

## The Difference of Ki-67 Expression on Patient with Thyroid Papillary Carcinoma Non Metastases and Regional Metastases on Dr. Soetomo General Hospital Surabaya

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### Abstract

The incidence of thyroid carcinoma has continued to increase over the past few years. In the United States, the incidence is increasing at an annual ratio of 5.4% for men and 6.5% for women. Based on medical records at Dr. Soetomo Regional Hospital in 2014-2018, 433 patients underwent surgery for thyroid cancer, 190 (43.8%) had total thyroidectomy and 109 (25.2%) total thyroidectomy with Functional Neck Dissection. Thyroid cancer with regional lymph node metastases and distant metastases require more difficult and complicated actions which are quite high in cost of functional neck dissection, radical neck dissection or internal radiation. On the other hand, we found that the properties of Ki-67 can be used as markers of carcinoma proliferation, so we would like to find out whether examining Ki-67 can be a predictor in metastatic cases of thyroid cancer. The study was an observational analytic study with a cross sectional study design. The general research subject data were collected and the histopathological results were recorded. Ki-67 immunohistochemistry was examined in the Anatomy Pathology laboratory of Soetomo General Hospital. In this study, a total of 30 research subjects were obtained. Patients with the most age were over 50 years old with 14 patients (46.7%), aged 25-50 years were 10 patients (33.3%), and aged <25 years were 6 patients (20.0%) with an average age  $47.57 \pm 2.867$  years. From the 30 study subjects, 15 patients underwent total thyroidectomy and FND (50%) and 15 patients underwent total thyroidectomy (50%). From this study, the mean expression of Ki-67 in thyroid cancer patients was  $14.00\% \pm 2.904\%$ . The minimum value of Ki-67 expression is 1% and the maximum value of Ki-67 expression is 60%. Based on the data, a statistical test was performed with Pearson ChiSquare, that statistically significant differences were obtained with a value of  $p = 0.029 (<0.05)$ . Regional lymph obtained cut off point value is 15%. From this study a significant difference was found between the high Ki-67 expression in non-metastases and regional lymph node metastases in patients with papillary thyroid carcinoma ( $p = 0.029$ ). The higher the level of expression of Ki-67 in papillary thyroid carcinoma patients, the higher the possibility of regional lymph node metastases

**Keywords:** Thyroid Carcinoma, Regional Metastasis, Ki-67, Thyroidectomy

### 1.0 INTRODUCTION

The incidence of thyroid carcinoma has continued to increase over the past few years. In the United States, the incidence increases with an annual ratio of 5.4% in men and 6.5% in women. Based on GLOBOCAN 2018, thyroid carcinoma accounts for 5.1% of the total incidence of new malignancy in 2018, with a mortality rate of 0.43 per 100,000 people. In Indonesia, in 2018 thyroid carcinoma ranked 11th most malignancy (3.3%) (GLOBOCAN, 2018). Based on medical record data at Dr. Soetomo Regional Hospital in 2014-2018, as many as 433 patients underwent surgery for thyroid carcinoma, as many as 190 (43.8%) of them performed total thyroidectomy and 109 (25.2%) of them underwent total thyroidectomy with Functional Neck Dissection (Suharyo, 2018).

Thyroid cancer is a relatively rare cancer, but it is the most common form of malignancy in the endocrine field. Risk factors that may be the cause of malignancy include gender, age, evolutionary time of goitre, presence of dominant nodules in multinodular goitre, history of previous thyroid surgery, history of radiation in the neck region, living in endemic areas of goitre, hypothyroidism, calcification of the goitre. Diagnosed through ultrasonography, and a family history of thyroid disease (Botrugno, 2011). Therapeutic strategies for thyroid disease include regular assessment and follow-up, medical treatment related to hyperthyroidism or hypothyroidism status, interventional therapy, and surgery. Surgical treatment includes sub-total or near-total thyroidectomy, and total thyroidectomy (Askitis et al., 2013)

Papillary Thyroid Carcinoma (PTC) constitutes more than 90% of new cases of thyroid cancer. Regional lymph node metastases often occur in the early stages of papillary thyroid cancer and initially appear in the middle region. Lymph node metastasis signifies an advanced stage in papillary thyroid cancer, so it can be used as a predictor of poor prognosis (Liu, 2017).

Functional Neck Dissection (FND) has been considered the gold standard for surgical treatment of lymph node metastases in the neck. Functional neck dissection involves the removal of all groups of ipsilateral neck lymph nodes extending from the mandibular corpus superiorly, to the inferior clavicle, anteriorly from the lateral border of the sternohyoid muscle, hyoid to the anterior border of the trapezius muscle with preservation of the spinal accessory nerve, to the internal jugular vein and anterior to the lateral border of the sternohyoid muscle, hyoid to the anterior border of the trapezius muscle with preservation of the spinal accessory nerve, the internal jugular vein and the anterior muscle sternocleidomastoid. Neck lymph node groups from level I to V are included in this dissection (Ardiansyah, 2015). Ki-67 is a type of protein that has been widely used as a proliferation marker for human tumor cells for decades.

Ki-67 plays an important role in the process of cell proliferation (Sun and Kufman, 2017). Ki-67 is present in the cell nucleus which greatly influences the rate of cell proliferation. This protein is expressed in cells that are proliferating (G1, S, G2 and M phases), and not expressed in resting cells (G0 phase). The expression of Ki-67 is related to the presence of proliferative activity in the intrinsic cell population of the carcinoma which may be used as a marker of tumor aggressiveness. In colorectal cancer, a high level of Ki-67 expression means that there is also a high proliferation of intrinsic colorectal cancer malignant cells (Mulyawan, 2019). In cervical cancer Ki-67 is a marker of the level of proliferation which is a marker of the level of aggressiveness of cervical cancer. (Ancuta et al., 2009). In thyroid cancer, found differences in Ki-67 expression between benign nodules and malignant nodules. The more virulent a nodule is, the higher the expression level of the Ki-67 tumor.

From research in Surabaya, benign thyroid nodules express Ki-67 with a weak score of 58.8%, a moderate score of 11.8% and a high score of 0%. While in malignant thyroid tumors without metastasis, found a weak Ki-67 score was 0%, moderate score was 62.5% and high score was 37.5%. In malignant thyroid tumors with metastasis, the weak Ki-67 score was 22.2%, the moderate score was 11.1% and the high score of Ki67 was 66.7%. It can be seen that a high Ki-67 number in thyroid cancer indicates a higher malignancy in the tumor (Suharyo, 2018). Assessment of Ki-67 protein expression by immunohistochemically methods is very likely to be used to assess tumor cell proliferation activity. However, the use of Ki-67 protein as a prognostic factor is still controversial. (Hertati, et al., 2014). Based on data from patients with thyroid cancer in RSUD Dr. Soetomo Surabaya, we found that thyroid cancer with regional lymph node metastases and distant metastases requires more difficult and complex treatment and requires quite high costs such as functional neck dissection, radical neck dissection or internal radiation. On the other hand, researchers found that the expression properties of Ki-67 can be used as a marker of carcinoma proliferation, so researchers want to find out whether the examination of Ki67 can be a predictor factor in cases of metastatic thyroid cancer. So in this study prospective search will be conducted to compare the level of Ki-67 expression in post-thyroidectomy thyroid cancer patients with post-total thyroidectomy and functional neck dissection at Dr Soetomo General Hospital, Surabaya.

## 2.0 METHODS

This study was observational analytic with cross sectional study design. Data collection for thyroid cancer patients who met the study inclusion and exclusion criteria. The process of taking subjects for the study of patients with thyroid cancer begins with data from the Head and Neck Surgeon Secretariat at Dr. Soetomo General Hospital Surabaya. Furthermore, general data of research subjects such as name, age, and gender were recorded. Then from the data, the histopathological results were recorded and a Ki-67 immunohistochemically examination was carried out in the Anatomy Pathology laboratory of Dr. Soetomo General Hospital. Tissue that has been in the form of a smear slide that has been fixed with alcohol 96% is carried out further examination with Ki-67 immunohistochemistry by following a fixed procedure. Data analytic is using the SPSS 22.0 program. Data is presented in the form of frequency distribution tables and cross tabulations. Data from independent and dependent variables in the form of nominal data will be tested using the chi-square test.

3.0 RESULTS

In this study, a total of 30 research subjects were obtained. Patients with the most age were over 50 years old with 14 patients (46.7%), aged 25-50 years were 10 patients (33.3%), and aged <25 years were 6 patients (20.0%) with an average age  $47.57 \pm 2.867$  years. Of the 30 study subjects, 15 patients underwent total thyroidectomy and FND (50%) and 15 patients underwent total thyroidectomy (50%).

Table 1 Characteristic of Research Subjects

Subject Characteristics		Frequency	Percentage (%)	Average
Sex	Female	20	33,3	
	Male	10	66,7	
Age	< 35 years	6	20,0	47,57 ± 2,867
	35 - 50 years	10	33,3	
	> 50 years	14	46,7	
Treatment	Total Thyroidectomy + FND	15	50,0	
	Total Thyroidectomy	15	50,0	

From 30 research subjects, 15 people (50%) had regional lymph node metastases and 15 (50%) non-metastatic regional lymph nodes.

Table 2 Distribution of Metastases in Research Subject

Metastases	Frequency	Percentage (%)
Lymph node Metastases	15	50.0
Non Lymph node Metastase	15	50.0
Total	30	100.0

It is obtained from this study, the mean expression of Ki-67 in thyroid cancer patients was 14.00% + 15.905%.

Table 3 Average of Ki-67 Expression in Thyroid Cancer Patient

	N	Range	Minimum	Maximum	Mean	Std.
	Statistic	Statistic	Statistic	Statistic	Statistic	Deviation
Ki-67	30	59	1	60	14.00	2.904
						15.905

From the data cross tabulation between sex and the presence of regional lymph node metastases, it was found that female sex had more regional lymph node metastases (30.05%), while male sex was more non-metastatic (20.05%).

Table 4 Cross Tabulation Between Sex and The Presence of Regional Lymph Node Metastasis

Subject Characteristics		Lymph Node Metastases	Non Lymph Node Metastases	Total
Sex	Male	4 13,3%	6 20,0%	10 33,3%
	Female	11 36,7%	9 30,0%	20 66,7%
	Total	15 50,0%	15 50,0%	30 100,0%
Age	< 35 years	4 13,3%	2 6,7%	6 20,0%
	35 - 50 years	5 16,7%	5 16,7%	10 33,3%
	> 50 years	6 20,0%	8 26,7%	14 46,7%
	Total	15 50,0%	15 50,0%	30 100,0%

From this study, in the metastases group there were 4 male patients (13.3%) and 11 female patients (36.7%). In the non-metastatic group, 6 male patients (20%) and 9 female patients (30.0%) were found. From the statistical test with Pearson Chi-Square, there was no statistically significant difference with  $p = 0.439 (> 0.05)$ . This shows that the sex distribution in the two groups is the same. From this study, in the metastasis group there were 4 patients (13.3%) less than 35 years of age, 35-50 years of age 5 people (16.7%), and more than 50 years of 6 people (20%). In the non-metastatic group, there were 2 patients (6.7%) aged 35-50 years (16.7%), and more than 50 years (8.7%). From the statistical test with Pearson Chi-Square, there were no statistically significant differences with  $p = 0.621$ . This shows that the age distribution in the two metastatic and non-metastatic groups is the same.

From this study, the average expression of Ki-67 in thyroid cancer patients was  $14.00\% \pm 2.904\%$ . The minimum value of Ki-67 expression is 1% and the maximum value of Ki-67 expression is 60%. Based on the data, a statistical test was conducted with Pearson ChiSquare, that a statistically significant difference was obtained with a value of  $p = 0.029 (< 0.05)$ . This shows that there are differences in the expression of Ki-67 in thyroid cancer patients with regional lymph node metastases and non-metastases.

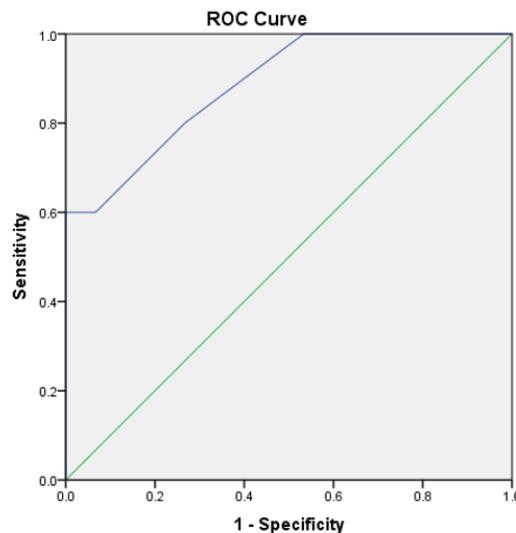


Figure 1. ROC Curve

In this study, from the results of Ki-67 expression in the regional lymph node metastasis and non-metastatic regional lymph node groups the cut-off point value was 15%.

#### 4.0 DISCUSSION

This study uses a comparative test method using an observational analytic research design with a cross sectional study design which is expected in this study to compare differences in the level of expression of Ki-67 in patients with non-metastases and regional metastases thyroid cancer. In this study, the research subjects included were patients diagnosed with thyroid cancer in Dr. Soetomo General Hospital from 2012 to 2018 who met the inclusion and exclusion criteria. The subjects will be divided into two groups. The first group were thyroid carcinoma patients with regional lymph node metastases, while the second group were patients who did not experience metastases. The variables examined in this study were the expression of Ki-67 as an independent variable and non-metastases / regional lymph node metastases thyroid cancer as the dependent variable. This study was conducted with a total of 30 patients and grouped into 15 thyroid cancer patients with regional lymph node metastases and 15 thyroid cancer patients who did not experience metastasis.

In this study, a significant difference was found between high Ki-67 expression and the possibility of metastasis in patients with papillary thyroid cancer ( $p = 0.029$ ) with mean Ki-67 expression of 14.00%. The study conducted by Miyauchi et al. in 2013 showed a high Ki-67 expression associated with a poor prognosis in patients with breast cancer and prostate cancer. In a study conducted by Pan et al. in 2017, that patients with over-expression of Ki-67 / MIB-1 have poor survival in thyroid cancer. This is consistent with the results obtained in this study, namely in patients who have high Ki-67 expression will tend to be metastatic. In addition, the 2013 Ranjbari study also stated that Ki-67 can predict distant metastases and survival in tumors, whereas in the case of papillary thyroid cancer Ki-67 can be used as an indication of the prognosis of disease spread and poor survival and affect therapeutic planning in patients.

In order to invade other tissues and metastases, cancer cells experience changes due to mutations include loss of inter-cell contact due to reduced expression of E-cadherin (ICAM), changes in signaling integrin, matrix upregulation of metalloproteinase and downregulation of tissue inhibitors of MMP, impairing immunity with tumor killing cells, and angiogenesis with upregulation of VEGF and angiopoietin. Cell proliferation can be stimulated by intrinsic growth factors, lesions, cell death, and even mechanical deformation of tissues. Cells that are proliferating develop through a series of phases called the cell cycle. The cell cycle consists of the growth phase of para synthesis 1 (G1), the phase of DNA synthesis (S), the growth phase of para mitosis 2 (G2), and the phase of mitosis (M), the resting cell is in a physiological state (G0) (Kumar, Abbas, Aster; 2017).

Ki-67 is a type of proliferation marker protein for tumor cells. The expression of Ki-67 is related to the presence of proliferative activity in the intrinsic cell population of the carcinoma which may be used as a marker of tumor aggressiveness. Ki-67 is found in the DNA region in the initial phase of G1 in the cell cycle. Ki-67 appears only in the growth and division phases in the cell cycle (G1, S, G2, and M), but does not appear in the resting phase (G0). Thus, Ki-67 is a good marker of proliferation in cancer cells that have aggressive cell growth and division. Specifically, Ki-67 expression starts in G1 phase and increases in S phase and reaches its highest expression in metaphase (phase M). In anaphases and telophases, Ki-67 expression begins to decrease (Dzulkifli, FA; Mashor, MY; and Jaafar, H; 2018). Cell proliferation activity is an important factor for evaluating the biological characteristics of cancer cells. This is key in tumor development when the balance between cell death and cell proliferation is disrupted. Immunohistochemistry is used to detect cell cycle specific antigens and this method can determine cell proliferative activity (Pan et al., 2017).

The method for evaluating tumor growth that is often used is immunohistochemistry namely Ki-67 in tumor tissue. Ki-67 is an antigen associated with cell proliferation that is found at all stages of the cell proliferation cycle except the G0 phase. The Ki67 protein undergoes phosphorylation and dephosphorylation during mitosis, while it is also sensitive to proteases and regulated by proteolytic pathways. Moreover, the structure of Ki67 is similar to some proteins involved in the cell cycle regulation. Ki67 is not in the resting phase (G0), but in the G1 phase, it starts to appear in the nucleus. Then, in the S and G2 phases, Ki67 protein expression gradually increases and peaks during the M phase, followed by a rapid decline during the final M phase.

Ki-67 is in the perinuclear region in the G1 phase. During interphase, Ki-67 localizes to the dense fibril components of the nucleoli. During mitosis, the diffuse nucleolus staining pattern reveals its relationship to chromatin. One important function of Ki-67 is to localize the components of the granular nucleus to the mitotic chromosome, and therefore play an important role in the segregation of nucleoli between daughter cells (Menon, 2019).

Ki-67 functions as a surfactant that allows chromosome motility and its interaction with the mitotic spindle, thereby preventing the collapse of chromosomes into chromatin mass after nuclear envelope decomposes. Ki-67 also has a role in rRNA transcription. These functions can explain the relationship between cell proliferation and Ki-67 protein expression. Recent studies have shown that Ki-67 does not directly correlate with cell proliferation, but is involved in the compacting and heterochromatin organization likely by the histone methylation complex needed for the maintenance of heterochromatin. With the expression of Ki-67 which is highly expressed in cancer cells, it indicates that there is a proliferation in high numbers as well, which indicates a higher malignancy in cancer. In cancer cells, the process of mitosis from the beginning of the G1 phase to the S phase goes so fast that the growth of the cancer cells becomes numerous because of the massive mitosis or rapid spin cell division which triggers the production of Ki-67 continuously and in large quantities so as to make the level of expression of Ki-67 becomes very fast and accumulates excessively. This is different in normal cells where mitosis and division do not run as fast as cancer cells. This makes the Ki-67 production does not increase rapidly or is still within normal limits. Both of these are what distinguishes between Ki-67 in cancer cells and Ki-67 in normal cells (Menon, 2019).

Several studies have found a correlation between Ki67 expression and malignant tumor metastases. From research in Surabaya it was found that the more virulent a nodule was, the higher the expression level of the Ki-67 tumor. From this study, benign thyroid nodules expressed Ki-67 with weak scores as much as 58.8%, moderate scores as much as 11.8% and high by 0%. Is on Malignant thyroid tumor without metastasis, found a weak Ki-67 score was 0%, moderate score was 62.5% and high score was 37.5%. In malignant thyroid tumors with metastasis, the weak Ki-67 score was 22.2%, the moderate score was 11.1% and the high score of Ki-67 was 66.7%. It can be seen that a high number of Ki-67 in thyroid cancer indicates a higher malignancy in the tumor (Suharyo, 2018). Other studies show that Ki67 has a role in thyroid cancer. Increased ki67 is associated with an increase in tumor size, membrane invasion and cervical lymph node metastases. With the intensity of expression and the increased positive Ki67 rate is accompanied by an increased invasion ability of the tumor by membranes and lymphatic metastases. So it can be concluded that the expression of Ki67 is closely related to tumor invasion and metastasis, Ki67 positive in thyroid cancer may have a stronger invasion ability, more susceptible to metastasis. (Zhou et al., 2015) A study on the molecular mechanism of Ki-67 revealed that Ki-67 played an important role in the initial steps of ribosomal RNA synthesis and that the Ki-67 protein might be related to various signaling pathways, which might answer some questions why Ki-67 is associated with inflammation (Tang et al., 2018).

In a study conducted by Nakamura, it was observed that E-cadherin is often re-expressed in cancer cells that form the focus of metastases in the involved lymph nodes. A similar observation was described as 'E-cadherin expression' in a previous study that studied PTC and follicular carcinoma of all stages. According to the latest findings, this step of re-expression of E-cadherin is considered to represent mesenchymal-to-epithelial transformation (MET), a reversal from EMT (epithelial to mesenchymal). This phenotypical reversal is considered to be a step towards creating conditions suitable for cancer cells where they can survive and form a metastatic focus in the environment other than the main site. E-cadherin expression in metastatic lymph nodes was commonly observed in patients with primary E-cadherin-negative PTMC in this study, indicating that MET is important for the formation of metastatic lesions. There is no regulation of proliferative activity at the metastatic site, as indicated by the low Ki-67 index in this cancer cell, indicating that the majority of PTMC metastatic foci in the lymph nodes involved show an indolent phenotype, similar to the main site. (Nakamura, 2013) So that in some thyroid cancer patients with metastasis can have low Ki-67 expression.

In this study, the cut-off point of Ki-67 expression in the metastases and non-metastases groups was obtained by 15%. This is like the research conducted by Miyauchi in 2013, which is a cut-off point of

10%. If Ki-67 is used to exclude patients with slow tumor proliferation from the chemotherapy protocol, the cut-off value used is 10% (Ranjbari and Rahim, 2013).

## 5.0 CONCLUSION

From this study, a significant difference was found between the high Ki-67 expression and the tendency for lymph node metastasis in patients with papillary thyroid cancer ( $p = 0.029$ ). The higher the level of Ki-67 expression in papillary thyroid cancer patients, the higher the possibility of regional lymph node metastases. Further research needs to be done on other related variables as factors that influence the presence of regional lymph node metastases in papillary thyroid cancer patients.

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