

Research Article

The role of cell cycle regulatory protein p53 in Follicular neoplasms of thyroid with hurthle cells

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Received: 27 March 2016

Accepted: 14 May 2016

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ABSTRACT

Background: The disease biology of Follicular neoplasms of thyroid with hurthle cells is poorly understood. Very few studies in literature have addressed the role of p53 in these neoplasms. The aim of the present study is to analyze the histomorphological features of Follicular neoplasms with hurthle cell change and to evaluate the role of p53 in their tumor biology.

Methods: 32 cases of Follicular neoplasms of thyroid with focal and pure hurthle cell change over a period of 2.5 years were studied histologically and immunohistochemically using p53 antibody (Biogenex). They included 20 follicular adenomas with focal hurthle cells, 10 pure hurthle cell adenomas and 2 hurthle cell carcinomas.

Results: All cases showed nuclear p53 positivity in hurthle cells. Muller-Hocker et al criteria was used for frequency scoring. Out of the 32 cases, 12 cases of pure hurthle cells showed score 3, Remaining 20 cases of follicular adenomas with focal hurthle cell change showed score 3 in cases with >50% hurthle cells, score 2 in cases with 20-40% hurthle cells and score 0 in cases with <10% hurthle cells.

Conclusions: The study showed a good correlation of p53 protein expression with tumor progression and aggressiveness. Hence this indicates that molecular alterations in p53 pathway play a role in tumor biology in Follicular neoplasms of thyroid with hurthle cell change.

Keywords: Follicular neoplasms with hurthle cells, p53, Frequency score, Tumor biology

INTRODUCTION

Hurthle cells are eosinophilic, follicular derived cells that are associated with a variety of non neoplastic and neoplastic lesions of thyroid. The exact significance of hurthle cells in the thyroid lesions remains a matter of great debate.¹

Out of these lesions, Hurthle cell tumors of thyroid constitute a rare group of neoplasms for which the disease biology is poorly understood. The diagnosis and malignant potential of these tumors have received a great deal of attention in recent years.

Hurthle cell tumors represent 4.5-10% of all primary thyroid epithelial neoplasms. The reported incidence of

Hurthle cell carcinoma is 0.4 to 10 percent of all thyroid carcinomas.²

According to the World Health Organization (WHO), these neoplasms are considered a variant of follicular carcinoma of the thyroid and are referred to as follicular carcinoma, oxyphilic type.³

According to Armed Forces Institute Of Pathology Hurthle cell carcinoma should be separated into a category of thyroid neoplasms different from true follicular cancer.³

The etiology of the hurthle cell tumors is unknown. The precise cellular derangements that lead to the abnormal accumulation of mitochondria in oncocytes and tumor

development are obscure. Alterations of nuclear genetic material (marked aneuploidy) and of mitochondrial DNA are a feature of thyroid oncocyctic tumors.²

The Hürthle cell is a follicular-derived cell, which has a cytoplasm characterized as “swollen” This swelling is due in large part to the presence of numerous mitochondria in the cellular cytoplasm. Hurthle cell is also called oxyphilic cell, askanzy cell and oncocyctic cell; the benign conditions with hurthle cells include autoimmune thyroiditis, multinodular goitre, Graves’ disease and patients treated with head and neck irradiation and systemic chemotherapy and as an ageing phenomenon. The neoplastic conditions include Hurthle cell neoplasms, Oncocyctic variant of papillary thyroid carcinoma, Warthin-like variant of papillary thyroid carcinoma and Oncocyctic variant of medullary carcinoma thyroid.⁴

Hurthle cell neoplasms range from benign tumors that disappear spontaneously to tumors with widespread metastasis.

A pivotal issue in the treatment approach to these tumors is the correlation of diagnostic histopathological criteria and tumor biology. The diagnosis of hurthle cell carcinoma can be challenging and diligent scrutiny of multiple histopathological sections is required to define the nature and extent of capsular and vascular invasion, the hallmarks of malignancy for this disease. This has prompted many studies to examine the biology of hurthle cell neoplasms on a molecular level.¹

Mutations in the p53 tumor suppressor gene are among the most frequently detected mutations in human cancer. A number of studies have reported an increased prevalence of p53 mutations in poorly differentiated and undifferentiated thyroid carcinomas. However, only few studies have addressed the role of p53 gene expression in oncocyctic neoplasms.¹

The aim of the present study is to analyse the histomorphological features of follicular neoplasms of thyroid with pure and focal hurthle cell change, to evaluate the expression of the cell cycle regulatory protein p53 and to compare the results of the study with other studies in literature.

METHODS

A prospective study of 32 cases of follicular neoplasms of thyroid with pure and focal hurthle cell change was done on cases collected at Osmania General Hospital over a period of 2.5 yrs from May 2007 to November 2009.

The specimens were fixed in 10% buffered formalin, grossed; sections were taken from appropriate representative sites. The sections were then processed in automated tissue processor and embedded in paraffin

wax, 4 microns thick sections were cut, stained with Haematoxylin and Eosin, mounted and then examined by light microscopy.

Areas showing diffuse infiltration of oxyphil cells were selected and only cells possessing abundant eosinophilic granular cytoplasm either forming follicles or compact groups on routine hematoxylin-eosin stained slides were considered as oxyphilic cells.

2–3 μ thick sections were stained for immunohistochemistry with a standard avidin-biotin complex method using monoclonal antibody against p53 (Biogenex). Intra ductal carcinoma of breast was taken as positive control and negative controls were obtained by omitting the primary antibody and incubating the slides in primary diluent. In addition 20 of the cases also contained normal thyroid tissue which was evaluated as a normal control.

Brown staining of nucleus is taken as positive result. The results were expressed as the percentage of positive cells per 1000 thyroid follicular cells. Only cells with an evidence of nuclear staining were considered positive. The criteria proposed by Muller-Hocker et al were used to score the staining pattern.

Frequency score

- 0 - no reactive cells or very few single scattered cells immunostained
- 1 - up to 10% reactive cells
- 2 - more than 10% and less than 50%
- 3 - more than 50% reactive cells

The intensity score was rated as weak (grade 1), moderate (grade2) and strong (grade3).

RESULTS

Total number of thyroid specimens received at Osmania General Hospital during the period was 112 out of which 32 were Follicular neoplasms with both pure and focal Hurthle cell metaplasia.

Table 1: Frequency score.

% of Hurthle cells	no of cases	% of cases	frequency score
100%	12	37.50%	3(82%)
50	3	0.90%	2(42%)
20-40%	8	25%	1(8%)
10%	9	28%	0(focal)

Out of the 32 cases, 20 cases were follicular adenomas with focal hurthle cell change, 10 were hurthle cell adenomas and 2 were hurthle cell carcinomas (Figure 1).

All the cases were examined grossly and microscopically.

Table 2: Comparison of p53 positivity.

% of Hurthle cells	Ranikanthan and Jasim et al % of positivity	Present study (% of p53 positivity)	% of Hurthle cells
100%	52%	82%	100%
50%	11%	42%	50%
20-40%	2-6%	8%	20-40%
10%	Negative	Focal	10%

Grossly out of the 20 cases of Follicular adenoma with Hurthle cell change, 16 cases showed a single well circumscribed lesion surrounded by a thin capsule involving one lobe of thyroid. 4 cases showed multiple nodules surrounded by a thin complete capsule, the nodules involving both lobes of thyroid. the average size of the tumors is 5x2x1cm the cut section in all the cases

showed grey white homogenous nodule with foci of pale tan to brown areas; one case showed areas of cystic degeneration.

Table 3: Intensity score.

	Muller-Hocker et al			In Present Study		
	1	2	3	1	2	3
Oncocytic Adenoma	67%	27%	6%	100%		
Oncocytic Carcinoma	33.3 %	53.3 %	13.3%	100%		

All the 10 cases of Hurthle cell adenomas showed a single solid nodule which was encapsulated completely by a thin complete capsule. The average size of the tumor is 5x3x2 cm; cut section showed a characteristic pale tan appearance.

Table 4: Frequency score.

	Muller-Hocker et al					In Present Study			
	0	1	2	3		0	1	2	3
Oncocytic Adenoma (20)	25%	60%	15%	-	Oncocytic Adenoma (10)	-	-	-	100%
Oncocytic Carcinoma (17)	12%	41%	29%	18%	Oncocytic Carcinoma (2)	-	-	-	100%

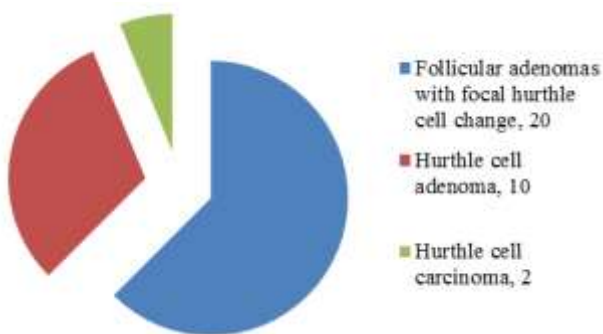


Figure 1: Distribution of cases.

The 2 cases of Hurthle cell carcinoma showed a single nodule which was partially encapsulated; the average size of the tumor was 6x4x3 cm. Cut section showed a variegated appearance with areas of hemorrhage and necrosis. One of the cases showed areas of myxoid degeneration.

On light microscopy, cases of Follicular adenoma with hurthle cells showed a thin fibrocollagenous capsule separating the adenoma from the normal uninvolved thyroid tissue which was compressed. The tumor is composed of relatively uniform appearing colloid filled follicles. The individual follicular epithelial cells showed moderate amount of eosinophilic cytoplasm with regular

vesicular nuclei with inconspicuous nucleoli. Foci of hurthle cells with abundant eosinophilic granular cytoplasm, enlarged vesicular nuclei with prominent eosinophilic nucleoli are seen these cells are seen lining some of the follicles and at places are seen in trabecular pattern. The stroma showed small to large vascular spaces (Figure 2).

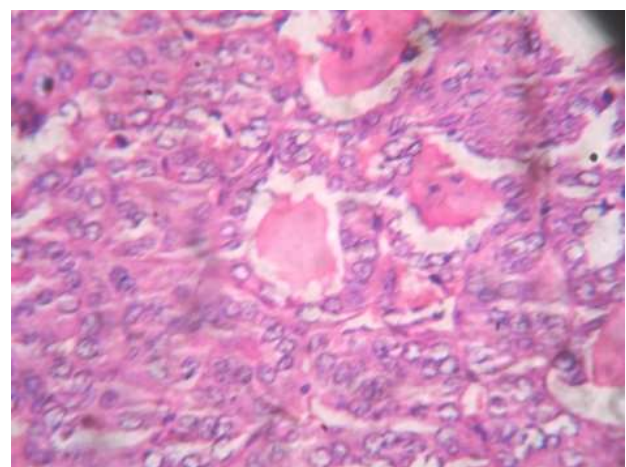


Figure 2: Follicular adenoma with focal hurthle cells (H&E x 40).

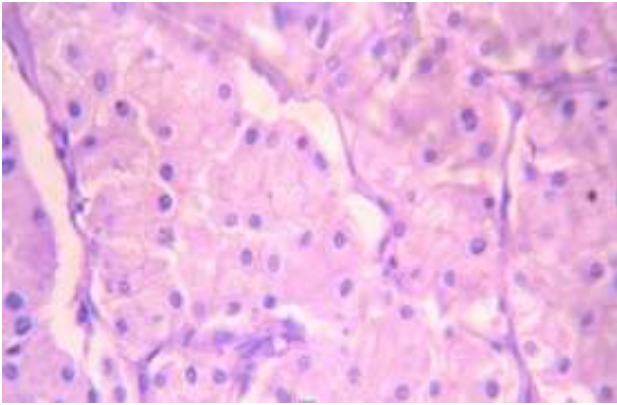


Figure 3: Hurthle cell adenoma (H&E x 40).

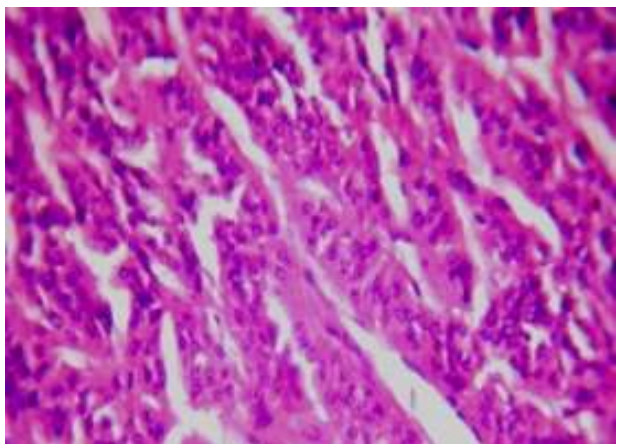


Figure 4: Hurthle cell carcinoma in trabecular pattern (H&E x 40).

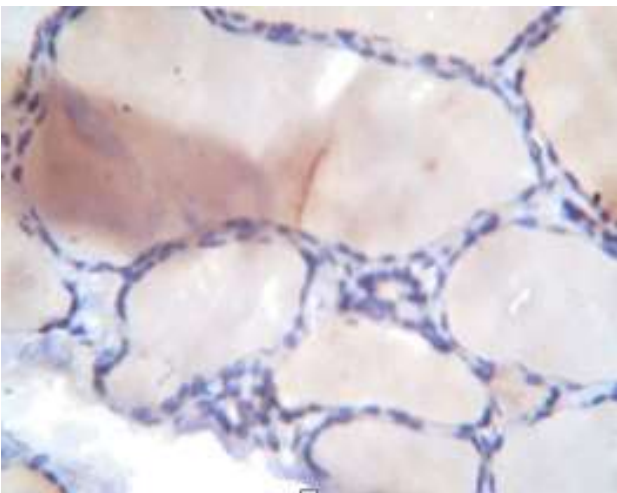


Figure 5: Immunohistochemistry for p53 in normal thyroid follicular cells).

Cases of Hurthle cell adenoma on microscopy also showed a thin fibrocollagenous capsule separating the tumor from the adjacent normal compressed thyroid tissue. The tumors were composed of hurthle cell which constituted more than 75% of the cells. The cells were seen in trabecular pattern (Figure 3).

Cases of Hurthle cell carcinoma showed a thick fibrocollagenous capsule separating the tumor from the adjacent normal compressed thyroid tissue. The tumor is composed of hurthle cells which constituted more than 75% of the cells. The cells showed moderate pleomorphism and an increase in the nuclear-cytoplasmic ratio. These cells were seen in trabecular pattern and at places as solid sheets. Atypical mitotic figures and areas of necrosis seen; capsular and vascular invasion is seen and one of the cases showed areas of myxoid degeneration (Figure 4).

Paraffin blocks of 32 cases of Follicular neoplasms with pure as well as focal hurthle cell change were taken and subjected to immunohistochemical study with P53 antibody. Only nuclear positivity in hurthle cells was considered positive. Frequency scoring was done according to Muller-Hocker et al criteria.

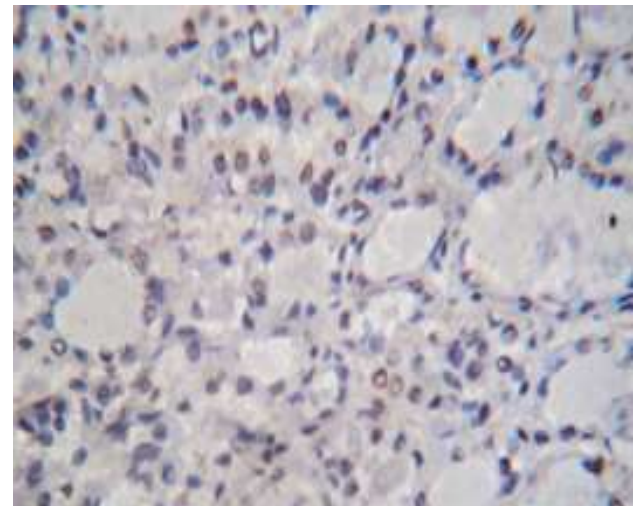


Figure 6: Immunohistochemistry for p53 in Follicular adenoma with focal hurthle cells showing score 1.

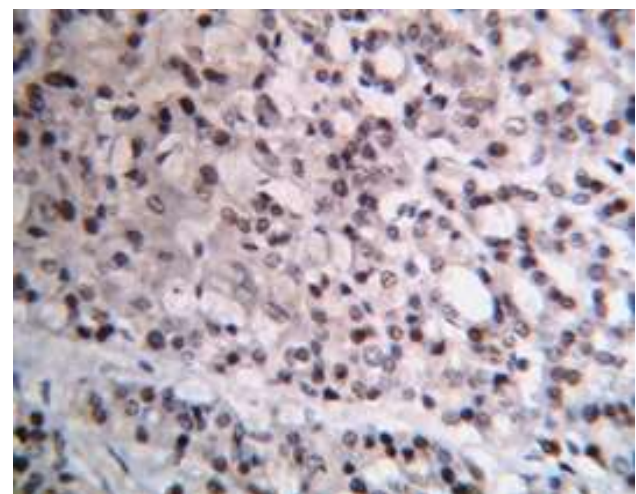


Figure 7: Immunohistochemistry for p53; hurthle cells showing score 2.

Out of the total 32 cases taken:

- 12 cases of pure 100% hurthle cells (37.5%) showed a frequency score of 3 (>50%) with intensity of staining being moderate (grade2) in hurthle cell adenomas and strong (grade3) in hurthle cell carcinomas (Figure 8).
- 3 cases with 50% hurthle cells (0.9%) showed frequency score of 2(10-50%) with moderate (grade 2) intensity (Figure 7).
- 8 cases with 20-40% hurthle cells (25%) showed frequency score of 1 with moderate (grade 2) intensity of staining (Figure 6).
- 9 cases with less than 10% hurthle cells (28%) showed frequency score of 0 with weak (grade 1) intensity.

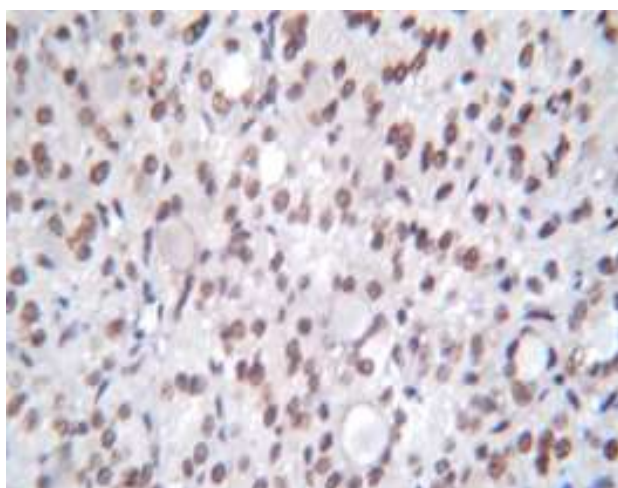


Figure 8: Immunohistochemistry for p53; hurthle cells showing score 3.

The normal thyroid tissue was non-reactive for p53 antibody (Figure 5).

All tissues used as positive controls showed specific immunoreactivity for p53; negative controls did not show any specific staining for the antibody (Table 1).

DISCUSSION

Hurthle cell tumors are rare epithelial thyroid neoplasms with variable biological behaviour. Despite well-defined histopathological criteria, the biology of this disease is poorly understood.¹

In the present study we encountered 112 cases of thyroid lesions out of which 32 cases were follicular neoplasms with pure and focal Hurthle cells.

The incidence of these cases at Osmania General Hospital is 28.5% of thyroid lesions. The exact significance of hurthle cells in the thyroid remains a matter of great debate. Although there were many theories (Heimann et

al, Gardner) regarding the morphogenesis of this cell, recent ultrastructural studies have established that they are transformed follicular cells (Lennox, Horn-Feldman et al, Heimann et al). Oxyphilic cells used to be thought to represent a degenerative change resulting from some unknown cellular injury. This belief although once widely accepted, has been contraindicated by enzyme histochemical studies (Tremblay and Pearse) demonstrating a very high mitochondrial activity in oxyphilic cells and oxidative enzyme system such as DPN diphorase, succinate dehydrogenase, and TPN-linked isocitrate dehydrogenase (Tremblay and Pearse).⁵

Most authors suggest that this cell represents a degenerative or metaplastic response to follicular epithelial cell damage.⁵ Studies have shown that hurthle cells are less active than follicular cells and they show limited thyroglobulin production however they do contain high levels of oxidative enzymes.⁶

The treatment approach to these tumors, especially for the clinically aggressive variant of widely invasive hurthle cell carcinoma, may be improved through the correlation of criteria reflecting histomorphology and tumor biology.

In addition investigation of molecular changes in tumors representing the entire disease spectrum may enhance our understanding of mechanisms involved in thyroid tumorigenesis.¹

Mutations in p53 tumor suppressor gene are among the most frequently detected mutations involved in the tumor biology of many human cancers. A number of studies have reported an increased prevalence of p53 mutations in poorly differentiated and undifferentiated thyroid carcinomas. However, few studies have addressed the role of p53 gene expression in oncocytic thyroid neoplasms.¹

The present study aims at analysing the biological behaviour of Hurthle cell tumors based on the protein expression profile of p53, a cell cycle regulatory protein.

p53 protein half-life is short and expression levels are low in normal cells and therefore immunohistochemistry cannot detect these wild- type p53 levels.

In cancer cells, most p53 mutations lead to products that accumulate in the nuclei and can be demonstrated by immunohistochemistry. Positive immunostaining represents the stable protein product of a mutated p53 gene that has lost its cell cycle regulatory function.p53 immunoreactivity in tissues is usually indicative of a mutation of p53 gene which is linked to malignant transformation of the cell.⁷

In the present study, cases with pure 100 % hurthle cells i.e. hurthle cell adenomas and hurthle cell carcinomas showed the highest nuclear p53 expression frequency

score of 3. Cases with a lesser number of hurthle cells showed a lower frequency score. Cases with 50% hurthle cells showed frequency score of 2 with moderate intensity, cases with 20-40% hurthle cells showed frequency score of 1 with moderate intensity of staining, cases with less than 10% hurthle cells showed frequency score of 0 with weak intensity. Out of the 20 cases of follicular adenomas with hurthle cell change, in only 1 case the follicular epithelial cells of the adenoma showed a frequency score of 1 (<10% nuclear positivity) whereas in the remaining 18 cases the follicular epithelial cells were non-reactive for the p53 antibody. Normal thyroid tissue in all the sections was non-reactive to the p53 antibody.

This shows that as the percentage of hurthle cells in the thyroid tumor increases, the percentage of hurthle cells showing positive reactivity for p53 protein also increased. The study also showed that there is higher expression of p53 in hurthle cells compared to that of follicular epithelial cells in cases of Follicular adenomas with hurthle cell change.

The present study correlated well with the study conducted by Ranikanthan and Jasim et al. They studied 25 cases of pure as well as focal hurthle cell change in thyroid adenomas and they found that majority of the hurthle cells were p53 positive and adenomas with increased percentage of hurthle cells had an increased percentage of p53 staining. The follicular epithelial cells in Follicular adenoma were negative for p53 expression (Table 2).⁷

Joseph Muller-Hocker et al analysed 20 oncocytic adenomas and 17 oncocytic carcinomas of the thyroid and found that 75% of adenomas and 88% of carcinomas show staining for p53. They found that the intensity of nuclear staining was higher in the carcinoma group than in adenoma group. They concluded that p53 is more prevalent in oncocytic carcinomas than in adenomas indicating that this factor may be involved in tumor progression (Table 3, 4).

Kohli A et al studied the expression of p53 and bcl-2 in 18 Hurthle cell adenomas and 8 hurthle cell carcinomas and compared them with 16 follicular adenomas. They found that only 1 follicular adenoma showed grade 1 (<10%) p 53 staining while the remaining 15 were negative. 15/18 of hurthle cell adenomas and all 8 of hurthle cell carcinomas showed p53 expression of varying grades. They concluded that there appears to be an inverse relationship between p53 and bcl-2 expression in thyroid follicular neoplasms; a higher expression of p53 and lower levels of bcl-2 in hurthle cell neoplasms may have biological and clinical implications.

In the study conducted by Hoos A et al nuclear p53 expression was present only in 7 cases in a cohort of 69 Hurthle cell tumors. However, in their cohort, overexpression of mdm-2, a p53 binding protein that

inactivates p53 function was a frequent event. This finding puts the relevance of different members of the p53 pathway for thyroid tumorigenesis into perspective. They suggested that mdm-2 inhibits wild type p53, thus contributing to the proliferative potential of these cells.¹

It has however been questioned whether p53 is an independent marker of prognostic significance. The study conducted by Muller-Hocker et al concluded that p53 is associated with tumor progression and aggressiveness and not so much with oncogenesis.⁸

Other investigators have stressed that positive nuclear reaction for p53 protein is always linked to mutations in the p53 gene. However, as the grade of the thyroid tumor malignancy increases a positive correlation has been observed between the presence of mutations within p53 gene and positive reaction for overexpression of p53 protein.⁹

The following are the conclusions made from the study:

- In Hurthle cell neoplasms, molecular alterations in p53 pathway are associated with tumor progression and aggressiveness.
- The present study showed a good correlation of p53 protein expression with tumor aggressiveness, the score being highest in Hurthle cell carcinoma and lowest in Follicular adenomas with focal hurthle cell change.
- However the exact role of this cell cycle regulator in these neoplasms is still not clear.
- Hence many more molecular genetic studies are needed to elucidate the biological significance of the abnormal expression of p53.

ACKNOWLEDGEMENTS

Our sincere and grateful thanks to Dr. K. R. K. Prasad, Head of Department of Pathology, Osmania Medical College, Hyderabad for his guidance and to all my colleagues and technical staff who helped me in this study.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: Not required

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Cite this article as: Nagoti S, Anunayi J, Patel M. The role of cell cycle regulatory protein p53 in Follicular neoplasms of thyroid with hurthle cells. *Int J Sci Rep* 2016;2(5):99-105.