Expression of P53 in Clinically Diagnosed Solitary Thyroid Nodules

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Abstract

Background: Solitary thyroid nodule has provoked increased concern owing to higher incidence of malignancy in it compared to multinodular goiter. Expression of p53 in solitary thyroid nodules has a significant impact on early diagnosis and better treatment strategy of thyroid carcinoma.

Method: Total 40 cases of solitary thyroid nodules were evaluated by immunohistochemical staining for mutant p53 expression. The study was performed in Sir Salimullah Medical College, Dhaka (from January, 2019 to December, 2020). Statistical analyses were carried out by using SPSS version 22 for Windows. A descriptive analysis was performed for all data.

Results: In this study, the mean age was 33.38 ± 13.11 and male to female ratio was 1:9. p53 expression was observed to be found more in relatively smaller tumor and most of the differentiated tumors. Among malignant lesions, the predominant histologic type was papillary thyroid carcinoma (85%) including follicular variant and usual type. All carcinomas (100%) were well differentiated. Most of malignant lesions (92.6%) showed positive p53 expression.

Conclusion: Patients with p53 expression are related to malignant thyroid nodules. So, p53 immunoexpression is an important genetic marker for differentiating benign and malignant thyroid nodules.

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Introduction

Solitary thyroid nodule (STN) is defined as a palpable discrete swelling within an otherwise apparently normal thyroid gland.[1] STNs are diagnosed in 4%-8% of adults by palpation and in 13%-67% of adults when ultrasound detection is used.[2] STN has provoked increased concern owing to higher incidence of malignancy in it as compared to multinodular goiter.[3] It is necessary to differentiate patients with benign STN from malignant ones for early treatment strategy and better patient management.

Among all the cancers of thyroid,characteristic nuclear changes are necessary for the diagnosis of papillary thyroid carcinoma (PTC), however when present focally along with papillary structures they cause diagnostic dilemma in distinguishing it from other thyroid lesions.[4] Currently, the standard diagnosis depends on the histomorphologic examination of routine Haematoxylin and Eosin (H&E) stained slides, but interobserver or intraobserver disagreements in the diagnosis of papillary and follicular thyroid lesions are well known and documented in literature.[5]

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Various newer immunohistochemistry (IHC) markers are being described and validated for differentiating benign STN from malignant ones and follicular variant of papillary carcinoma (FVPTC) from follicular carcinoma (FCa) or follicular adenoma (FA).[6] However, unfortunately most of these markers still lacking accuracy are costly and/or difficult to implement in a clinical practice.[7,8] In the present study, IHC marker p53 was analyzed to differentiate between benign and malignant surgically resected STN along with their utility in the identification of PTC.

STN cases diagnosed on USG, clinically and on gross thyroid specimen examination were studied which include dominant/hyperplastic nodule in MNG, colloid nodule, FA and its variants and FCa, PTC and its variants. Thyroid lesions with multiple nodules or diffuse hyperplasia and tumors/lesions which are not of follicular epithelial cell origin were excluded from the present study.

p53 has been described as the guardian of genome because of its role in conserving stability by preventing gene mutation. The p53 gene (also known as TP53) is a tumor suppressor gene located on short arm of chromosome 17p13.1 that regulates cell cycle progression, DNA repair, cellular senescence and apoptosis.[1]

Mutation of p53 gene gives rise to abnormal protein with a long half life rendering it to be easily detected by immunohistochemistry. However, IHC is simple and is consistent with other techniques. Detection of p53 protein in malignant cells has been associated with poor clinical outcome and reduced survival in several tumor type.[9]

The aim of the current study was to see the expression of mutant p53 protein to differentiate between benign and malignant surgically resected STN. So far known, there is no previous study on solitary thyroid nodule incorporating p53 expression and its utility in differentiating benign and malignant tumors in Bangladesh.

Objective

The objective of the study was to find out the role of p53 expression in differentiating benign and malignant solitary thyroid nodules.

Methods

It was a Cross-sectional and analytical study carried out from January 2019 to December 2020 at Sir Salimullah Medical College & Mitford Hospital and other teaching hospitals in Dhaka city.

The studied materials were formalin fixed paraffin embedded blocks of solitary thyroid nodules, taken from 40 patients were diagnosed histopathologically as benign and malignant thyroid lesions in the pathology department of Sir Salimullah Medical College Hospital and other teaching hospitals in Dhaka. Specimens were processed routinely for Haematoxylin and Eosin stain and analyzed under microscope. Histological clasiifications were recorded according to World Health Organization (2017) classification. Immunostainingwas done by using Dako Autostainer plus at the immunohistochemistry laboratory, department of Pathology, Square Hospital. For p53 immunostain, positive control was taken from sections of diffuse glioma. To validate the stain negative control was taken from sections of normal thyroid

tissues by omitting the primary antibody. The relation between p53 expression and the clinicopathological variables were analysed by the Chi-square test. The p-value < 0.05 were considered significant. The SPSS software, version 22.0 was used for data analysis. p53 immunostained sections were examined under light microscope. Cells were considered positive for p53 when a clear cut brown staining observed in the nucleus. The cases which showed <5% nuclear positivity were taken as p53 negative whereas those which showed ≥5% nuclear positivity were considered as positive.[10,11]

Evaluation of p53 expression
Negative: <5% cells are positive
Positive: ≥5% cells are positive

Results

The purpose of current study was to see the expression of mutant p53 protein to differentiate between benign and malignant surgically resected solitary thyroid nodules. Total 40 patients were included in this study. Among the patients, 13 cases were with benign lesions and 27 cases were malignant lesions who were diagnosed in the department of Pathology in Sir Salimullah Medical College and other teaching hospitals of Dhaka during specified time period. All slides were reviewed by two Pathologists at department of Pathology in SSMC. All cases of thyroid carcinoma were graded according to WHO grading criteria and staged according to TNM system. The expression of p53 was observed and counted.The results were compared by statistical analyses among the different groups and presented in a tabulated form.

Table I: Distribution of the study cases according to age (n=40)

Age (years)	Number of cases	Percentage
≤20	9	22.5
21-30	11	27.5
31-40	8	20.0
41-50	9	22.5
51-60	3	7.5
Mean±SD	33.38	±13.11
Range (min,max)	12	,60

It was observed that the distribution of the study cases according to age had 11 cases (27.5%) belonged to age 21-30 years. The mean age was 33.38±13.11 years which ranged from 12 to 60 years. (Table I).

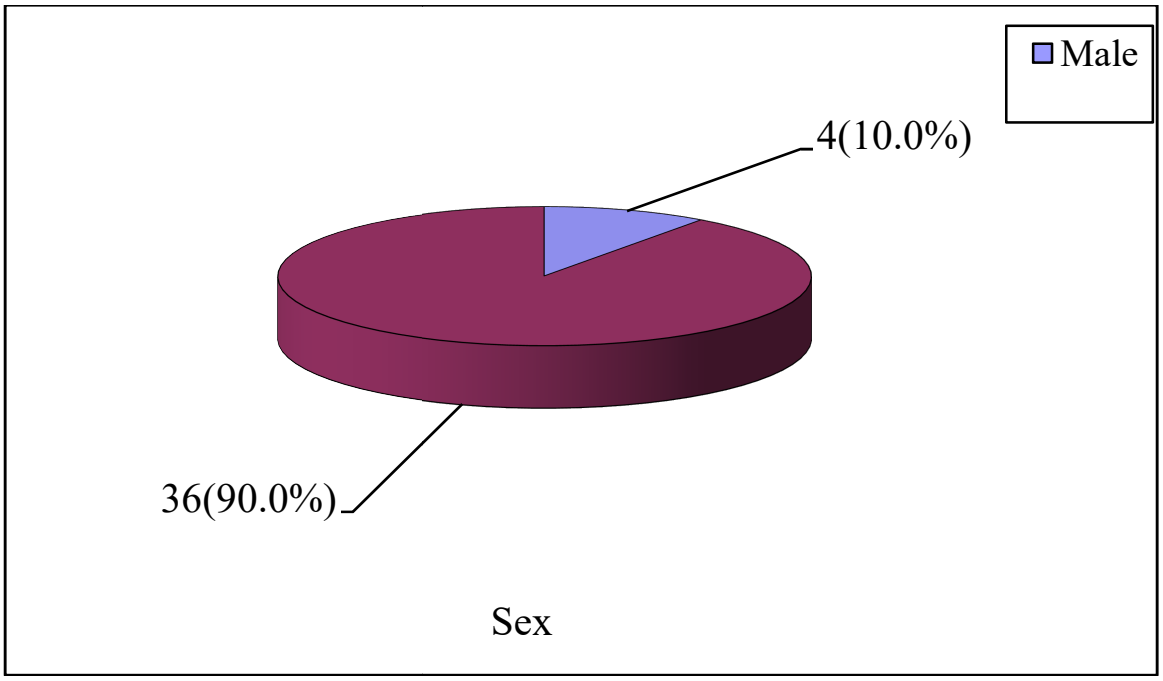

Figure 1. Pie chart showing distribution of study cases according to sex(n=40)

The Pie chart showing the distribution of the study cases according to sex that 36 cases (90%) were female and 4 cases (10%)were male (Figure 1).

Table II: Distribution of the study cases according to size of solitary nodule (cm) (n=40)

Size of solitary nodule (cm)	Number	%
<2	9	22.5
2-4	27	67.5
>4	4	10.0
Mean±SD	2.74	±1.18
Range(min,max)	1.5	6.5

It was observed that the distribution of the study cases according to size of solitary nodule had 27 cases (67.5%) belonged to size of solitary nodule 2-4 (cm). The mean size of solitary nodule 2.74±1.18 (cm) which ranged from 1.5 to 6.5 (cm) (Table II).

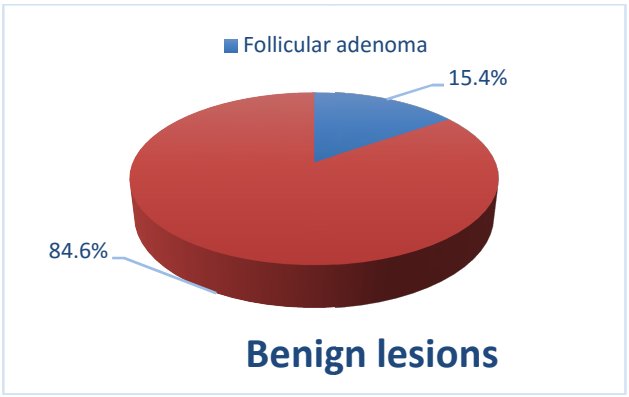

Figure 2. Pie chart showing benign lesions of the study cases (n=13)

It was observed in Pie chart showing the distribution of study cases according to benign lesions that 11 cases (84.6%) had nodular goiter followed by 2 cases (15.4%) of follicular adenoma (Figure 2).

Table III: Distribution of the study cases according to types of malignant lesions (n=27)

Malignant lesions	Number of cases	Percentage (%)
Papillary carcinoma	23	85.2
Follicular carcinoma	4	14.8

It was observed that the distribution of the study cases according to malignant lesions had 23 cases (85.2%) of papillary carcinoma and 4 cases (14.8%) of follicular carcinoma (Table III).

Table IV: Distribution of the study cases according to p53 expression (n=40)

p53 expression	Number of cases	Percentage
Negative	15	37.5
Positive	25	62.5

It was observed in the distribution of the study cases according to p53 expression that 25 cases (62.5%) showed positive p53 expression and 15 cases (37.5%) showed negative p53 expression (Table IV).

Table V: Distribution of benign and malignant lesions according to age of study cases (n=40)

Age (years)	Benign lesions (n = 13)	Malignant lesions (n = 27)
≤20	2	7
21-30	2	9
31-40	5	3
41-50	3	6
51-60	1	2

It was observed that the distribution of benign and malignant lesions according to age of study cases had 9 cases (33.33%) belonged to age 21-30 years of malignant lesions and 2 cases (15.4%) of benign lesions (Table V).

Table VI: Association of p53 expression with size of solitary nodule of the study cases (n=40)

Size of solitary nodule (cm)	p53 expression				*p* value
	Negative (n=15)		Positive (n=25)		
	n	%	n	%	
<2	1	6.7	8	32.0	
2-4	12	80.0	15	60.0	
>4	2	13.3	2	8.0	
Mean±SD		3.3±1.28		2.03±0.82	0.0005[s]
Range(min-max)		1.5,6.5		1.5,45	

s = significant
p value reached from ANOVA test

The association of p53 expression with size of solitary nodule was observed in study cases.
The mean size of solitary nodule was 3.3±1.28 cm in negative p53 expression and 2.031±0.82 cm in positive p53 expression. The difference was statistically significant ($p<0.05$) (Table VI).

Table VII: Distribution of p53 expression according to benign lesions of the study cases (n=13)

p53 expression	Nodular goiter (n=11)		Follicular adenoma(n=2)	
	n	%	n	%
Negative	8	100.0	2	100.0

It was observed in distribution of p53 expression with benign lesions that all cases (100.0%) had negative p53 expression (Table VII).

Table VIII: Association of p53 expression with malignant lesions of the study cases (n=27)

p53 expression	Papillary carcinoma (n=23)		Follicular carcinoma (n=4)		*p* value
	n	%	n	%	
Negative	2	8.7	0	0.0	0.539^{ns}
Positive	21	91.3	4	100.0	

ns=not significant
p value reached from Chi-square test

The association of p53 expression with malignant lesions was observed in the study cases. It was observed that 21 cases (91.3%) had positive p53 expression in papillary carcinoma and 4 cases (100%) had positive p53 expression in follicular carcinoma. The difference was statistically not significant (p>0.05) (Table VIII).

Table IX: Association of p53 expression with benign/malignant lesions of the study cases (n=40)

p53 expression	Benign lesions (n=13)		Malignant lesions (n=27)		*p* value
	n	%	n	%	
Negative	13	100.0	2	7.4	0.001^{s}
Positive	0	0.0	25	92.6	

s=significant
p value reached from Chi-square test

The association of p53 expression with benign/malignant lesions was observed in the study cases. It was observed that 13 cases (100.0%) had negative p53 expression in benign lesions and 2 cases (7.4%) had negative p53 expression in malignant lesions. The difference was statistically highly significant (p<0.05) (Table IX).

Figure 3. Photograph showing gross picture of a papillary carcinoma(Case no:17)

Figure 4. Photograph showing gross picture of a follicular carcinoma(Case no:16)

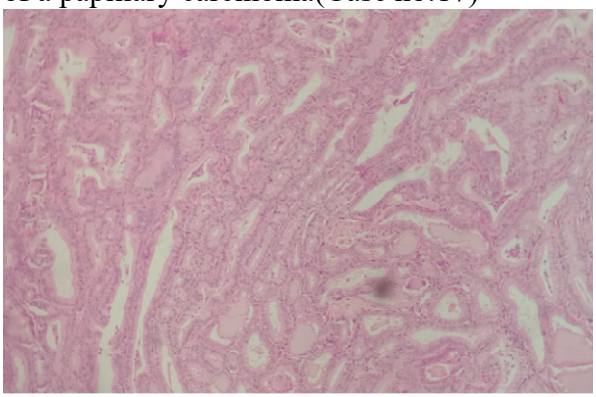

Figure 5. Photomicrograph showing well differentiated papillary carcinoma of thyroid(Case no: 20,H&E,100x)

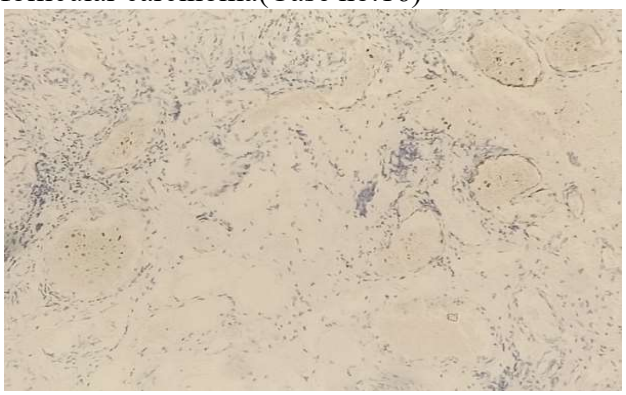

Figure 6. Photomicrograph showing positive p53 expression in papillary carcinoma (Case no: 20,IHC for p53,100x)

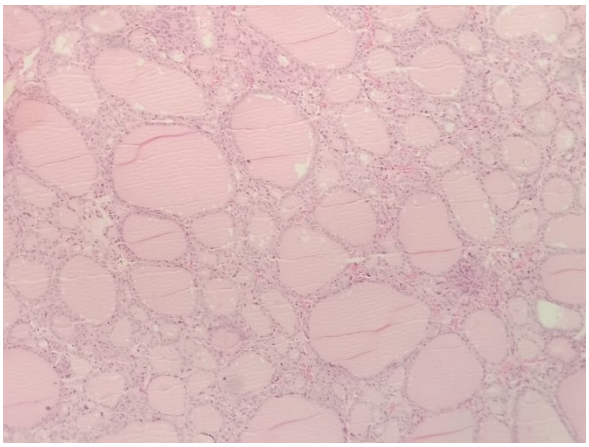

Figure 7. Photomicrograph showing papillary carcinoma,follicular variant of thyroid (Case no: 8, H&E, 100x)

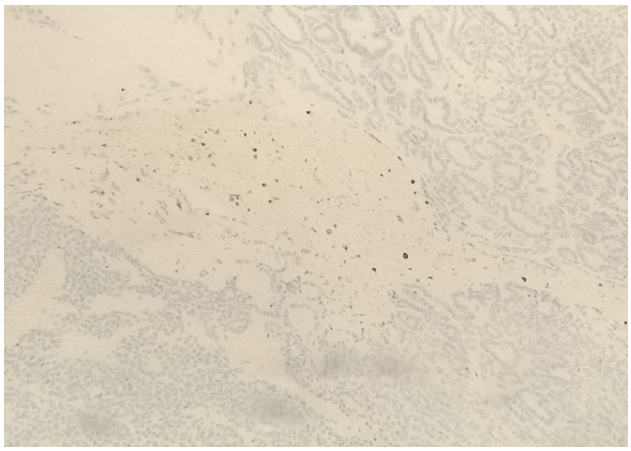

Figure 8**.** Photomicrograph showing positive p53 expression in papillary carcinoma, follicular variant of thyroid (Case no: 8, IHC for p53, 100x)

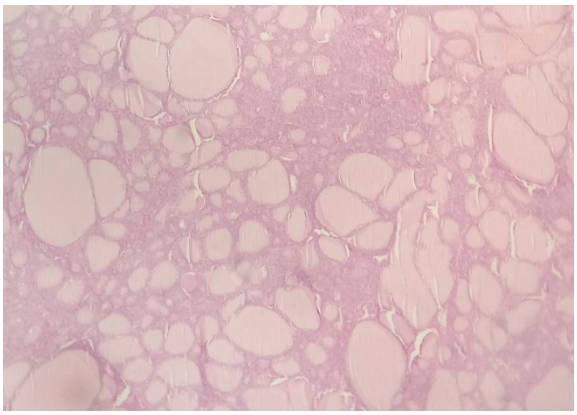

Figure 9. Photomicrograph showing follicular carcinoma of thyroid (Case no : 22, H&E, 100x)

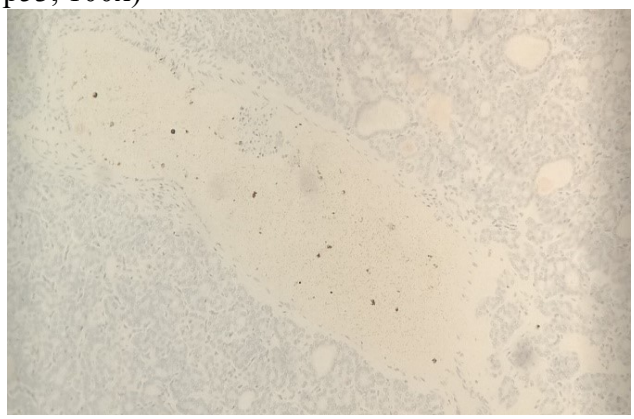

Figure 10**.** Photomicrograph showing positive p53 expression in follicular carcinoma (Case no: 22, IHC for p53, 100x)

Discussion

Solitary thyroid nodules are diagnosed in 4%-8% of adults by palpation and in 13%-67% of adults when ultrasound detection is used.[2] Thyroid cancers occur in approximately 5% of all thyroid nodules independent of their size.[2] A female predominance has been noted among patients who develop thyroid carcinoma in the early and middle adult years. The incidence in women is about 6%.In contrast,cases presenting in childhood and late adult life are distributed equally among males and females.Majority of thyroid carcinomas are derived from the thyroid follicular epithelium and of these,the vast majority are well differentiated lesions.[1]

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In this study,total 40 cases were included.The age ranged from 12 to 60 years and the mean age was 33.38 ± 13.11.Most of the study subjects were between 21 to 30 years of age group(11 cases,27.5%) followed by 41 to 50 years (9 cases, 22.5%) and less than 20 years of age group(9 cases,22.5% each).There was another group 31 to 40 years of age(8 cases,20%).The fifth group was 51 to 60 years of age(3 cases,7.5%).There was no case below 12 years or above 60 years.

Among 40 cases, below 20 years age group had 7 cases (25.9%) of malignant lesions and 2 cases (15.4%) of benign lesions. Among the age group 21-30 years, 9 cases (33.33%) had malignant lesions and 2 cases (15.4%) had benign lesions. Among the age group 31-40 years, 3 cases (11.11%) had malignant lesions and 5 cases (38.5%) had benign lesions. Among the age group41-50 years, 6 cases (22.22%) had malignant lesions and 3 cases (23.0%) had benign lesions. Among the age group 51-60 years, 2 cases (7.4%) had malignant lesions and 1 case (7.6%) had benign lesion.

In this current study,4 cases(10%) were male and 36 cases(90%) were female.And male female ratio was 1:9.In a previous study, it was showed that p53 expression was significantly higher in papillary thyroid carcinoma found in male patients.[11]

Among 40 cases,25 cases(92.6%) showed positive p53 expression.Among them, 8 cases (32.0%) were less than 2cm,15cases(60.0%) were 2 to 4cm and 2 cases (8.0%) were above 4cm of solitary nodular size. Fifteen cases showed negative p53 expression.Among them,1case(6.7%) was below 2 cm, 12 cases (80%) were 2 to 4 cm and 2 cases (13.3%) were above 4cm of nodular size. The mean size of solitary nodule was 3.3±1.28(cm) in negative expression and 2.03±0.82 cm in positive expression of p53. The difference was statistically significant.It was seen that patients with mutant p53 positive group showed a greater tendency to smaller nodules than larger ones.This finding is consistent with Marcello et al. (2013) who reported that p53 was more often seen staining smaller tumours(<2cm) than tumors larger than 4 cm ($p<0.01$).[12] He also found that p53 was more frequent in solitary nodules than in multifocal tumours($p=0.0286$) and tended to appear more frequently in encapsulated tumours than in those without capsule. Although the statistical comparison showed a marginal p-value ($p=0.0762$).[12]

Among the 13 cases of benign lesions, 11 cases(84.6%) were nodular goiter and 2 cases(15.4%) were follicular adenoma. All the 13 cases(100%) showed negative expression of p53. This finding is consistent with the study of Dwivedi et al. (2016) who found negative p53 expression in 70.65% cases of benign solitary nodules.[10]

Out of the 27 cases of malignant lesions,23 cases (85.2%) were papillary carcinoma and 4 cases (14.8%)were follicular carcinoma. Among the 23 cases of papillary carcinoma, 21 cases(91.3%) showed positive and 2cases(8.7%) showed negative p53 expression. Out of 4cases of follicular carcinoma, all cases (100%) showed positive expression of p53. This finding is consistent with Dwivedi et al.(2016) who found positive p53 expression in 85.71% cases of follicular carcinoma and 86.11% cases of papillary carcinoma.[10] Shin et al.(2014) showed p53 overexpression in 47.3% of the papillary thyroid carcinoma cases.[13] In that study, the diagnostic sensitivity and specificity were 85.0% and 72.7% , respectively.[13]

Among the 27 cases of malignant lesions, all were found as differentiated. Among the differentiated carcinoma, 25 cases(92.6%) showed positive and 2 cases (7.4%) showed negative p53 expression. So it is observed the high tendency of well differentiated carcinoma for positive p53 expression from the above discussion. Dwivedi et al.(2016) found positive p53 expression in 86.21% differentiated thyroid carcinoma.[10] Choudhury et al (2011), Lee(2013), Marcello(2013) and Shin et al.(2014) have reported that the detection of p53 protein was a significant and independent prognostic indicator in differentiated thyroid carcinoma.[11-14]

Conclusion

The present study was intended to find out the association of p53 protein,which is associated with TP53 gene mutation with solitary thyroid nodule. It was found that p53 positive expression tended to be related with malignant thyroid nodules. Thyroid carcinoma patients with p53 positive expression are related to relatively smaller thyroid nodules and well differentiated thyroid carcinoma. The IHC method is acceptable for detection of p53 mutation due to it's reliability and feasibility. The technique may be expected to serve as a new genetic marker for differentiating benign and malignant thyroid nodules. So, p53 expression in solitary thyroid nodules could be useful for early treatment strategy and better patient management.

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