Significant differences in p53 and BRAFV600 mutations among histological type of thyroid carcinoma in Indonesia

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Abstract

Introduction: Thyroid carcinoma is the most common endocrine cancer, where 80% of all thyroid carcinomas are of the Papillary Thyroid Carcinoma type with a 5-year survival rate of more than 95%. A series of molecular markers that can improve diagnostic and prognostic scoring systems are currently in use.

Aim: This study wanted to see the differences in P53 mutations based on the histological type of thyroid carcinoma in H. Adam Malik General Hospital Medan.

Methods: This study was an observational analytic study using a cross-sectional design, carried out at the Division of Surgical Oncology, Department of Surgery and at the Anatomical Pathology Laboratory of RSUP H. Adam Malik Medan, with a sample of 64 people diagnosed with thyroid carcinoma. The data obtained was then presented descriptively in the form of a narrative, proportion distribution table, and statistical analysis wasa conducted with Kolmogorov Smirnov($p<0.05$).

Results: Respondents had an average age of 51.88 years and the female sex was found to be at most 41 patients (63.1%). There are 53 patients (82.8%) with a tumor size >5 cm , 43 patients (67.2%) with neck lymph nodes and 59 patients (92.2%) were found without metastases and thyroid cancer histopathology was found mostly with papillary type in 42 patients (65.6%). Respondents were most often found without P53 mutations as many as 44 patients (68.8%) with p-value <0.001.

Conclusion: There were significant differences in P53 mutations based on the histological type of thyroid carcinoma in H. Adam Malik General Hospital Medan

Keywords: p53 mutation, histological type, thyroid carcinoma

1. Introduction

Thyroid carcinoma is a common endocrine malignancy that includes several histological subtypes including papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC), medullary thyroid cancer (MTC) and anaplastic thyroid cancer (ATC). Approximately 80% of all thyroid carcinomas are PTC and are usually curable and have a 5-year survival rate of over 95%. In contrast, ATC is rarer and is one of the more aggressive solid tumors with a median survival of 3-5 months.[1]

Thyroid carcinoma is the most common endocrine cancer, accounting for 1.0%-1.5% of all new cancers diagnosed annually in the United States, and its incidence has been steadily increasing in the last three decades worldwide. In Italy, thyroid carcinoma is the second most common cancer in women under the age of 45.[2,3]

Based on Riset Kesehatan Dasar in 2019, the proportion of cancer cases in the entire population at Dharmais Cancer Hospital was 3.78%, while the proportion was 2.68% in men and 4.64% in women.[4]

Data regarding the number of cases of thyroid carcinoma in Medan has not been widely published. Based on a hospital study in 2015, a profile of thyroid carcinoma patients was obtained at the H.Adam Malik General Hospital in Medan. In the period January 2013 - December 2015 as many as 288 patients were diagnosed with thyroid carcinoma and most experienced it in the age group 55 - 64 years, namely 31 people (32.0%). The highest frequency distribution of histopathological features of thyroid carcinoma patients was papillary thyroid carcinoma with 45 people (46.4%) and the lowest was medullary thyroid carcinoma with 3 people (3.1%).[5] Epidemiology of the anaplastic type has a median survival rate of 4 months and a 6-month survival rate of 35%, while disease-specific mortality is 98%-99%.[6]

A series of molecular markers that can improve diagnostic and prognostic scoring systems are currently in use. The tumor suppressor protein P53, encoded by the TP53 gene, plays a role in controlling cell proliferation and linking several cellular functions.[7] From P53 activation, several biological responses occur including apoptosis, cell cycle arrest, DNA repair, differentiation or cell senescence. The P53 gene is a truly multifunctional protein and is specifically involved in complex interactions with various species (DNA, RNA, protein and cell metabolism).[8,9] Mutations in the P53 gene can cause loss of tumor suppressor function by altering the function of the P53 protein as an apoptosis induction factor. and as a transcription factor.[10] This requires access to multidisciplinary care and molecular testing of tumors to improve survival. Such as targeted therapy for patients with BRAFV600E mutations with combination therapy of dabrafenib and trametinib.[11,12]

In terms of immunohistochemical examination to assess P53 mutations in thyroid carcinoma, this can be done at H. Adam Malik General Hospital so that later it is expected to be a basis and useful in determining the choice of therapy in thyroid carcinoma patients, where currently targeted therapy has been developed for other types of cancer accompanied by P53 mutation. From the description above, it appears that examination of P53 mutations in thyroid carcinoma patients is very important. This can be used as initial data for determining appropriate management and developed in subsequent studies. In addition, this research can be done and has never been done in Medan and even Indonesia.

2. Methods

This research is an observational analytic study using a cross-sectional design to analyze the differences in P53 mutations in the histological type of thyroid carcinoma in H. Adam Malik General Hospital Medan. This research was conducted at the Surgical Oncology Division of the Department of Surgery and at the Anatomical Pathology Laboratory of H. Adam Malik General Hospital, Medan. The research was initiated after obtaining approval from the ethical committee. The population in this study were all patients diagnosed with thyroid carcinoma at H. Adam Malik General Hospital, Medan. The sample in this study is part of the population who received treatment from August 2021 to December 2021 which met the inclusion and exclusion criteria. The sampling method used is non-probability sampling, namely the consecutive sampling technique of 64 samples.

The inclusion criteria for this study were thyroid carcinoma patients and had complete data including age, cancer stage, histological type of cancer, histopathological grading of cancer and still stored paraffin blocks of cancer tissue to assess P53 mutations. Exclusion criteria for this study were patients suffering from other malignancies, patients with other chronic diseases, patients suffering from immune system disorders and patients who were not willing to participate in this study. Data was collected from secondary data, namely by recording medical record status at the Division of Surgical Oncology, Department of Surgery, H. Adam Malik General Hospital Medan, data was recorded according to the variables studied.

This study required several equipment and reagents, namely patient medical records and patient

research status, consent forms to participate in the survey, paraffin blocks for patients who had a histopathological examination and tools for immunohistochemical analysis: Immunohistochemical visualization system, tissue cutting machine (microtome), silanized slides. The working procedure of immunohistochemistry on paraffin blocks of thyroid cancer patients stored at the Anatomical Pathology Section of H. Adam Malik General Hospital Medan includes cutting the paraffin blocks using a Leica 2125 RM microtome with a thickness of 3m, then affixing them to object glass that has been coated with poly-lysin. Then the researchers incubated in an incubator at 400C for 1 night and deparaffinized (xylol I, xylol II, xylol III) for 2 minutes. We rehydrated (absolute alcohol, 95% alcohol, 80% alcohol, 70% alcohol) for 2 minutes each, washed with distilled water for 5 minutes, endogenous peroxidase blocking with 3% H2O2 for 20 minutes, rinsed with distilled water for 10 minutes and rinse with phosphate buffer saline (PBS) twice for 5 minutes each. We soaked it with 0.01 M citrate buffer, pH 6.0, then heated it in the microwave for 10 minutes, initially with high heat (800C) until it boiled, then with medium heat (500C) for 5 minutes, cooled to room temperature, For 30 minutes. Wash in PBS pH 7.4 2 times, each for 5 minutes, and drip 100 l of blocking solution for 5 minutes. Drop 100 l of primary antibody (P53), which has been diluted with a dilution of 1:50 (according to the antibody data sheet), for 30 minutes at room temperature or overnight at 40°C.

We washed in PBS pH 7.4 2 times for 10 minutes each; we dripped Biotinylated Anti Polyvalent for 15 minutes. Wash in PBS pH 7.4 2 times for 5 minutes each, leak streptavidin peroxidase for 10 minutes, wash in PBS pH 7.4 2 times for 5 minutes each, drip DAB for 10 minutes, and wash in running water for 5 minutes. We counterstained with Hematoxylin Mayer for 4 minutes, then washed with running water for 5 minutes. Dehydration (80% alcohol, 96% alcohol, absolute alcohol, absolute alcohol) for 3 minutes each, clearing (xylol I, xylol II, xylol III) with 3 minutes each and mounting with a cover glass. The data obtained was then presented descriptively in the form of a narrative, proportion distribution table, and statistical analysis to look for differences in p53 mutations according to the histological type of thyroid cancer with Kolmogorov Smirnov test in the SPSS ver.24 program.

3. Results

This research is an observational analytic study using a cross-sectional design to analyze the differences in P53 mutations in the histological type of thyroid carcinoma at H. Adam Malik General Hospital Medan. The number of samples included was 64 samples. Data collection was carried out from February to June 2022.

Table 1. Frequency Distribution of Research Respondents (n=64)

Characteristics		
Age		
Mean+SD (Min-Max)	51.88+12.922 years (13-78 years)	
Gender (n, %)		
Man	24	36.9%
Woman	41	63.1%
Histopathological Type (n, %)		
Follicular	12	18.8%
Papillary	42	65.6%
Medullary	5	7.8%
Anaplastic	5	7.8%

Tumor size (n, %)		
< 5cm	11	17.2%
≥5cm	53	82.8%
Cervical lymph node (n, %)		
Found	21	32.8%
Not found	43	67.2%
Distant metastases (n, %)		
Found	59	92.2%
Not found	5	7.8%

In Table 1 the researcher presents data related to the frequency distribution of research respondents, numerical data is displayed in mean + SD (min-max), where the average respondent is 51.88 years old with the youngest age range being 13 years and the oldest being 78 years. Categorical data is presented in terms of frequency and percentage, where the gender variable shows that 41 female respondents (631%) and 24 male respondents (36.9%). The histopathology of thyroid cancer was found mostly with papillary type in 42 patients (65.6%), followed by follicular type in 12 patients (18.8%). For the medullary and anaplastic types each amounted to 5 patients (7.8%).

Cancer stadium is assessed based on the TNM classification system recommended by the UICC (International Union Against Cancer of the World Health Organization)/AJCC (American Joint Committee on Cancer). TNM Classification of Thyroid Cancer Based on the AJCC Cancer Staging Manual, 8th Edition for Primary Tumor variable (T) found 53 patients (82.8%) with tumor size >5 cm and 11 patients (17.2%) with tumor size <5 cm. For the nodule variable (N), 43 patients (67.2%) had lymph nodules and 21 patients (32.8%) had lymph nodules. Regarding the variable Metastases (M), 59 patients (92.2%) were found without metastases and 5 patients (7.8%) with metastases.

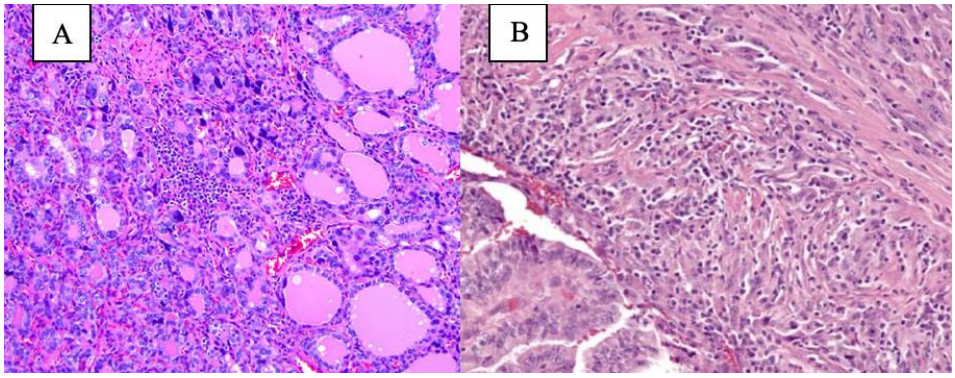

Figure 1. Histopathological picture of thyroid carcinoma A. With P53 mutation B. Without P53 mutation (wild type) (Magnification 400x)

Table 2. Distribution of P53 Mutation Frequency in Thyroid Carcinoma Patients (n=64)

Variable		
P53 Mutation (n, %)		
No mutations	44	68.8%
Mutation	20	31.3%

Based on Table 2, 44 patients (68.8%) were found without P53 mutations and 20 patients (31.3%) were found to have P53 mutations.

Table 3. Distribution of P53 Mutation Frequency in Follicular Thyroid Carcinoma Based on Demographic Characteristics (n=12)

Variable	Follicular Thyroid Carcinoma (n, %)
Age	
<40 years	2 (16.7%)
40-50 years	2 (16.7%)
50-60 years	5 (41.7%)
>60 years	3 (25%)
Gender	
Man	4 (33.3%)
Woman	8 (66.7%)
P53 Mutation	
No mutations	8 (66.7%)
Mutation	4 (33.3%)

Table 3 shows the type of follicular thyroid carcinoma with age <40 years as many as 2 people (16.7%), 40-50 years as many as 2 people (16.7%), 50-60 years as many as 5 people (41.7%) and > 60 years as many as 3 people (25%). Female sex was most commonly found in the type of follicular thyroid carcinoma as many as 8 people (66.7%) and men as many as 4 people (33.3%). Regarding the P53 mutation, 8 people (66.7%) were found without the P53 mutation and 4 people (33.3%) were found with the P53 mutation.

Table 4. Distribution of P53 Mutation Frequency in Papillary Thyroid Carcinoma Based on Demographic Characteristics (n=42)

Variable	Papillary Thyroid Carcinoma (n, %)
Age	
<40 years	9 (21.4%)
40-50 years	7 (16.7%)
50-60 years	14 (33.3%)
>60 years	12 (28.6%)
Gender	
Man	14 (33.3%)
Woman	28 (66.7%)
P53 mutation	
No mutations	31 (73.8%)
Mutation	11 (26.2%)

In Table 3 shows the type of papillary thyroid carcinoma, age <40 years were 9 people (21.4%), 40-50 years were 7 people (16.7%), 50-60 years were 14 people (33.3%) and >60 years were 12 people (28.6%). Female sex was most commonly found in the type of papillary thyroid carcinoma as many as 28 people (66.7%) and men as many as 14 people (33.3%). Regarding the P53 mutation, there were 31 people (73.8%) without mutations and 11 people (26.2%) had mutations.

Table 5. Distribution of P53 Mutation Frequency in Medullary Thyroid Carcinoma Based on Demographic Characteristics (n=5)

Variable	Medullary Thyroid Carcinoma (n, %)
Age	
<40 years	2 (40%)
40-50 years	1 (20%
50-60 years	1 (20%)
>60 years	1 (20%)
Gender	
Man	4 (80%)
Woman	1 (20%)
P53 mutation	
No mutations	4 (80%)
Mutation	1 (20%)

Table 5 shows the type of medullary thyroid carcinoma, related to the age variable, showing that 2 people (40%) are <40 years old, 1 person (20%) 40-50 years old, 1 person (22%) 50-60 years old and >60 years as many as 1 person (20%). There were 4 men (80%) for the type of medullary thyroid carcinoma and 1 woman (20%). Regarding the P53 mutation, there were 4 people (80%) without the mutation and 1 person (20%) with the mutation.

Table 6. Distribution of P53 Mutation Frequency in Anaplastic Thyroid Carcinoma Based on Demographic Characteristics (n=5)

Variable	Anaplastic Thyroid Carcinoma (n, %)
Age	
<40 years	0 (0%)
40-50 years	0 (0%)
50-60 years	2 (40%)
>60 years	3 (60%)
Gender	
Man	1 (20%)
Woman	4 (80%)
P53 mutation	
No mutations	1 (20%)
Mutation	4 (80%)

Table 6 shows the type of anaplastic thyroid carcinoma, aged 50-60 years as many as 2 people (40%) and >60 years as many as 3 people (60%). There were 4 men (80%) for the type of anaplastic thyroid carcinoma and 1 woman (20%). Regarding the P53 mutation, there were 1 person (20%) without the mutation and 4 people (80%) with the mutation.

Table 7. Distribution of P53 Mutation Frequency Based on Histological Type of Thyroid Carcinoma (n=64)

Histological Type	P53 mutation		p-value
	No mutations (n, %)	By mutation (n, %)	

Follicular	8 (18.2%)	4 (20%)	
Papillary	31 (70.5%)	11 (55%)	<0.001a
Medullary	4 (9.1%)	1 (5%)	
Anaplastic	1 (20%)	4 (20%)	

[a]Kolmogorov-Smirnov test

Analysis of differences in P53 mutations based on histological type of thyroid carcinoma with a p value of <0.001 which can be interpreted found differences in P53 mutations based on histological type of thyroid carcinoma in H. Adam Malik General Hospital Medan.

4. Discussion

The P53 gene has been shown to play a role in the negative control of cell proliferation and in controlling signaling cascades that are important in DNA repair and apoptosis. Previous studies have shown that P53 mutations may play an important role in the transformation and progression of thyroid tumors to malignancy(Bai, Kakudo, Jung, 2020). Several studies have found P53 mutations in poorly differentiated and undifferentiated thyroid carcinomas, but this does not occur in benign tumors, and is only found in some well-differentiated thyroid cancers. The presence of P53 mutations in late stages of tumor development contributes to metastasis. In addition, mutations that occur in the P53 gene are associated with chemosensitivity, radiosensitivity, prognosis, and antitumor immune responses in several types of cancer.[11,12]

P53 mutations have been reported to occur with high frequency in other types of cancer, including lymphoma, leukemia, lung cancer, pharyngeal cancer, breast, liver, bone, bladder, ovarian, and brain cancer. Ledent recently observed that mice bearing thyroglobulin-SV40 on the thyroid tumor antigen developed anaplastic subtypes known to associate with and possibly functionally inactivate P53. This study hypothesizes that P53 mutations play a role in the development of aggressive human thyroid carcinoma and determine differentiation. In a previous study examining the DNA of benign and malignant thyroid neoplasms, 4.6% of poorly differentiated thyroid carcinoma cells were found, for the previously reported mutation in the P53 gene.[13,14]

Regarding the variable P53 mutation, 44 patients (68.8%) had no mutations and 20 patients (31.3%) had mutations. The results of this study are in line with previous studies which found total mutations in the P53 gene, partial mutations and no mutations in thyroid carcinoma with a proportion of 13.2%, 17.1% and 69.7% for each type of methylation.[15] In a cohort study in South China (2010-2020) of 54 anaplastic thyroid carcinoma patients using Whole-Exome Sequencing (WES), the most common types of mutations were P53 (48%), BRAF (24%), PIK3CA (24%), and TERT (21%).[15,16]

In Romei's study in Italy in 2017, 10 out of 21 samples from the anaplastic thyroid carcinoma group had a P53 mutation (47.6%) followed by TERT mutations (42.8%), PTEN (19%), BRAF (19%), N -RAS (9.5%). In the poorly differentiated thyroid carcinoma group, there were no samples with P53 mutations. The types of mutations found were TERT (33.3%), BRAF (19%), N-RAS (4.7%), AKT (4.7%) and PIK3CA (4.7%). In this study, 64% of patients with stage IV were found in the anaplastic thyroid carcinoma group, whereas only 5.9% of patients with poorly differentiated thyroid carcinoma group had stage IV. Multiple mutations in somatic genes may have a role in the clinicopathology of thyroid carcinoma patients.[17] Research is also needed on the types of mutations experienced by patients with advanced stages.

A Nikitski study in Pennsylvania published in 2021 showed that 86% of resected nodules with isolated P53 mutations (single mutations) were mostly follicular adenomas (benign tumors), whereas 82% of thyroid nodules with P53 mutations were accompanied by other gene mutations (multiple mutations). was a thyroid malignancy (p = 0.001). Molecular pathogenesis involving early hit genetic changes by BRAF, RAS followed by late hit

genetic changes by TERT, P53, P53 mutations may not only act as a tumor suppressor gene but also have a role as an oncogene that changes tumor properties.[18]

In Pozdeyev's study of 583 poorly differentiated thyroid carcinomas and 196 anaplastic thyroid carcinomas, only 10% of P53 mutations were found in papillary thyroid carcinoma, 12% of P53 mutations were found in follicular thyroid carcinoma, in papillary thyroid carcinoma type follicular variant (Hurthle Cell) P53 mutations were found as much as 20%, whereas in the type of anaplastic thyroid carcinoma found P53 mutations as much as 65%. The authors state that mutations in the P53 gene are low or almost absent in early-stage tumors and without neck lymph node metastases, inactivation of the P53 gene is found, as a result of genetic changes or epigenetic inhibition, which is often found in advanced thyroid carcinoma.[19,20]

There is no relationship between P53 gene mutations and histological subtypes explained by Kanapathipillai's 2018 study that methylation of the DNA promoter is essential for total inactivation of the gene, although a small proportion of unmethylated alleles may be able to induce gene expression. In primary tumor cells, super high methylation (SHM) represents complete methylation in cancer cells. The results of previous studies by Hincza concluded that SHM in the TP53 gene might functionally cause P53 dysfunction.[21,22]

In a study conducted by Arena et al in 2021, changes in the P53 methylation profile at alleles -139 and -132 revealed either methylation of both alleles (total methylation), or only methylation at one allele (partial methylation) which was seen at a lower gene level.[22] From these results, it can be surmised that complete methylation involving both alleles may indicate a higher tendency for the development of malignant tumors. In addition, hypermethylation of the promoter region of the TP53 tumor suppressor gene is usually associated with impaired gene activity, and can cause an inhibitory process, which in turn causes a dysfunction.[21,22]

The weakness of this study is that it uses a retrospective design and uses secondary data. This research is also a single centered study which was only conducted at the H. Adam Malik General Hospital in Medan. Assessment of P53 mutations was carried out qualitatively, not quantitatively which could affect the results of the study. External factors can also damage proteins on anatomical pathology slides such as temperature, storage, humidity, and fungi. This highlights the importance of processing, fixation and transfer of paraffin blocks. Efforts to avoid bias in this study have been carried out by sorting the appropriate paraffin blocks and all preparations were read by two Anatomical Pathologists, as well as re-examination of the 15 initial preparations after all samples had been examined.

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