break under exposure to various agents like hypoxia, ethanol, etc. (Dillon, Burrow and Wang, 2010)

The other gene mutation was the focus of investigations, as it may be an indicator for the prognosis and therapy in the treatment of PTC, in addition to its value as a diagnostic factor, this gene is BRAF mutation. The mutation of this gene is the area of intensive investigation (Kakarmath et al., 2016). Since the first reports describing the BRAF mutation in melanoma, glioma, colorectal, ovarian, lung, and liver cancers and sarcoma cells, numerous studies have been published correlating. The mutation of BRAF is more specific for epithelial derivatives tissue, particularly papillary thyroid carcinoma PTC and poorly differentiated thyroid carcinoma. BRAFis the B-type Raf kinase, it is located on chromosome7, it is a potent activator of the mitogen-activated protein kinase/extracellular-signal-regulated kinase (MEK-ERK) pathway. thiamine transversion to adenine at nucleotide position 1799 (T1799A) in exon 15 is the most common hotspot mutation in the BRAF gene. This causes a conversion of valine to glutamate of amino acid 600 in the BRAF protein, creating a constitutively active BRAF kinase, which has been proven to be an oncogene in human cancer (Garnett and Marais, 2004).

II. PATIENTS AND METHODS:

The cross sectional study was done on 45 patients with papillary thyroid carcinoma. The age range was from 26 to 50 years, 4 males and 41 females. The study was carried out at Al-Hilla Teaching Hospital and in a number of private laboratories in Babil Province. The technique was used to detect DNA sequence by using fluorescent probes. The study was based on taking a thyroid tissue specimen in addition to retrieving paraffin blocks from the central laboratory of the teaching hospital and from private laboratories and performing conventional hematoxylin and eosin stain and genetic study.

The manufacturer’s guide was used to detect RET pronecoge chromosomal abnormalities, FISH CytoCell RET Breakapart (10q11.21) from (Oxford Gene Technology -USA), and BRAF gene mutation detection kit were used. The procedure used following the manufacturer instructions.

III. RESULTS

RET mutation in patients with papillary carcinoma of thyroid gland

Out of 45 patients, 15 showed RET gene mutation accounting for 36.6 %. Comparison of proportion of RET mutation between male and female patients was shown in table 1. All male patients (100.0 %) had RET gene mutation, whereas, only 36.6 % of females had the RET gene mutation and the difference was statistically significant (p<0.05). The mutation of RET gene was either in the form of deletion or in the form of rearrangement. Out of 45 patients, 11 had RET gene deletion accounting for 24.4 %. Comparison of proportion of RET deletion between male and female patients is shown in table 2. All males (100.0 %) had the RET gene deletion, whereas, 7 (17.1 %) of female patients had the RET gene deletion and the difference was significant (p = 0.002). Out of 45 patients, 11 had RET gene rearrangement accounting for 24.4 %. Comparison of proportion of RET rearrangement between male and female patients was shown in table 3. None of males (0.0 %) had the RET gene rearrangement, whereas, 11 (26.8 %) of female patients had the RET gene rearrangement and the difference was not significant (p = 0.558) figure 1.
Figure 1: Thyroid gland section of patient with PTC, using FISH technique detecting the rearrangement of RET gene (yellow circles). X400

BRAF mutation in patients with papillary carcinoma of thyroid gland

Out of 45 patients, 14 had BRAF gene rearrangement accounting for 31.1%. Comparison of proportion of BRAF rearrangement between male and female patients was shown in table 4. All males (100.0%) had the BRAF gene mutation, whereas, 10 (24.4%) of female patients had the BRAF gene rearrangement and the difference was significant ($p = 0.007$) figure 2.

Figure 2: Thyroid tissue of patient with PTC, FISH technique to detect BRAF gene mutation (blue squares). Deletion of proximal 5' of chromosome 7 (yellow arrow head). X400
Table 1: Comparison of proportion of RET mutation between male and female patients with papillary thyroid carcinoma

<table>
<thead>
<tr>
<th>RET mutation</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive, n (%)</td>
<td>19(42.2%)</td>
<td>4(100.0%)</td>
<td>15(36.6%)</td>
<td>0.026 F S</td>
</tr>
<tr>
<td>Negative, n (%)</td>
<td>26(57.8%)</td>
<td>0(0.0%)</td>
<td>26(63.4%)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; F: Fischer exact test; S: significant at p ≤ 0.05

Table 2: Comparison of proportion of RET deletion between male and female patients with papillary thyroid carcinoma PTC

<table>
<thead>
<tr>
<th>RET deletion</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive, n (%)</td>
<td>11(24.4%)</td>
<td>4(100.0%)</td>
<td>7(17.1%)</td>
<td>0.002 F S</td>
</tr>
<tr>
<td>Negative, n (%)</td>
<td>34(75.6%)</td>
<td>0(0.0%)</td>
<td>34(82.9%)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; F: Fischer exact test; S: significant at p ≤ 0.05

Table 3: Comparison of proportion of RET rearrangement between male and female patients with papillary thyroid carcinoma PTC

<table>
<thead>
<tr>
<th>RET rearrangement</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive, n (%)</td>
<td>11(24.4%)</td>
<td>0(0.0%)</td>
<td>11(26.8%)</td>
<td>0.558 F NS</td>
</tr>
<tr>
<td>Negative, n (%)</td>
<td>34(75.6%)</td>
<td>4(100.0%)</td>
<td>30(73.2%)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; F: Fischer exact test; NS: not significant at p > 0.05

Table 4: Comparison of proportion of BRAF mutation between male and female patients with papillary thyroid carcinoma PTC

<table>
<thead>
<tr>
<th>BRAF mutation</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive, n (%)</td>
<td>14(31.1%)</td>
<td>4(100.0%)</td>
<td>10(24.4%)</td>
<td>0.007 F S</td>
</tr>
<tr>
<td>Negative, n (%)</td>
<td>31(68.9%)</td>
<td>0(0.0%)</td>
<td>31(75.6%)</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; F: Fischer exact test; S: not significant at p ≤ 0.05

IV. DISCUSSION

In the current study, out of 45 patients, 15 showed RET gene mutation accounting for 36.6 %. All male patients (100.0 %) had RET gene mutation, whereas, only 36.6 % of females had the RET gene mutation and the difference was statistically significant (0.026). The mean frequency of RET mutation in sporadic papillary carcinoma is 20%-30% in adults, rising up to 45-50% in pediatric and young patients, and being highest (50-80%) in patients with a history of accidental or therapeutic radiation exposure (Rabes et al., 2000; Sadetzki et al., 2004). Accordingly, it appears quite reasonable to believe that the pathomorphologically identical papillary carcinomas must have followed different routes of carcinogenesis characterized by different clinical manifestation. In the last decades, there was a significant increase in the number of PTC, so one of the possibilities was the influence of radiation like that due to Chernobyl nuclear power plant disaster (LiVolsi et al., 2011). Knowing that RET mutation has been proven to be a good marker for its influence (Pundaet et al., 2018). In the present study, the mutation of RET gene was either in the form of deletion or in the form of rearrangement. Out of 45 patients, 11 had RET gene deletion accounting for 24.4 %. All males (100.0 %) had the RET gene deletion, whereas, 7 (17.1 %) of female patients had the RET gene deletion and the difference was significant (p = 0.002). In addition, in this study, out of 45 patients, 11 had RET gene rearrangement accounting for 24.4 %. None of males (0.0 %) had the RET gene rearrangement, whereas, 11 (26.8 %) of female patients had the RET gene rearrangement and the difference was not significant (p = 0.558). Rearrangement of the tyrosine kinase
receptor gene (RET gene named RET/PTC) is the most common structural genetic alteration which, however, shows great geographical variability ranging from 0 to 80% in different studies (Pundaet al., 2018).

Papillary thyroid carcinoma (PTC) is the most frequent thyroid cancer and consists in a well-differentiated carcinoma, originating from thyroid follicular cells and associated to exposure to ionizing radiation (Sherman, 2003; Williams, 2008). Consistently, typical molecular features of PTCs are chromosomal aberrations generated as a consequence of ionizing radiation-induced double-strand breaks and unfaithful repair. In particular, PTCs display chromosomal rearrangements of chr. 10q, causing the rupture of the RET gene and its fusion to heterologous genes due to unfaithful repair (Carlomagno, 2012). The partner genes encode heterogeneous proteins all containing protein-protein interaction domains such as coiled-coil motifs (Nikiforov et al., 2011). RET/PTC1 and RET/PTC3 represent over 90% of all RET/PTC rearrangements identified so far. In both cases, the chromosomal aberration consists in a paracentric inversion of the long arm of chromosome 10 where, together with RET the corresponding fusion partner of RET/PTC1, CCDC6 (H4) and of RET/PTC3, NCOA4 (RFG, ELE1, ARA70) map (Carlomagno, 2012). RET/PTC3 is mainly associated with radiation-induced carcinomas and is frequently found in more aggressive PTC variants such as the solid-follicular or the tall cell histotypes. The other 11 RET/PTC isoforms are very rare and have been found only in few cases of radiation-induced PTCs (Carlomagno, 2012).

In our study, RET mutation was seen in all males and this may explain the poor prognosis of thyroid cancer in males in comparison with females; however, this issue is still controversial. According to previous studies, it is still controversial whether male gender a poor prognostic factor in cases of PTC (Nilubol et al., 2013; Lee et al., 2017). A meta-analysis demonstrated that male gender was a strong prognostic factor, and increased the risk of recurrence (Guo et al., 2014).

In the current study, out of 45 patients, 14 had BRAF gene rearrangement accounting for 31.1 %. All males (100.0 %) had the BRAF gene mutation, whereas, 10 (24.4 %) of female patients had the BRAF gene mutation and the difference was significant (p = 0.007). The BRAF V600E mutation has been observed in 18% to 87% of thyroid cancers (Xing, 2005; Trovisco et al., 2006). It is most commonly present in PTC, and some forms of poorly differentiated thyroid cancer and anaplastic thyroid cancers that coexist with, or arise from, PTC (Begum et al., 2004). Both in vitro studies and transgenic models of BRAF suggest that the BRAF V600E mutation promotes thyroid cancer progression and is associated with invasive thyroid cancer phenotype (Knauf et al., 2005; Mesa et al., 2006). On the basis of these findings, several investigators have evaluated whether the presence of a BRAF V600E mutation in thyroid cancer is associated with an aggressive tumor phenotype. Some studies suggest an association between the presence of BRAF V600E mutation and poor prognostic factors, such as older age, male gender, extrathyroidal tumor invasion, lymph node and distant metastases, higher tumor stage, and even higher rates of recurrent disease. However, several investigators have not found the presence of the BRAF V600E mutation to be associated with aggressive thyroid cancer phenotype (Kebebew et al., 2007).

REFERENCES:


