Chelonian Conservation And Biology

Vol. 19 No. 1 (2024) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2024.01(1).644.664

ASSOCIATION OF HISTOPATHOLOGICAL CHARACTERISTICS AND TERT (TELOMERASE REVERSE TRANSCRIPTASE) IMMUNOEXPRESSION WITH METASTATIC RATE IN CUTANEOUS MALIGNANT MELANOMA

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ABSTRACT

Cutaneous malignant melanoma (MM) is a malignant tumor with a high mortality rate. Histopathological characteristics are prognostic predictive factors of cutaneous MM, in which tumor thickness >2 mm and mitotic rate ≥5/mm^2 correlate with a worse survival rate. A mutation in MM can occur at telomerase reverse transcriptase (TERT) promoter region, which leads to unlimited cell proliferation. Telomerase also increases metastatic risk. This study aims to determine the association between histopathological characteristics and TERT immunoexpression with metastasis in cutaneous MM. The study samples are 30 metastatic and 30 non-metastatic cutaneous MM in the Anatomical Pathology Department FKUI/RSCM, from January 2011 to July 2023. Histopathological characteristics (tumor thickness, mitotic index, lymphovascular invasion, and perineural invasion) were assessed, and anti-TERT antibodies were used for immunohistochemistry staining. Histopathological characteristics and TERT immunoexpression data were analyzed to determine their association with metastasis. Histopathological features that correlate significantly with metastasis are tumor thickness >2 mm (p=0.006) and mitotic index ≥5 mitosis/mm^2 (p=0.008). Multivariate analysis showed a significant association between high TERT immunoexpression and metastasis in cutaneous MM (p<0.001, aOR=56.1). This study concludes that high TERT immunoexpression increases the metastatic rate in cutaneous MM. Greater tumor thickness and a higher mitotic index are associated with metastasis in cutaneous MM.

Keywords: Cutaneous Malignant Melanoma; Immunohistochemistry; Metastasis; TERT

1. INTRODUCTION

Malignant melanoma (MM) is a malignant melanocytic lesion that can be develop in the skin, eyes (ocular), and mucosa. Skin (cutaneous MM) is the most common location, with an incidence rate of 3.8/100,000 in men and 3/100,000 in women. Meanwhile, the incidence of ocular MM is 5/1,000,000 people, and mucosal MM is found less frequently, approximately 1% of all MM cases (Bolick & Geller, 2021). According to the Global Cancer Observatory data in 2020, there were 324,635 new cases of MM, causing 57,043 deaths each year (International Agency for Research on Cancer, 2020). MM has a highly aggressive nature with unpredictable progression, and its mortality is related to metastatic events. Cutaneous MM without metastasis is categorized as stage I-II, with regional metastasis as stage III, and distant metastasis (brain, lung, liver, digestive tract) as stage IV. The 5-year survival rate for cutaneous MM without metastases (stages I-II) is 99.4%, for cutaneous MM with regional metastases (stage III) is 68%, while for cutaneous MM with distant metastases (stage IV) is only 29.8% (Elder et al., 2020).

Histopathological features can predict the prognosis of MM patients, and certain characteristics are known to be associated with a worse prognosis. Calomarde et al. (Calomarde et al., 2019) found that lymphovascular invasion, tumor thickness >2 mm, and mitosis $\geq 5/mm^2$ increase the risk of metastasis. Perineural invasion is a weak prognostic factor but it increases the rate of local recurrence (Hyams et al., 2019; Namikawa et al., 2018). In addition to those factors, TERT (telomerase reverse transcriptase) promoter mutations were also found to increase the risk of metastasis in MM (Calomarde et al., 2019). MM is a tumor with a high frequency of TERT promoter mutations, approximately 67-85% (Jafri et al., 2016).

MM is a malignant neoplasm with a high mutational burden. Initially, most benign melanocytic lesions have MAPK pathway mutations such as BRAF p.V600E. If second hit mutation occurs, such as TERT promoter mutations, the lesion can further develop into malignancy. TERT promoter mutations will increase telomerase expression, an enzyme that causes cells to proliferate without telomere shortening (Elder et al., 2018; Harou et al., 2019). Macerola et al. (Macerola et al., 2015) found that TERT promoter mutations are associated with greater tumor thickness and a higher mitotic index, whereas Calomarde et al. (Calomarde-Rees et al., 2019) found that MM patients with TERT promoter mutations have 2.9 times higher risk of metastasis.

TERT promoter mutations are found only in tumor cells, so therapy targeting telomerase has a good potential because of its high specificity. One of the telomerase inhibitors which currently being developed is imetelstat, and was proved to improve patient survival. Another modalities related to TERT promoter mutations are being researched, namely gene editing and ETS (E26 Transformation Specific) transcription factor inhibition therapy, which also plays a role in telomerase expression (Guo et al., 2022; Guterres & Villanueva, 2020). Currently, molecular testing is the primary modality for detecting TERT promoter mutations; however, this examination is not widely available and relatively expensive. TERT immunohistochemistry (IHC) examination is an alternative modality with a more affordable availability and cost; however, TERT immunoexpression has not been extensively researched (Moosvi et al., 2021).

TERT promoter mutations play an important role in MM tumorigenesis and are associated with the rate of metastasis. Given the high mortality rate of MM, especially related to metastasis, a research is needed to determine the association between histopathological characteristics and TERT immunoexpression with metastatic rate of cutaneous MM. Hopefully, this research can become an initial study that explaines TERT immunoexpression as a prognostic indicator regarding the metastatic rate of cutaneous MM.

2. THEORETICAL REVIEW

MM can occur in the skin (91%), ocular (5%), mucosal (1%), or unknown location (2%). MM of the skin (cutaneous MM) is a malignant tumor that originates from skin melanocyte, cell that produces melanin pigment. Cutaneous MM can develop from benign melanocytic lesions (nevus), but can also develop de novo (Shields et al., 2015; Shreberk-Hasidim et al., 2023). MM develops due to the accumulation of mutations that enhanced cellular growth signals and suppress suppressor signals. One of the mutation cause is ultraviolet radiation, termed cumulative sun damage (CSD). CSD causes point mutations, mostly transitions of cytosine (C) to guanine (G) (Elder et al., 2018).

The prognosis of cutaneous MM patients mainly depends on the occurrence of metastases because the therapeutic options for advanced/metastatic cases are limited. The rate of metastasis can be predicted by several histopathological features, including tumor thickness, mitotic index, and lymphovascular invasion (Macerola et al., 2015). Tumor thickness was grouped according to Breslow, which categorize tumor thickness into ≤1 mm; 1.01-2 mm; 2.01-4 mm; and >4 mm. Kim et al. (Kim et al., 2019) found that Breslow thickness >2 mm increased the incidence of metastasis. In addition to tumor thickness, Calomarde et al. (Calomarde-Rees et al., 2019) found that mitotic index ≥5/mm^2, and the presence of vascular invasion also increases the incidence of metastasis. Namikawa et al. (Namikawa et al., 2018) discovered that perineural invasion was not significantly associated with metastasis but increased the rate of local recurrence. Other prognostic factors are assessed by molecular testing. Researchers found that TERT promoter mutations increase the risk of metastasis and are associated with greater tumor thickness and a higher mitotic index (Calomarde-Rees et al., 2019; Macerola et al., 2015).

Somatic cells will eventually become senescent because telomeres shorten during cell division. In embryonic stem cells, hematopoietic cells, and germ cells, telomere shortening are prevented by telomerase, so that cells can continue to divide. Normally, telomerase is suppressed in differentiated cells and inhibited in somatic cells leading to telomeres shortening over time. This telomerase activity is regulated by TERT gene promoters (Guo et al., 2022; Viceconte et al., 2017). The hTERT gene is 42 kb long and located on chromosome 5 with 15 exons. In MM, TERT promoter mutations occur in noncoding regions and are the most common non-coding region mutations compared to other gene mutations. TERT promoter mutations are associated with increased TERT mRNA expression, telomerase activity, and a poor prognosis (Guo et al., 2022; Viceconte et al., 2017).

The most frequent mutations of melanocytic lesions occur in the kinase signaling pathway, especially in BRAF. Approximately 90% of somatic mutations are substitutions of thymidine (T) to adenine (A) at codon 600 (p.V600E) (Macerola et al., 2015). TERT promoter mutations in MM often coexist with BRAF v600e mutation (Del Bianco et al., 2020). Seven TERT promoter mutations have been identified in MM, which are -124, -146, -124/125, -138/139, -136, -100, and -57 sequence mutations. The most common mutation found on chromosome 5 is the substitution of cytosine (C) to thymidine (T) at sequence –124 (called the C228T mutation) and at sequence –146 (called the C250T mutation). In tumorigenesis, the ETS family of transcription factors is the key regulator for malignant tumor cell proliferation and invasion (Guo et al., 2022). Increased telomerase transcription prevents telomeres shortening during cell division, resulting in unlimited cancer cells proliferation (mitosis). Cutaneous MM patients with TERT promoter mutations were found to have a higher mitotic index with a worse prognosis (Calomarde-Rees et al., 2019; Guo et al., 2022).

MM patients with TERT promoter mutations have 2.8 times higher rate of metastasis (Calomarde-Rees et al., 2019). Molecular analysis of the C228T and C250T "hotspot" mutations found that 50-85% of MM with metastasis have TERT promoter mutations, whereas only 29-37% MM without metastasis have these mutations (Horn et al., 2013). Tumor cell needs to undergo complex process to metastasize, including epithelial-mesenchymal transition, invasion of surrounding tissue, penetration of the basement membrane, avoidance of apoptosis, dissociation from the tumor mass, intravasation into blood or lymphatic vessels, and extravasation. TERT promoter mutations will increase TERT transcription, which lead to increased metastatic potential. This enzyme increase matrix metalloproteinase (MMP) and transforming growth factor β1 (TGFβ1) expression, resulting in tumor invasion and metastasis (Hannen & Bartsch, 2018; Wagstaff et al., 2022).

Immunohistochemical examination can be used to detect hTERT protein at cellular level. TERT antibodies will stain cell cytoplasm and/or the nucleus (Hiyama et al., 2001). Ma et al. (Ma et al., 2019) found that TERT protein expression is a strong indicator of telomerase reactivation and active cell proliferation. Woo et al. (Woo et al., 2023), who studied TERT immunoexpression in 35 MM patients, found that IHC testing has 100% sensitivity and 57% specificity in detecting TERT gene amplification. Meanwhile, Moosvi et al. (Moosvi et al., 2012), who studied TERT immunoexpression in Glioma, found that the IHC examination has 61.1% sensitivity and 69.2% specificity in detecting TERT promoter mutations.

3. METHODS

This research is an analytical case control study to evaluate TERT immunoexpression in metastatic and non-metastatic cutaneous MM. It was conducted at Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia/RSUPN Dr. Cipto Mangunkusumo from September to October 2023. The research was conducted with permission from the Medical Faculty Research Ethics Committee number KET-733/UN2.F1/ETIK/PPM.00.02/2023. The

target population is all cases of cutaneous MM. The accessible population is patients who have been diagnosed by histopathological examination at the Department of Anatomical Pathology FKUI/RSCM during the period of January 2011 to July 2023. Cases were selected by consecutive sampling. Based on the sample size calculation, the total cases required is 60.

The inclusion criteria for this study were all specimens of patients diagnosed histopathologically as cutaneous MM in the Department of Anatomical Pathology FKUI/RSUPN Dr. Cipto Mangunkusumo during the period of January 2011 to July 2023. The exclusion criteria for this study were specimens of patients in which paraffin blocks were not found in the archives, and tumors that were not taken in entirety by excision, due to difficulty in assessing total tumor thickness. The independent variables in this study were TERT immunoexpression, tumor thickness, mitotic index, lymphovascular invasion, and perineural invasion. The dependent variable in this study was the occurrence of metastasis.

TERT immunoexpression were assessed in the cytoplasm and nuclei of tumor cells.(Gomatou et al., 2022) This assessment was done using light microscopy in a semiquantitative manner based on H-score. H-score is calculated by the quantity (percentage) and intensity of IHC staining in the cytoplasm and nuclei of tumor cells. TERT immunoexpression stainings were graded as negative (0), weak (1+), moderate (2+), and strong (3+), using stratum spinosum keratinocytes and eccrine gland luminal cells as reference for intensity (figure 3.2).(Cho et al., 2021) Evaluation were conducted by researcher and supervisor in a blinding manner.(Indonesia, 2023; Zach, 2021)

The immunohistochemistry staining was assessed in the hot-spot area staining of tumor cells. The staining quantity (percentage) and intensity of each case will be assessed in 500 tumor cells with imageJ program. Statistical analysis is then performed to analyze ROC curve, to determine H-score cut-off value for high or low TERT immunoexpression.

The datas obtained were processed using statistical analysis to calculate adjusted Odds Ratio (aOR) by SPSS (Statistical Package for the Social Sciences) version 25.0. Mitotic index, lymphovascular invasion, and perineural invasion are presented categorically. Tumor thickness is presented numerically and categorically. TERT immunoexpression data was calculated using H-score and further categorized into high and low expression. Bivariate statistical analysis was carried out on each variable using Chi-square tests. If test requirements for Chi-square were not met, Fisher's Exact was used as the alternative test. The result was considered statistically significant if the p value is <0.05 with 95% confidence interval. Variable with a p value of <0.2 in bivariate analysis will be further analyzed with logistic regression test multivariate analysis. Statistical analysis of the association between TERT immunoexpression and tumor thickness is presented with numerical data. Data distribution/normality was assessed using the Kolmogorov-Smirnov test. Statistical analysis on numerical variables was carried out by a mean comparison test in 2 unpaired groups, using the unpaired t-test if data distribution is normal or Mann-Whitney U test if data distribution is not normal.

4. RESULTS

A total of 30 cases of cutaneous MM with metastases and 30 cases of cutenaous MM without metastases were found in the archives from the period of January 2011 to July 2023. Subjects were selected from cases that met the inclusion criteria and did not meet the exclusion criteria. The average age of the subjects was 52 years old, with an equal gender proportion, consisting of 29 men (48.3%) and 31 women (51.7%). The mean age, gender, and tumor location can be seen in table 1.

Table 1. Clinical characteristics of research subjects

Characteristics	Results
Average age ± SD	52±13.66 years old
≥65 years old	7 (11.7%)
<65 years old	53 (88.3%)
Gender	
Male	29 (48.3%)
Female	31 (51.7%)
Location	
Head and Neck	30 (50%)
Torso	10 (16.7%)
Upper Extremities	5 (8.3%)
Lower Extremities	15 (25%)

Histopathological characteristics including tumor thickness, mitotic index, lymphovascular invasion, and perineural invasion were assessed. The findings were then analyzed to determine their association with the occurrence of metastasis. From the results of statistical analysis, it was found that tumor thickness >2 mm and mitotic index ≥5/mm^2 were significantly associated ($p < 0.05$) with metastasis in cutaneous MM. Meanwhile, lymphovascular and perineural invasion were also associated with increased metastasis rate, but were not statistically significant.

Table 2. Histopathological characteristics of research subjects

Variables	Groups		Total	Odds Ratio (95% CI)	P value
	Metastasis	No Metastasis			
Tumor thickness					
>2 mm	29 (96.7%)	21 (70%)	50 (83.3%)	12.43 (1.46-105.73)	0.006**
≤2 mm	1 (3.3%)	9 (30%)	10 (16.7%)		
Mitotic index					
≥5/mm^2	17 (56.7%)	7 (23.3%)	24 (40%)	4,3 (1.41-13.07)	0,008**
<5/mm^2	13 (43.3%)	23 (70%)	36 (70%)		
Lymphovascular invasion					
Positive	7 (23.3%)	3 (10%)	10 (16.7%)	2.74 (0.64-11.82)	0.166
Negative	23 (76.7%)	27 (90%)	50 (83.3%)		
Perineural invasion					
Positive	5 (16.7%)	1 (3.3%)	6 (10%)	5.8 (0.64-53)	0.195
Negative	25 (83.3%)	29 (96.7%)	54 (90%)		

**p<0,01

TERT immunoexpression was assessed in both study groups (cases with and without metastases). TERT immunoexpression is considered positive if there was staining in the cytoplasm and/or nuclei of tumor cells. TERT expression was assessed in a blinded manner, then H-score were calculated using imageJ program. ROC (Receiver Operating Characteristics) curve analysis was performed, to determine the cut-off value for distinguish high and low expression.

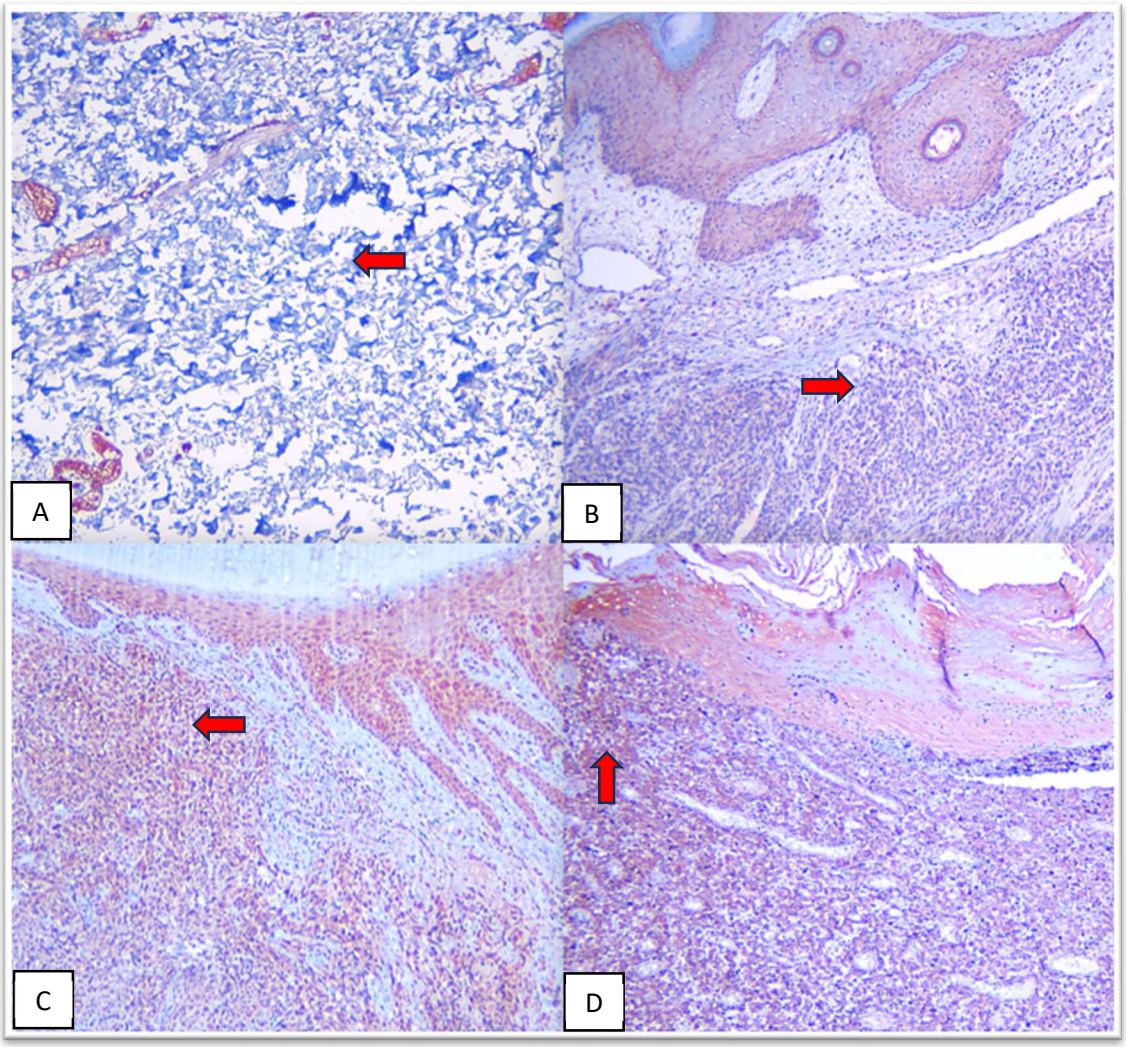

Figure 1. Results of TERT immunohistochemistry staining on tumor cells

Description: A. TERT expression with predominantly negative staining (HE, 100x). B. TERT expression with predominantly weak staining intensity (HE, 100x). C. TERT expression with predominantly moderate staining intensity (HE, 100x). D. TERT expression with predominantly strong staining intensity (HE, 100x).

TERT immunoexpression was calculated semiquantitatively based on the percentage and intensity of staining in the cytoplasm and/or nuclei of tumor cells. TERT will stain positive in the keratinocytes of epidermal stratum spinosum. Immunoexpression was considered negative if there was no staining, weak intensity if the staining was weaker than the staining intensity of stratum spinosum keratinocytes, medium intensity if the staining is equal to the staining intensity of stratum spinosum keratinocytes, and strong intensity if the staining is stronger than the staining intensity of stratum spinosum keratinocytes. Examples of the staining intensity is shown in figure 1.

ROC curve analysis was performed to determine the H-score cut-off value to categorize the TERT immunoexpression into high and low expression. The recommended cut-off value is 131.7 with 83.33% sensitivity and 80% specificity (figure 2). Case with H-score above the cut point (>131.7) was categorized as high expression, and with H-score less than/equal to the cut point (≤131.7) was categorized as low expression.

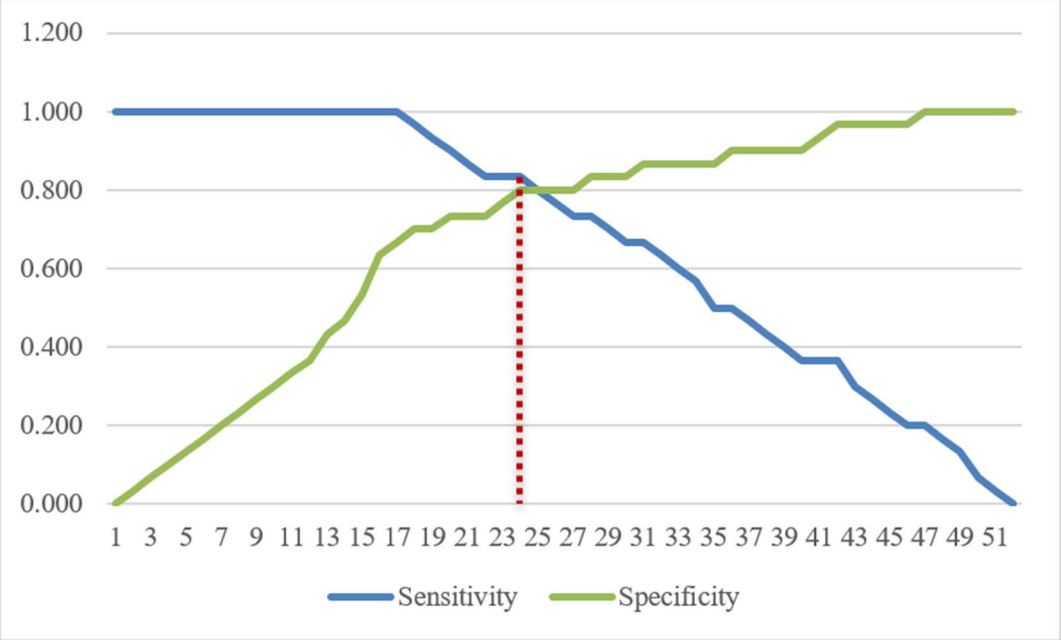

Figure 2. H-score sensitivity and specificity curve of TERT immunoexpression to predict the cutaneous MM metastases.

TERT immunoexpression result of each case was categorized into high or low expression using H-score cut off-point. In cases with high TERT immunoexpression, the median tumor thickness was 8.5 mm, whereas in cases with low immunoexpression the median value was 5.5 mm (table 3). The data had an abnormal distribution, so Mann-Whitney test was used for analysis. There was a statistically significant difference between the tumor thickness of cases with high immunoexpression compared to low immunoexpression (p=0.033).

Table 3. Tumor thickness comparison of high and low TERT immunoexpression

Variable	Tumor thickness (mm)	P value
	Median (min-max)	
TERT Immunoexpression		0.033*

High (H-score >131.7)	8.5 (2-20)
Low (H-score ≤131.7)	5.5 (2-18)

*p<0,05

High TERT immunoexpression was associated with mitotic index ≥5/mm^2 (OR 1.863), although not statistically significant (p=0.245). The association between TERT immunoexpression and mitotic index can be seen in table 4.

Table 4. Association of TERT immunoexpression with mitotic index

Variable	Mitotic index		Odds Ratio (95% CI)	P value
	≥5/mm^2	<5/mm^2		
TERT Immunoexpression				
High (H-score >131.7)	15 (46.9%)	17 (53.1%)	1.863 (0.649-5.345)	0.245
Low (H-score ≤131.7)	9 (32.1%)	19 (67.9%)		

High TERT immunoexpression was found in 26 (81.3%) cases of cutaneous MM with metastasis, whereas in cases without metastasis only in 6 samples (18.7%). Chi-square bivariate analysis showed a statistically significant relationship between high TERT immunoexpression and metastasis (p<0.001).

Table 5. TERT immunoexpression in cutaneous MM with and without metastases

Variable	Metastasis		P value
	Yes	No	
TERT Immunoexpression	26	6	<0.001***

High (H-score >131.7)	(81.3%)	(18.7%)
Low (H-score ≤131.7)	4 (14.3%)	24 (85.7%)

***p<0.001

Multivariate logistic regression analysis was subsequently performed to determine independent predictors for metastasis. In previous bivariate analysis, all independent variables had a p value of <0.2, therefore all of them were subsequently included in multivariate logistic regression test. The mutivariate analysis result concluded that high TERT immunoexpression was an independent predictor of metastasis in cutaneous MM with aOR 56.1 and a significant p value (p<0.001). This means that tumors with high TERT immunoexpression have 56-fold risk of metastasis compared to tumors with low TERT immunoexpression. The results of the multivariate analysis are presented in table 6.

Table 6. Multivariate logistic regression analysis for metastasis prediction

Characteristics	**Metastasis**		
	Adjusted Odds Ratio	**95% CI**	**P value**
High TERT immunoexpression	56.1	7.6-412.2	<0.001***
Tumor thickness >2 mm	29.1	0.7-1,109.5	0.07
Mitotic index ≥5/mm^2	5.1	0.78-34	0.089
Lymphovascular invasion	5.3	0.4-83.7	0.235
Perineural invasion	1.4	0.04-45.1	0.85

***p<0.001

5. DISCUSSION

MM is a malignant tumor with a high mutational burden, which incidence increases with advancing age. The highest incidence of cutaneous MM is at 55 years old. (Böer-Auer et al., 2022) Mutu et al. (Mutu et al., 2022) who studied 56 cutaneous MM patients found that the average age of the patients was 57 years old. In this study, the average age was 52 years old, not

much different from previous studies. As humans get older, they undergo biological changes and genetic damage accumulates. MM occurs due to a complex interaction between genetic and environmental factors, including exposure to ultraviolet radiation. This causes the incidence of MM to increase as human get older. The latent period from initiation of carcinogenesis to clinical manifestation is usually more than 10 years. (Memon et al., 2021; Ribeiro et al., 2018) Global Cancer Observatory data shows that in 2020, there were 173,844 new cases of cutaneous MM in men and 150,791 new cases in women. In this study, patient's gender was equal, consists of 48.3% men and 51.7% women. This is similar to Morgese's et al. (Morgese et al., 2020) study of 1,023 patients which found that 47.6% patients were men and 52.4% were women. The metastatic cases of this study consists of 16 (53.3%) regional lymph node metastases and 14 (46.7%) distant organ metastases.

Bivariate analysis result showed that the histopathological characteristics that had statistically significant association with metastasis were tumor thickness >2 mm ($p=0.006$) and mitotic index ≥5 mitoses/mm^2 ($p=0.008$). Meanwhile, the presence of lymphovascular and perineural invasion had no statistically significant association with metastasis. Thompson et al. (Thompson et al., 2011) found that tumor thickness and mitotic index are independent predictors of survival rate in cutaneous MM patients. Ghasemi et al. (Ghasemi Basir et al., 2018) found that tumor thickness is among the most important prognostic factors. In MM pathology specimen, tumor thickness should always be measured and included in the histopathologic report, to determine patient management and prognosis. The excision margin recommended for tumors with <2 mm in thickness is 10-20 mm, while for thicker tumors (2-4 mm) is 20 mm. In tumors with >4 mm in thickness, the recommended excision margin is 20-30 mm. In this study, statistical analysis showed that tumor with >2 mm thickness is associated with metastases with odds ratio (OR) of 12.43. This means that the rate of metastasis in cutaneous MM with >2 mm tumor thickness are increased by 12-fold. Portinari et al. (Portinari et al., 2018) who studied 289 cutaneous MM patients found that cutaneous MM with >1 mm in thickness was 3.43 times more likely to metastasize than tumor with ≤1 mm in thickness. Meanwhile, the metastatic risk in patients with tumor thickness >2 mm is increased by 9.32 times. Only 1 case (3.3%) of metastatic MM in this study was ≤2 mm in thickness. In this particular case, the tumor size was found to be exactly 2 mm thick. However, a high mitotic index was found (≥5/mm^2) as well as lymphovascular and perineural invasion.

Mitotic index, a histopathological characteristic that reflects tumor cell proliferation, is the second most important predictor of survival after tumor thickness. Each 1/mm^2 increase in mitotic index is correlated with a 0.8 mm increase in tumor thickness. (Ghasemi Basir et al., 2018) Cutaneous MM with a mitotic index of ≥5/mm^2 are significantly associated with the incidence of metastasis and reduced patient survival rates. (Calomarde-Rees et al., 2019; Egger et al., 2019; Thompson et al., 2011) In this study, the results showed significant association between mitotic index ≥5/mm^2 and metastasis with an OR of 4.3. This means that the risk of metastasis is increased by 4-fold in cutaneous MM patients with a mitotic index of ≥5/mm^2. Namikawa et al. (Namikawa et

al., 2018) who studied 1,898 MM patients, found that tumors with a mitotic index of ≥5/mm^2 were 8.65 times more likely to develop metastasis.

Lymphovascular invasion is one of the prognostic factors that needs to be evaluated in cutaneous MM. (Bobos, 2021) Lymphovascular invasion is a part of the tumor metastatic cascade. Previous researches on the association of lymphovascular invasion with metastasis have shown various results. The significance of lymphovascular invasion as a single prognostic predictor factor remains unclear. (Rose et al., 2011; Suresh et al., 2020) Egger et al. (Egger et al., 2019) found that cutaneous MM with lymphovascular invasion was 3.3 times more likely to develop metastases. Meanwhile Rose et al. (Rose et al., 2011) did not find a significant association between lymphovascular invasion and metastasis. In this study, lymphovascular invasion has no statistically significant association with metastasis. This lack of significance may be caused by small number of samples with positive lymphovascular invasion (16.7% of the total sample). The incidence of lymphovascular invasion finding in MM is lower than the incidence of lymph node metastasis. Rose et al. (Rose et al., 2011) found that lymphovascular invasion was detected only in 0-6% cases, while lymph node metastases were detected in 19-47% cases. The fewer number of lymphovascular invasion findings compared to lymphn node metastasis may be caused by the difficulty of detecting one in routine histopathological examination.

Perineural spread is one of the pathway by which tumors can metastasize. (Marek et al., 2018) Varey et al.(Varey et al., 2017) who studied 671 MM patients with neural invasion found that neurotropism was not associated with recurrence or survival rates. Meanwhile, Namikawa et al. (Namikawa et al., 2018) found that perineural invasion increased local recurrence rates, although it had no significant association with metastasis. In this study, it was found that perineural invasion had no statistically significant association with metastasis.

Increased telomerase activity accelerates the growth of MM. Guo et al. (Guo et al., 2022) found an association between increased telomerase activity with a worse prognosis, vascular invasion, high mitotic index, and increased Breslow thickness. Bustos et al. (Bustos B et al., 2019) also found associations between TERT promoter mutations with increased tumor thickness and mitotic activity. In this study, tumors with high and low TERT immunoexpression had statistically significant differences in tumor thickness ($p=0.033$). Tumors with high immunoexpression had a mean tumor thickness of 9.81 mm and a median of 8.5 mm, while tumors with low immunoexpression had a mean tumor thickness of 6.71 mm and a median of 5.5 mm. This is similar to Macerola's et al. research (Macerola et al., 2015) who discovered MM with TERT promoter mutations had a significantly different mean tumor thickness ($p=0.029$) compared to tumors with wild-type TERT promoter (5.5 mm versus 2.1 mm). Hugdahl et al.(Hugdahl et al., 2018) also found that MM with TERT promoter mutations were associated with an increase in tumor thickness, although not statistically significant ($p=0.10$). Nagore et al.(Nagore et al., 2016) found that MM with rapid growth (≥0.5 mm/month) had a higher frequency of TERT promoter mutations, twice as much as MM with slow growth (<0.5 mm/month).

Tumor cells with TERT promoter mutations have increased telomerase activity resulting in unlimited proliferation. Rapid growing MM is not only associated with increased Breslow thickness, but also associated with lymph node metastasis. (Nagore et al., 2016) This study result showed that cutaneous MM with high TERT immunoexpression was 1.8 times more likely to have a high mitotic index (≥5/mm^2), although not statistically significant (p=0.245). This is similar to Hugdahl's research. (Hugdahl et al 2018) who discovered MM with TERT promoter mutations had a higher mitotic index (median 6.6/mm^2) than wild-type TERT (median 4.7/mm^2), but was not statistically significant (p=0.18). Macerola et al. (Macerola et al., 2015) found that MM with TERT promoter mutations had a higher mitotic index (5.6/mm^2) compared to wild-type (2,8/mm^2) with statistically significant difference (p=0.041). On the other hand, Thomas et al.(Thomas et al., 2020) found that TERT promoter mutations were not associated with Breslow's thickness and mitosis.

TERT promoter mutations causes increased expression of telomerase enzymes. This process is one of the most common mechanism for cell immortalization. This enzyme causes cells to avoid cellular senescence and can undergo neoplastic transformation. TERT promoter mutation inflicts neoplastic transformation in two phases. In the first phase, there is an increase in TERT expression which results in avoidance of cellular senescence. Meanwhile, in the second phase, there is genomic instability and increased telomerase activity. In MM with TERT promoter mutations, transcription activity is increased two to fourfold compared to MM with wild-type TERT promoter status. (Andrés-Lencina et al., 2019; Calomarde-Rees et al., 2019; Colebatch et al., 2019; Lamb et al., 2013) TERT promoter gene mutations in cutaneous MM are associated with metastasis and poor survival rates. (Andrés-Lencina et al., 2019; Calomarde-Rees et al., 2019; Colebatch et al., 2019; Lamb et al., 2013) Lencina et al.(Andrés-Lencina et al., 2019) who studied 287 cutaneous MM patients found TERT promoter mutations increases the incidence of relapse and death due to MM. Calomarde-Rees al.(Calomarde-Rees et al., 2019) in 1,177 cutaneous MM patients found that tumors with TERT promoter mutations were 2.9 times more likely to develop metastasis. In this study, high TERT immunoexpression is more commonly found in cases with metastasis (81.3%) than without metastasis (18.7%). The results of statistical analysis showed a significant association between high TERT immunoexpression and metastasis (p <0.001).

Based on the ROC curve, the recommended H-score cutoff value for high and low expression is 131.7 with 83.3% sensitivity and 80% specificity. Multivariate logistic regression analysis showed that high TERT immunoexpression was an independent predictor of metastasis with aOR of 56.1. This means that the risk of metastasis increases by 56-fold in cutaneous MM with high TERT immunoexpression. Hugdahl et al.(Hugdahl et al., 2018) research in cutaneous MM found that TERT immunoexpression was significantly associated with worse survival rates, with an H-score cut-off value of 100 for high and low expression. Elders et al.(Elders et al., 2012) research in glioblastoma patients also found that high TERT immunoexpression was significantly associated with poor prognosis. Meanwhile, Cho et al.(Cho et al., 2021) who studied TERT immunoexpression in 30 acral MM patients with and without metastases found that strong TERT

expression was associated with worse survival rates, although not statistically significant. This may be caused by small sample size of that study.

The most common MM subtype found in this study was nodular MM. In this subtype, high TERT immunoexpression was found as much as 75% of cases. Hugdahl et al. (Hugdahl et al., 2018) who studied 194 nodular MM also found TERT promoter mutations in 68% of this subtype. Heidenreich et al. (Heidenreich et al., 2014) also discovered that TERT promoter mutations was frequently found in nodular MM (55.3%). Nodular MM is a tumor with vertical growh pattern. Macerola et al. (Macerola et al., 2015) found TERT promoter mutations in 86% nodular MM, whereas in MM with a radial growth pattern only 36% of superficial spreading MM and none of acral lentiginous MM had the mutation. Meanwhile in this study, high TERT immunoexpression was found in 50% case of acral lentiginous MM and superficial spreading MM, a smaller proportion compared to nodular MM.

TERT promoter mutations are often considered the second hit mutation which play a key role in the malignant transformation of benign melanocytic lesions. (Harou et al., 2019) TERT assessment at the protein level has not been extensively studied, but this modality can determine telomerase activity related to TERT promoter gene status. The results of previous studies have found that TERT promoter mutations are significantly associated with a worse prognosis. Hopefully TERT immunohistochemistry can be used as a more affordable alternative examination to predict the risk of metastasis and prognosis of cutaneous MM patients.

CONCLUSION

Based on this study, it can be concluded that high TERT immunoexpression increases the rate of metastasis in cutaneous MM. There is a statistically significant association between the histopathological characteristics of tumor thickness and mitotic index with metastasis in cutaneous MM. There is also an association between the histopathological characteristics of lymphovascular invasion and perineural invasion with the metastasis in cutaneous MM, although not statistically significant.

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