Iraqi Journal of Cancer and Medical Genetics

Correlation Between Tumor Suppressor Gene P53 In Some Iraqi Patients With Thyroid Carcinoma By Immunohistochemical Assay and In Situ hybridization Method

Abd-Alameer N. Galoub Al-Rekabi[1], Noor Al-Huda Ali A.H. Saeed[1]

1 *Dept. of Biology, College of Science, University of AL-Mustansiriya*

Abstract:

The present study was designed to compare between two methods (Immunohistochemistry and In Situ hybridization) to investigate the relationshipe between tumor suppressor gene (P53) in some samples of some Iraqi patients with thyroid tissue cancer from different site (papillary, follicular and lymph node). A series of 30 patients with thyroid cancer who had undergone thyroidectomy. All these samples were collected during (2011-2012) . The histologic types included 21 well differentiated papillary , follicular and lymph node carcinomas,and nine poorly differentiated carcinomas.By using Immunohistochemistry , P53 was detected in eighteen out of thirty (60%) , whereas at In Situ hybridization , it was detected in fourteen out of thirty (46.6%), Moreover the positive results of the tumor supprewwor gene (p53) was related in highly significant to each of age, gender, stage and histologic grade of tumor.It is conclude from that immunohistochemistry and in situ hybridization for detection of p53 could be useful in the clinical evaluation of patients with thyroid carcinoma. And suggested that p53 mutations are involved in thyroid carcinogenesis and may play an important role in the malignant transformation of thyroid cancer.

Key word: *Thyroid cancer, Tumor suppressor gene (p53), Immunohistochemistry, Insitu hybridization*

Introduction:

Mutations in the tumor suppressor gene P53 are the most common mutations found in human cancers (1).It has been reported as a tumor suppressor gene whose inactivation by mutations has been noted in a variety of human malignancies, more than 95% of these alterations are missense mutation which are scattered in the central part of the gen(2). Although these mutations lead to the the inactivation of the biological properties of the p53 protein, they also have dramatic consequences in term of p53 stability (3).

Inactivation of p53 tumor suppressor activity during tumor development is a process of accumulation of its genetic abnormalities (4).Mutation in p53 can be precede allele loss or vice versa (5). In both circumstances p53 function is only partially inactivated because one alelle of p53 gene is affected. Tumor suppressor gene inactivation involving both alells, usually by mutation in one alelle and loss of the other, results of the complete loss of p53 function(6).

Thyroid carcinoma is a common malignant tumor of endocrine gland and most common cancer in head and neck. Recen studies indicated that the genesis of thyroid cancer is closely correlated with suppressor gene (7). Zou, et al reported that p53 mutations are involved in thyroid carcinogenesis and may play an important role in the malignant transformation of thyroid cells as well as thyroid tumor progression. Tumor suppressor gene P53 expression in a large series of thyroid tumor specimens suggest that, although not mutant p53 activity may be inhibited in thyroid cancer by other mechanisms. Increased p53 protein levels were observed by immunohistochemistry not only in anaplastic and poorly differentiated thyroid cancer , but also in well differentiated cancers in the absence of any p53 mutations (8,9,10).

Corresponding Address:
Noor Al-Huda Ali A.H. Saeed
Dept. of Biology, College of Science, University of AL-Mustansiriya
Email: nnhuda@yahoo.com

Material and Methods:

The current study involved of thirty patients with thyroid cancer (mean age 40 years) ranged between (23-64) years , male-female ratio 1:2 with 10 men and 20 women. They were collected randomly from Teaching laboratories\ Medical city in Baghdad. Whom already diagnosed as a (papillary , follicular and lymph node) of thyroid cancer by specialist , compared with 10 apparently healthy control where their ages and sex were matched to patients group. P53 suppressor gene and p53 protein were determined in specimens using Insitu hybridization and Immunohistochemistry methods and performed as recommended in leaflet with kits.

- DNA probe Hybridization \ Detection System : Highly – Sensitivity In Situ kit. A complete hybridization and immunodetection system were purchased from Maxim Biotech , USA . cat. No. (IH – 60001), (IHD – 00S0).
- Mouse anti-Human p53 (tumor suppressor protein, oncogene protein) from Us Bio;ogical code (P1001 – 32 C) for (ISH).
- Detection system for p53 protein :-

Universal Dakocytomation labeled streptavidin – Biotin 2 system , Horseradish peroxidase (LSAB – 2 system . HRP) ready to use detection system , code no. K0673 (CA.USA). For (IHC).

- Ready to use N- series primary antibody (Monoclonal mouse Anti- Human p53 protein clone : DO7 . code N 1581). Dako. Denmark.

Statistical analysis :

The ststistical analysis system – SAS (11) was used to effect of differences factors in study parameters. The chi-square $\chi 2$ test at the comparative between percentage in this study.

Results:

Resected specimens of thirty (30) cases of varied types of thyroid cancer were studied immunohistochemically and insitu hybridization using a monoclonal antibody to detect p53 protein and DNA hybridization was used to detect p53 suppressor gene.

The mean age of our patients was 40 years ranged between (23-64)years . The male to female ratio was 1:2 , with 10 men and 20 women .

1- Iraqi patients with thyroid carcinoma according to their age , tumor size , grade and gender in relation with p53 by (ISH).

As shown in table (1), there were highly significant correlation between tumor suppressor gene with thyroid cancer patients age (p=0.0026) , tumor site (p=0.0013), tumor grade (p=0.0001) and gender (p=0.0025) respectively at ($p < 0.01$). The positive rate of p53 was detect in 14 out of 30 (46.6%) for thyroid carcinoma patients by in situ hybridization.

2- Iraqi patients with thyroid carcinoma according to their age, gender, tumor site and tumor grade in relation with p53 in immunohistochemical method (IHC).

As we can see in table (2) the highly significant correlation between thyroid cancer patients age (p=0.0017), grade(p=0.0002), tumor site(p=0.0001) and gender (p=0.0003) respectively with p53 immunohistochemically at ($p < 0.01$). The positive results for p53 was 18 out of 30 (60%) of some Iraqi patients.The well differentiated rate is 21 out of 30 for all these cases,where as , the positive count for (IHC) was 12 out of 18 which is (66.67%), so in (ISH) the positive count was 7 out of 14 (50%). The poor differentiated positive rate was in (IHC) 6 out of 18 (33.33%) and at (ISH) 7 out of 14 which is (50%).

Table (1): Distribution patent of Iraqi patients with thyroid carcinoma according to their age, tumor site, gender and tumor grade of their leision in relation with p53 at insitu hybridization (ISH).

The factor	Number of	Patients (%)	P – value And $\chi 2$ - Value
	P53 (ISH) positive Total No.= 14	P53 (ISH) negative Total No.= 16	
Age 40 ≥ 40 <	9 (64.28 %) 35.72 %))5	50.00 %))8 50.00 %))8	P = 0.0026 χ^2=7.18
Tumor site Papillary Follicular Lymph Node	64.28 %))9 28.57 %))4 1(7.15 %)	62.50 %))10 4(25.00 %) 2(12.50 %)	P = 0.0013 χ^2 = 9.37
Tumor Grade Well poor	7(50.00 %) 50.00 %))7	87.50 %))14 2(12.50 %)	P = 0.0001 χ^2 = 9.84
Gender Male Female	28.57 %))4 71.43 %))10	6(37.50 %) 10(62.50 %)	P = 0.0025 χ^2 = 8.42

All these results at $p < 0.01$

Table (2): Distribution of thyroid carcinoma patients in relation with p53 protein in immunohistochemical (IHC) method according to their age, gender, site of tumor and tumor grade.

The factor	Number of	Patients (%)	P – value And χ^2 - Value
	P53 (IHC) positive Total No.= 18	P53 (IHC) negative Total No.= 12	
Age ≤ 40 > 40	12(66.67 %) 6(33.33 %)	5(41.17 %) 7(58.83 %)	P = 0.0017 χ^2 = 8.44
Tumor site Papillary Follicular Lymph Node	12(66.67 %) 6(33.33 %) 0(0.00%)	7(58.83 %) 2(16.67 %) 3(25.00 %)	P = 0.0002 χ^2 = 9.82
Tumor Grade Well poor	12(66.67 %) 6(33.33 %)	9(75.00 %) 3(25.00 %)	P = 0.0001 χ^2 = 10.39
Gender Male Female	6(33.33%) 12(66.67 %)	4(33.33 %) 8(66.67%)	P = 0.0003 χ^2 = 7.69

All these results at $p < 0.01$

Discussion:

The results shows there were highly significant correlation between p53 with thyroid cancer patients age, tumor site, tumor grade and gender at P< 0.01 . This finding results confirmed with (21) who reported that p53 protein was detected in (66.6%) of samples which was significantly higher at P< 0.01 . In contrast of (12) who found no relationship between p53 immunoreactivity and clinical study. In addition others(13) found that no correlation with tumor size ,age and sex. P53 mutations and their implicants tumorigensis attracted much attention over the past 20 years (14, 15 , 16). Highly significant results and the positivity of p53 protein is a good marker of tumor progression.

Several genetic hits have demonstrated to be prevalent in the evolution of thyroid cancer. P53 is one of the highest correlative genes with human tumor so far (7,12).

As we can see in this study, the most common type of malignancy was papillary carcinoma (63.3%) followed by follicular carcinoma (26.6%) and finaly lymph node detected in (10%) of our cases . These results similar to (17 , 18).

The mean age of our patients was 40 years ranged between (23-64)years , it is the same reported by (19 , 20)which was the median age 49 years and 38.4 years ranged between (20-71)years.

Although the number of cases was small , female appeared to be more frequent affected , female to male ratio was 2:1 , it was similar to (20) and (13) study that found female to male ratio 3:1 and 2.8:1 respectively . However, Al-Katib and Yasser, et al , reported an even higher female to male ratio of 4.8:1 , 8.4:1 in their cases.

The positive results of well differentiated in (IHC) was (66.6%) where as the positive of poor differentiated was (33.3%) this finding confirm with (8,9,10) which were found that p53 protein levels were observed by IHC not only in anaplastic and poorly differentiated thyroid cancer, but also in well differentiated cancers in tha absence of any p53 mutations (8,9,10). But it was disagree with (22) who found that p53 mutations are not frequent in thyroid cancer and are believed to be responsible mainly for cancer progression to poorly differentiated . The positive finding of well differentiated of (ISH) was (50%) as well as the positive poor differentiated was (50%). This study was in constant with (23) who reported that well differentiated carcinomas have exhibited the lowest p53 staining frequency the expression has been higher in poorly differentiated carcinomas .

These results refers to the mutations of the p53 gene which associated with the most aggressive histologic types of thy-

roid cancer such as poorly and well differentiated and the alteration of this gene represent a late genetic event in human thyroid cancer.

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العلاقة بين الجين الكابح للورم في بعض المرضى العراقيين المصابين بسرطان الغدة الدرقية باستخدام طريقة التصبيغ المناعي وتقنية التهجين بالموقع

عبدالامير ناصر غلوب الركابي، نورالهدى علي عبدالحسين سعي
قسم علوم الحياة/ كلية العلوم/ الجامعة المستنصرية

الخلاصة :

صممت هذه التجربة لدراسة المقارنة بين طريقتي التصبيغ المناعي والتهجين بالموقع. لاكتشاف العلاقة بين الجين الكابح للورم في بعض المرضى العراقيين المصابين بسرطان الغدة الدرقية.اخذت الخزعات النسيجية من مواقع مختلفة (الحليمات , الحويصلات والعقد اللمفاوية). اشتملت الدراسة على ثلاثين مريض مصابين بالغدة الدرقية ممن خضعوا لعملية ازالة الغدة الدرقية. جميع هذه العينات النسيجية جمعت خلال الفتره مابين 2012-2011 . شملت هذه الخزعات النسيجية على 21 عينة جيدة التمايز (الحليمات , الحويصلات والعقد اللمفاوية) و 9 عينات ضعيفة التمايز الورمي.

بأستخدام تقنية التصبيغ المناعي تم تشخيص الجين الكابح للورم في 18 عينة موجبة من اصل 30 عينة (60%) . بينما في طريقة التهجين بالموقع شخص في 14 خزعة موجبة من بين 30 عينة (46.6%) . لذا وجد ان النتائج الموجبة للجين الكابح للورم بأرتباط عالي المعنوية لكل من العمر والجنس ودرجة الورم وموقع الورم التي تم دراسته.

تم الاستنتاج بان طريقتي التصبيغ المناعي والتهجين بالموقع للتحري عن الجين الكابح للورم ذات اهمية للفحوصات السريرية للمرضى المصابين بسرطان الغدة الدرقية. ويعتقد ان الجين الكابح للورم المطفر متورط في السرطانات ويلعب دورا مهما بالتحول السرطاني للغدة الدرقية .