Immunohistochemical Expression of CD68, P53 and Bcl2 in Thyroid Tumors

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Abstract

Background/aim: The aim of this study was to determine the expression of CD68, P53 and Bcl2 in thyroid tumors and correlation between them**.**

Materials and Method: The expression of CD68, P53 and Bcl2 was examined by immunohistochemistry on paraffin-embedded tissues obtained from patients with benign and malignant tumors between 2010 and 2015.Thirty three malignant thyroid tumors of variable subtypes and 10 benign tumors. The extents of staining and intensity were scored semi quantitatively.

Results: CD68 have shown strong positive score expression in (28.9%) in PTC while score was found in (18.5%). The higher percentage of strong weak positive score expression of P53 was in PTC (6.1%) Followed by (3.03%) strong score in FTC, (3.03%) moderate score in benign and (3.03%) in MTC. The expression of Bcl2 was higher in benign tumors (20%) than in malignant tumors were PTC (9.1%) followed by FTC (3.03%), the strong positive expression seen in MTC (9.1).

Keywords: *Thyroid tumors, Immunohistochemistry, CD68, P53, Bcl2.*

Introduction

The commonest type of endocrine malignancy is thyroid cancer, although it's rare. Thyroid cancer accounting for about 1.5 of all newly diagnosed cancer in the United States, its occurrence being steadily increasing worldwide in the last three decades [1]. It is categorized into three main histological types: differentiated including "papillary, follicular and hurthle» «medullary» and «anaplastic» (aggressive undifferentiated tumor) [12]. Papillary thyroid carcinoma (PTC) represents 80.3% of malignant endocrine tumors and 65% of malignant thyroid tumors [12, 14] According to Iraqi cancer registry, it ranks the eight cancers from commonest ten cancers in general and the second cancer in female (Iraqi cancer registry, 2019). Combined with there are different cellular and molecular factors that plays a role in development and progression of thyroid cancer. One of the main cellular constituents in the stromal of many cancers is "tumor-associated macrophages" (TAM) [20]. Advanced staging and poor prognosis in many human cancers including thyroid cancers related to tumorigenic role of "TAM" [17].TAMs have an essential role in tumor advancement at different levels, including stimulating genetic instability, nurturing cancer stem cells pavement the way to metastasis , taming defensive adaptive immunity [1]. Transformations in the p53 gene are the most widely recognized in human cancer [11] and they express to the most frequent genetic changes in malignant transformation. P53 protein assumes an essential role in the regulation of the cell cycle. Wild sort p53 protein is capable of inhibiting cell proliferation and transformation and it has been observed to be latent in tumor cells [13]. This gene mutation is the most prevalent genetic alterations in human tumors and has been found in over 15% of thyroid neoplasm. The p53 gene mutations are promoting more aggressive cancers. Bcl2 protein is a modular of programmed cell death and is involved in both lymphoid and epithelial malignancies. The reporting of bcl2 protein expression in thyroid cancer has been sporadic and some have shown a down regulation of bcl2 in papillary carcinomas [5]. The present study aimed to evaluate level of CD68, P53 and Bcl2 expression in both malignant and benign thyroid

tumors, and to correlate the results with the variable clinicopathological parameters (taking in respect the age and sex of the patients combined with tumor types histopathological).

Materials and Method

Our study involved paraffin-embedded tissue blocks samples of 43 thyroid tumors patients, of which 33 thyroid carcinomas and 10 benign as control group. All these samples were collected from the Pathology Department of Teaching Laboratories /Medical city and Central Public Health Lab between the years 2010-2015. All Hematoxylin and Eosin stained tissue sections were reviewed by pathologist. Immunohistochemistry study was prepared on serial sections and the antibodies panel that has been used is shown in the table below (table 1).

Table 1: Antibodies used for immunohistochemical study

Antibody	Code	Clone	Antigen retrieval	Dilution	Source
CD68	M 0814	KP-1	pH.9	1:50-1:100	Dako/Denmark
P53	M 7001	DO-7	pH.9	1:25-1:50	Dako/Denmark
Bcl2	M 0887	124 clone	pH.9	1:50-1:100	Dako/Denmark

CD68, P53 and Bcl2 immunohistochemical staining.

Immunohistochemistry: Four µm sections were cut from formalin-fixed, paraffin-embedded tissue blocks and placed on polylysine-coated glass slides. After overnight packing at 65ºC, tissue sections were deparaffinized in xylene and rehydrated in descending grades of alcohol. Antigen retrieval was performed in a water bath for 15 min in 95 º C citrate buffer pH 9.0 for CD68, P53 and Bcl2**.** Endogenous peroxidase action was blocked by 3% hydrogen peroxide.The sections were incubated at room temperature for 1 hour with primary monoclonal antibodies.

Scoring of immunohistochemistry: The stained slides were observed microscopically by histopathologist using the p53, Bcl2 and semiquantiative criteria: 0= negative; 1+ = <33% area of positive staining; 2+ =34-66% area of positive staining; 3+ = >67% area of positive staining. Positive staining was also graded on intensity, 0 to 2+. A combined score of 0 to 6 was allocated. Tumors assumed a score 0 to 1 were classified as negative; those given a score of 2 were ordered as weakly positive; a score of 3-4 was regarded as moderately positive; and a score of 5-6 was considered strongly positive [25]

The proportion of CD68 cells in each tumor on each slide was assessed after staining. Based on a median value of 25%, weak (+) and strong positive (++) staining were defined as <25% and ≥ 25%, respectively [17].

Statistical Analysis

Data were analyzed by one -way analysis variance (ANOVA) followed by Fisher`s test for multiple comparison, using Statview version 5.0.Differences were considers "significant when p<0.05".Reversion analysis was done by analysis of covariance (ANCOVA) likewise by Statview version 5.0.

Results and Discussion

CD68

In this study, the results of immunohistochemical analysis for CD68 have shown in table 2, 3 and 4. As shown in table 2 there significant correlation between CD68 with thyroid patients age less than (45 years) (P=0.01) and (P=0.0001) with thyroid patients age more than 45 years.

In table 3 and 4 the results recorded depended 2 scores weak positive and strong positive. There is significant difference between score of CD68 and gender (P. value: 0.058). (Table 3)

Score of CD68 showed associated with gender in table 2, from total 29 female were (20 cases, 98.9%) weak positive and (9 cases, 31%) strong positive while from 9 male were (5 cases, 55.6%) weak and (4 cases, 44.4%) strong positive.

Table 4 showed CD68 score related with tumor type, PTC was appeared high associated with strong positive (11 cases, 28.9%) followed by MTC and Hurthle cell carcinoma in (1 case, 2.6) in same score. While the weak score was found in (7 cases, 18.5%) in benign tumors ,(10 cases, 26.4%) in PTC , (3 cases, 7.9%) in FTC and MTC and finally (1 case ,4.5)in ATC.

Table2: Distribution of thyroid carcinoma patients in relation with CD68 protein in immunohistochemical (IHC) method according to their age, gender and tumor type

factor	CD68 +	CD68 -
Age total N	N %	N %
<45 26	21 80.8	5 19.2
≥45 17	17 100	0 0
Sex N	N %	N %
Female 33	29 87.8	4 12.2
Male 10	9 90	1 10
Tumor type N	N %	N %
Benign 10	7 70	3 30
Malignant 33	31 93.9	2 6.1
Histotype 0	0 0	0 0
Papillary Ca 22	21 63.6	1 3.03
Follicular Ca 4	3 9.1	1 3.03
Medullary Ca 4	4 12.12	0 0
Anaplastic Ca 2	2 6.1	0 0
Hurthle cell 1	1 3.03	0 0

Table 3: Immunohistochemical score of CD68 related with gender

	CD68 IHC score			P.Value
	< 25 weak	≥25 strong		0.058
Sex (+ve n)	No. %	No. %	Total	
Female 29	20 69	9 31	100	
Male 9	5 56	4 44	100	
Total	125	75	200	

Table 4: Immunohistochemical score of CD68 related with tumor type

POSITIVE N (38)	CD68 IHC score	
Tumor type	**<25 weak**	**≥25 strong**
	No. %	No. %
Benign 7	7 18.5	0 0
Malignat	No. %	No. %
Papillary Ca 21	10 26.4	11 28.9
Follicular Ca 3	3 7.9	0 0
Medullary Ca 4	3 7.9	1 2.6
Anaplastic Ca 2	2 5,4	0 0
Hurthle cell 1	0 0	1 2.6

P53

Expression of P53 was related with age group, gender and tumor type summarized in table 5 Of 26 patients were less than 45 year, only (5 cases, 11.6%) were positive. These 5 cases were 4 female and 1 male which of them 1 benign tumor case, (2, 6.1%) PTC, (1, 3.03%) FTC and (1, 3.03%) MTC .

Table 6 showed significant difference between score of P53 and gender (p.value:0.001).

The results in table 7 show that high percentage of strong score was in PTC (2 cases, 6.1%),

Followed by 1 case 3.03% strong score in FTC, 1 case 3.03% moderate score in benign and 1 case 3.03% in MTC.

Table 5: Distribution of thyroid carcinoma patients in relation with P53 protein in immunohistochemical (IHC) method according to their age, gender and tumor type

factor	P53+	CDP53 -
Age total N	N %	N %
<45 26	5 19.2	21 80,8
≥45 17	0 0	17 100
Sex N	N %	N %
Female 33	4 12.1	29 87.9
Male 10	1 10	9 90
tumor type N	N %	N %
benign 10	1 10	9 90
malignant 33	4 12.1	29 87.9
histotype 0	0 0	0 0
Papillary Ca 22	2 6.1	20 60,6
follicular Ca 4	1 3.03	3 9.1
medullary Ca 4	1 3.03	3 9.1
Anaplastic Ca 2	0 0	2 6.1
Hurthle cell 1	0 0	1 3.01

Table 6: Immunohistochemical score of P53 related with gender

	P53 IHC score					P.value
Sex P N	**score 0 (-ve)** No. %	**2 weak** No. %	**3 moderate** No. %	**4 strong** No. %	**Total**	
Female 33	28 84	1 3	2 6	2 6	100	**0.001**
Male 10	9 90	1 10	0 0	0 0	100	
Total	174	13	6	6	200	

Discussion

The host´s immune response to the tumor represent by macrophage infiltrates in the context of or surrounding a variety of malignancies [4,7]. The "tumor-associated macrophages" (TAMs) had fundamental jobs in tumor development and metastasis of various cancers, including advanced thyroid malignant growth [17].This study demonstrated the presence of CD68+ in benign and malignant thyroid tumors. But all (7 cases, 18.5%) of CD68+ in benign were in weak score. While (11 out of 21)28.9% from PTC were strong positive score which came in similar with Qing et al.,(2012) who investigated TAM density in both benign thyroid tumors and PTC by CD68 immunostaining. They establish that overall density of TAM was higher compared with "thyroid goiter" and "follicular adenoma". On the contrary to previous studies, [17] pointed to the expression of TAM in 36 PTC Patient with L.N metastasis using immunohistochemical staining with anti-CD68 antibody. And Ryder et al.,(2008) who recorded a higher TAM thickness in ineffectively separated PTC and ATC This different was explained by the mixed cell populaces of macrophage with CD136 ,CD68 and undifferentiated monocyte/macrophage which are for all positive for CD68 ,these kinds of macrophages play special roles in the advancement and progression HNSC [18] Other study by [10] using CD68 immunohistochemistry, observed that TAM anticipated metastasis in a number of patients. Of 121 patients with PTC, 15% had TAMs (CD68) that seemed to have a phagocytic capacity on cancerous cells. These patients had fundamentally less blood vessel attack and remote metastasis and largely supplementary invasion lymphocyte and dendritic cells, than patients without a CD68 cells. Further, the findings of the current study are in similar with other study by [21] Who observed expression of p53 was more frequently in malignant than in benign lesions. Since p53 has been known to be rarely mutated in well differentiated tumors of the thyroid where an frequently expressed in poorly differentiated or undifferentiated tumors which are of different number, in our study, however there is a concerned of tumor suppressor gene where it plays an important role in cancer progression ,yet a serious studies should that it plays a role in early stages different types of cancers including thyroid cancers. We found a moderate immunoreactivity of Bcl2 in cytoplasm of epithelium of the benign and well-differentiated PTC and these findings are accordance with other studies by [26 , 22, 2 , 24]. .

Conclusion:

CD68 + macrophages are key players in thyroid tumors microenviroment leading to thyroid tumor development where its indirectly correlated with BCL2 family oncoproteins where antiapoptotic pathway is down regulated leading to increased aggregates of CD68+macrophages and that CD68+macrophages are positively correlated with p53 gene mutation expression where both markers can explain the biological behavior of tumor. BCL2&P53 have an inversed correlation since both have an opposite functions (p53 represent cell death gene while bcl2 is an antidote to a programmed cell death ,bcl2 was more expressed in benign tumor in our study than in malignant tumors unlike to p53

where its more expressed in WDT (PTC 6%followed by FTC3.03%) indicating that expression of p53 may be a late event in thyroid tumor genesis and that there's a mutual expression between both bcl2 & p53 in thyroid tumors.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry, Mustansiriyah University, Iraq, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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