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COMPRATIVE STUDY BETWEEN IMMUNOHISTOCHEMISTRY AND MOLECULAR METHOD IN DETECTION OF BRAF V600E MUTATION IN PAPILLARY THYROID CARCINOMA

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Abstract

Background: Papillary thyroid carcinoma is the most common endocrine and thyroid malignancy. The (BRAF V600E) mutation is the most common mutation in papillary thyroid cancer (PTC). The mutation is detected in about (60%) of the cases.
Patients with BRAF mutation have been linked to aggressive clinical behavior, high recurrence rate, metastasis, and failure of treatment
Aim of the study This study aims to find whether Immunohistochemisrty can substitute molecular analysis in detection of BRAF V600E mutation in papillary thyroid carcinoma. This will safely time, cost, and effort.
Patients and method This was a retrospective study, carried out in Basrah province, wax blocks were collected from histopathology labs during period from October through September 2023. Tissue sections were obtained from the paraffin blocks and submitted to routine hematoxylin and eosin stain. Then Immunohistochemical staining using primary monoclonal antibody for detection of BRAF mutation in papillary thyroid carcinoma.

After examination of H &E slides, the area of papillary thyroid carcinoma was determined and Then piece of tumor tissue with thickness of 5~10 um and a surface area between 0.5~1 cm^2. was taken from the wax block for DNA extraction and detection of BRAF mutation using Real-time PCR.

Results Thirty cases of papillary thyroid carcinoma were included in this study. Twenty-five case (83%) were female and five cases (16.66%) were male with mean age of 41.33+16.1 (range 16-81 years). Using real-time PCR, twelve cases (40%) were positive for BRAF mutation and eighteen cases (60%) were negative for BRAF mutation.

Using immunohistochemistry, thirteen cases (43.3%) were positive for BARF mutation & seventeen cases (56.7%) were negative for the mutation. The sensitivity of Immunohistochemistry in detecting BRAF mutation in papillary thyroid carcinoma is (66.7%) with specificity (72.2%). The positive predictive value is (61.5%) and the negative predictive value is (76.5%).

Conclusion The findings from this study reveal a discrepancy between Immunohistochemistry and real-time PCR in identifying the BRAF V600E mutation. It is important to note that Immunohistochemistry should not serve as a substitute for molecular methods in detecting this mutation. In fact, relying solely on Immunohistochemistry may not be reliable due to its lower sensitivity. This could potentially lead to increased time, cost, and effort in the diagnostic process

keywords: papillary thyroid carcinoma, BRAF, IHC, PCR

Introduction:

Papillary thyroid carcinoma (PTC) is a common malignant epithelial thyroid neoplasm with a good overall prognosis. It is characterized by set of nuclear features.[1] Papillary thyroid carcinoma can occur at any age. Most tumors are diagnosed in the third to fifth decades of life. Women are affected more frequently than men in ratios of (2:1 to 4:1).[2] It can spread easily and invade cervical lymph nodes while vascular invasion is less common. The life expectancy of papillary thyroid carcinoma is affected by age of the patient.[3&4]

Papillary thyroid carcinomas, when observed microscopically, have a central fibro vascular core lined by one or multiple layers of cells with crowded nuclei. The tumor cells characterized by clear, ground glass, empty nuclei, called (Orphan Annie eyed) and contain hypo dense chromatin, often overlapping .[2]

The BRAF protein is a serine /threonine kinase expressed by the BRAF gene on chromosome 7q34 [long arm of chromosome 7, region 3, band 4] that function with Ras–Raf-MEK-MAPK pathway. Normally it has important role in cell proliferation, differentiation, and programmed cell death.[5]

The aberrant activation of MAPK pathway is the driving force for development of papillary carcinoma of thyroid gland and its progression.[6]

The most common mutation that led to this aberrant activation is the*T1799A B-type Raf kinase (BRAF) mutation that occur in about 60% of papillary thyroid carcinoma .[7] The alteration occurring in BRAF gene is called (V600E) which alters the valine at position 600 in the protein to glutamic acid .[8]This mutation has been extensively studied, and significant progress has been made toward understanding its role in development of papillary thyroid cancer and clinical significance .[7]

Patients with BRAF mutation have been linked to aggressive clinical behavior, high recurrence and metastatic rate and failure of treatment (5). BRAF mutation can be positive in tumors other than papillary thyroid carcinoma like Melanoma, colorectal carcinoma, anaplastic thyroid carcinoma, papillary craniopharyngiomas.[9]

Molecular methods including real time polymerase chain reaction (PCR) is the standard for detection of BRAF mutation with sensitivity reach between 71% and 99% .[10&11]

Immunohistochemistry is a recent method for detection of this mutation using monoclonal antibody but it remains unclear whether it can replace molecular methods in clinical practice or not.[12]

Patients and Method

This is a cross sectional retrospective study, carried out in Basrah province. The data collected from Alsader Teaching Hospital and private laboratories during the period from October 2019 through September 2023. All cases of papillary carcinoma of thyroid gland diagnosed during the period of study were included in this study

Formalin fixed paraffin embedded blocks were collected. Three to five micrometers thickness sections were obtained and stained with routine hematoxylin and eosin stains. The slides were examined to confirm the diagnosis and to define the histological variants

Then additional sections using positively charged slides were provided for Immunohistochemical staining for BRAF V600E mutations.

The piece of tumor tissue in appropriate size were taken from the wax block for DNA extraction for detection of BRAF mutation using real time PCR.

Regarding Immunohistochemistry stains only definite positive or negative results were included.

Cut of value is >10% of moderate and strong intensity of cytoplasmic tumor

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cells are considered positive expression of BRAF, while < 10 of any intensity as well weak intensity in >10of tumor cells is considered negative expression.[13]

Looking for BRAF mutation from extracted DNA was performed using real-time polymerase chain reaction (PCR), using The AmoyDX BRAF V600 E Mutations Detection kit. The result was examined by histopathologists and geneticist.

The results were tabulated and analyzed using SPSS for windows, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Independent-samples t-test was used to investigate the significance of any statistical differences in quantitative data. The $\chi 2$-test was applied to investigate the association between qualitative data. P-value less than 0.05 was statistically significant.

Result

A total of thirty cases were included in the study, all diagnosed as papillary thyroid carcinoma. the mean age was 41.33 years (ranging from 16 to 81 years). Twenty-five cases (83.33%) out of thirty cases were females and five cases (16.66%) were males. Table I

Table I: Age (Years) and Sex of the study sample

Number	30
Age	
Mean± SD.	41.33± 16.1
Median (Min.-Max.)	39 (16-81)
Sex	
Female	25(83.33%)
Male	5(16.66%)

Annual distribution of papillary thyroid carcinoma

Nineteen cases (63%) of papillary thyroid carcinoma were diagnosed during 2022 and 2023, while only two cases (6.7%) were diagnosed in 2020 Table II.

Table II: The number and percentage of cases diagnosed in each year of study

Year	Number	Percentage
2019	4	13.3
2020	2	6.7
2021	5	16.7
2022	10	33.3
2023	9	30.0
Total	30	100.0

Nineteen cases (63.3%) were diagnosed as classic variant of papillary thyroid carcinoma. Four cases (13.3%) were diagnosed as follicular variant. Three cases (10.0%) were diagnosed as micro papillary variant. Two cases (6.75%) were diagnosed as encapsulated variant and Two cases (6.7%) were diagnosed as tall cell variant. Table III

Table III The number and percentage of histological variant of papillary thyroid carcinoma included in the study.

Variants	Number	Percentage
Classic	19	63.3
Follicular	4	13.3
Papillary micro carcinoma	3	10.0
Encapsulated	2	6.7
Tall cell variant	2	6.7
Total	30	100.0

Result of real time PCR :Twelve cases (40.0%) were detected as positive for BRAFV600E mutation with real-time PCR. Eighteen cases (60.0%) were detected as negative for BRAFV600E mutation with real-time PCR as in Table IV

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Table IV number and percentage of positive and negative BRAF mutation cases by real-time PCR.

rPCR	Number	Percentage
Positive	12	40.0
Negative	18	60.0
Total	30	100.0

Result of Immunohistochemisrty thirteen cases (43.3%) were positive for BRAFV600E mutation in immune histochemical staining. seventeen cases (65.7%) were negative for BRAFV600E mutation. Table V

Table V number and percentage of positive and negative BRAF mutation cases by Immunohistochemisrty (IHC).

IHC	Number	Percentage
Positive	13	43.3
Negative	17	56.7
Total	30	100.0

Immunohistochemisrty versus real time PCR in detection of BRAF mutation. The sensitivity, specificity, positive Predictive value, and negative predictive value for IHC were tested against the rPCR. The sensitivity of Immunohistochemistry in detecting BRAF mutation in papillary thyroid carcinoma is 66.7% with specificity 72.2%. The positive predictive value is 61.5% and the negative predictive value is 76.5%. Table VI

Table VI: Validity and predictivity of IHC against RPCR

		Real time PCR		
		Positive for BRAF	Negative for BRAF	
IHC	Positive for IHC	True positive (TP)= 8	False positive (FP)= 5	Positive predictive value =TP/(TP+FP) =8/ (8+5) =61.5%
	Negative For IHC	False negative (FN)= 4	True negative (TN)=13	Negative predictive value =TN/ (FN+TN) =13/ (4+13) =76.5%
		Sensitivity =TP/(TP+FN) =8/ (12) =66.7%	Specificity =TN/(FP+TN) =13/ (18) =72.2%	

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Consistency between Immunohistochemisrty and real time PCR
ROC curve shows that the consistency between the two tests covered an area of 30.6% only (Figure 1).

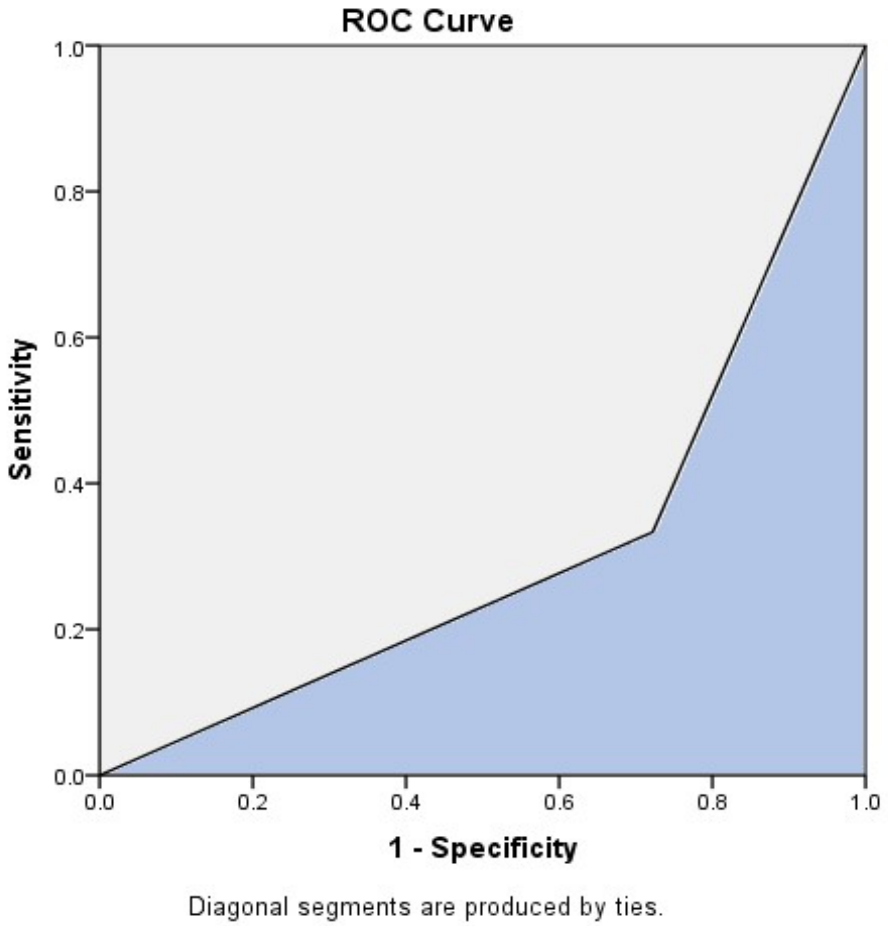

Figure (1): ROC Curve of testing IHC results against RPCR results

Area Under the Curve
Test Result Variable(s): IHC
Area= 30.6%

Discussion

Papillary thyroid carcinoma (PTC) is the commonest type of thyroid malignancy with overall good prognosis. It has variable gross appearance according to its different histological variants.[14] There is increase in the incidence of papillary thyroid carcinoma due to increased detection of small nodule through neck ultrasound and subsequent fine needle aspiration.[15&16]

BRAF V600 E is the commonest mutation occurring in papillary thyroid carcinoma. The detection of

BRAF mutation is important because it is related to local recurrence, metastasis and resistant to treatment(7).[7]

In this study the mean age was (41.33±16.1) year which was slightly higher than other studies in Iraq which reveals a mean age at diagnosis (36.9±11.17) years.[13]

In the present study, most of patients were females (83%) which is consistent with other local studies .[13,17&18]

In this study, there was increase in the number of papillary thyroid carcinoma cases in Basrah in the last two years of the study (2022 and 2023). This increase may be due to increase in detection rate by ultrasound or could be due to real increase in the incidence of papillary thyroid carcinoma in the last two years.

The increase detection rate of papillary thyroid carcinoma is due to increase patient's awareness.

The real time polymerase chain reaction (PCR) is regarded as the gold standard method for the detection of BRAF gene mutations.[10&11]The Immunohistochemistry method is widely used in pathology laboratories due to its lower cost, the shorter time required, and simple processing in compare to PCR. So, we thought about the use of Immunohistochemistry in detection of BRAF mutation to safe time, cost, and effort.

In the current study, the sensitivity, specificity, positive and negative predictive values were calculated for Immunohistochemisrty and compared with real time PCR in detection of BRAF mutation.

By Immunohistochemistry (IHC), BRAF mutation was detected in (66%) of papillary thyroid carcinoma cases included in this study. The positive predictive value of Immunohistochemistry that refer to probability of diseased person with positive result is true positive was (61.5%) and negative predictive value that refer to probability of person with negative result is true negative was (76.5%). These results are lower than other international study by Parker et al. (2020) who reported a sensitivity of (100%) and specificity of (40%).[19] Another study by Zagzag et al. (2013) reported sensitivity (89%) and specificity (100%).[20]

In this study the low sensitivity of Immunohistochemistry in detecting BRAF mutation may be due to small sample size and so it is recommended to perform extended study to evaluate the real sensitivity of Immunohistochemistry in detection of BRAF mutation.

In this study, the percentage of positive case for BRAF mutation using real time PCR is 40%, which is slightly lower than other international results from two

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different studies showing a percentage of 58% and 65% respectively.[12&20]

Not all papillary thyroid carcinoma express BRAFv600E mutation,[16,21&22] and therefore (60%) were detected as negative for BRAF mutation using real time polymerase chain reaction.

In current study the roc curve shows that consistency between Immunohistochemistry and real time PCR are only (31%).

This study shows that IHC cannot replace molecular analysis for detection of BRAF mutation. This result is not like other study done in same line that showed Immunohistochemistry can be useful without need for further molecular technique and this result may be due to small sample size.[23]

Conclusion

The findings from this study reveal a discrepancy between Immunohistochemistry and real-time PCR in identifying the BRAF V600E mutation. It is important to note that Immunohistochemistry should not serve as a substitute for molecular methods in detecting this mutation. In fact, relying solely on Immunohistochemistry may not be reliable due to its lower sensitivity. This could potentially lead to increased time, cost, and effort in the diagnostic process.

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Concept and design: 1
Data collection and analysis: 1,2,3
Responsibility for statistical analysis: 1
Writing the article: 1,2,3
Critical review: 1,2,3,4
Final approval of the article: 1,2,3,4

Each author believes that the manuscript represents honest work and certifies that the article is original, is not under consideration by any other journal, and has not been previously published.

Availability of Data and Material:

The corresponding author is prompt to supply datasets generated during and/or analyzed during the current study on wise request.

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