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The Comparison of p16 Protein Expression between Single and Multiple Seborrheic Keratosis

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ABSTRACT

Background. Seborrheic keratosis (SK) is a benign epithelial tumor that attacks adults, the elderly, and an increasing number of age-related lesions. Various risk factors influence the pathogenesis of SK, including genetic predisposition and sun exposure. Recently, SK associated with a theory of keratinocytes aging. The aging prevents unlimited and uncontrolled growth of damaged cells, thereby preventing degeneration, but on the other hand, increases the resistance of the cells to apoptosis. From previous studies, the expression of p16 protein increased substantially with age, thus enabling it to be a biomarker of cellular aging at the molecular level. This study was aimed to determine p16 protein expression between single and multiple seborrheic keratoses, the relationship between p16 protein expression in single and multiple SK, and p16 protein expression in the elderly. **Methods:** Observational study with a cross-sectional design was used to determine the comparison of p16 protein expression between single and multiple seborrheic keratoses was conducted from December 1, 2021, to January 31, 2022. According to the inclusion criteria, study participants with a total of 42 people were selected through the consecutive sampling method. Physical examinations, dermoscopic and histopathologic were carried out until the results were obtained. Then the data were analyzed using the Fisher Test, Chi-Square, with a 95% confidence interval ($p < 0.05$). **Results:** Most SK patients were found in the age range of 60-69 years (38%), male gender (54.8%), sun-related occasional 3-6 hour per day (50%), location of SK in sun-exposed areas (83.3%), the number of multiple lesions (69%), size 1-2 cm (57.1%), disease duration > 3 years (54.8%), and have no comorbid (69%), an acanthotic subtype of SK (40.5%). All single SK patients have a negative-weak expression of p16 protein (38.2%). Multiple SK patients have a moderate-strong expression of p16 protein (100%) and negative-weak expression of p16 protein (61.8%). Based on statistical analysis using Fisher Exact test has a statistically significant to p16 protein expression with number SK lesion (p-value 0,043). **Conclusion:** There is a significant correlation between p16 protein expression with single and multiple SK. The study has several limitations. The sample size was small. Other supporting examinations are needed in SK, including polymerase chain reaction (PCR).

1. Introduction

Seborrheic keratosis (SK) is the most common benign epidermal skin tumor, especially in middle and old age with a varied clinical picture, generally in the form of patches to brown plaques with a verrucous surface, can be single, multiple, and the size of the lesions enlarges with age.[1-3] Histopathological characteristics of SK were hyperkeratosis, acanthosis, papillomatosis, and pseudohorn cyst.[1,4] SK predilection

is generally in sun-exposed areas, especially the face and neck can also be found in areas not sun-exposed.[1,5,6]

Seborrheic keratosis can be found in Caucasian and Asian races, more men than women with Fitzpatrick skin type IV, increasing in older people more than 50 years. In a retrospective cohort study in Brazil (n=10,545), it was found that 89% of SK aged more than 80 years. Taylor (2017) got SK in Australia (n=191), with 52.4% SK at 51 years.[6] Peng et al. (2017) study in China (n = 400), getting SK at age > 50 years as much as 80-100%.[7] Epidemiological studies in Australia, UK, Netherlands, and Korea have shown an increase in the incidence, number, and size of SK with increasing age.[5,8]

The etiopathogenesis of seborrheic keratoses remains unclear. Various risk factors have been shown to influence the pathogenesis of SK, including sun exposure and genetic predisposition.[1] Ultraviolet (UV) radiation is an important genotoxic that induces keratinocyte cell stress, the main carcinogen of the skin.[9,10] Azazmeh et al. (2020), on cultured mouse keratinocytes after sun exposure, showed an increase in p16 protein expression and aging.[11] Recently, several researchers have supported the theory that the keratinocyte aging process plays a role in the pathogenesis of KS, aging cells disrupt the cell cycle due to the aging process of keratinocytes where cell damage occurs cell cycle regulatory genes do not control that, including proteins p16, p53, p21, and p27, induce cellular stress on keratinocytes leading to carcinogenesis in the skin.[12-14]

The clinical feature of SK has many variants; it can resemble premalignant tumors such as actinic keratosis and Bowen's disease and malignant tumors such as squamous cell carcinoma (SCC). There are six histopathological subtype variants of SK: acanthotic, hyperkeratotic, reticular/adenoid, clonal, irritated, and pigmented.[3,5] To differentiate between variants of SK with premalignant and malignant tumors, it is necessary to examine the IHC, including p16 protein.

The p16 gene is a tumor suppressor gene that belongs to the cell cycle guardian group.[12] The p16 gene regulates normal and tumorigenic squamous epithelial cells encoded in chromosome 9 locus 21. In several studies, increased expression of p16 protein plays a role in the regeneration of stem cells. When changes occur in these cells, they can play a role in epidermal homeostasis, aging, and tumour development.[15] In previous studies, the p16 gene was implicated in the development of sporadic or familial melanoma, glioma, lung carcinoma, and T cell leukemia. Recently, several studies have shown p16 protein as a prognostic biomarker for patients with SCC of the oropharynx and cervix.[9,11,12]

Based on a study by Nakamura et al. (2003), examination of p16 protein expression shows SK is a benign neoplasm with the accumulation of p16 protein in keratinocytes aging. Alexandrova et al. (2016), p16 protein expression in single and multiple SK (n=20), found strong p16 protein expression in multiple SK as many as seven samples (70%), and weak values in single SK as many as three samples (30%), multiple SK have a relation with strong p16 protein expression and comorbid diabetes mellitus (DM).[12] Kalegowda et al. (2017), examination of p16 protein expression compared clonal type SK (n=29), and Bowen's disease (n=13), obtained moderate-strong p16 protein expression in Bowen's disease of 100 %, while the expression of p16 protein was negative and weak in clonal type SK as much as 83% and 17%.16 This study was aimed to determine p16 protein expression between single and multiple seborrheic keratoses, the relationship between p16 protein expression in single and multiple SK, and p16 protein expression in the elderly.

2. Methods

This study is an analytical observational study with a cross-sectional design by comparing the expression of p16 protein in single and multiple SK. The study was conducted at the Outpatient Unit, Department of Dermatology and Venereology (DV), Dr. Mohammad Hoesin General Hospital Palembang during the period December 2021 to January 2022. Selection of participants based on inclusion criteria. Criteria inclusions are all patients with clinical and dermoscopical concordance with seborrheic keratosis,

all seborrheic keratosis patients over 25 years, and signing the informed consent after being explained. The independent variable was p16 protein expression. The dependent variable was single and multiple SK lesions. The covariables of this study were age, gender, location of SK, size, occupation, duration of illness, and comorbidities. This study has received approval from the ethics committee of Faculty of Medicine, Universitas Sriwijaya (No. 118/keprsmhfkunsri/2021).

3. Results

Forty-eight patients were diagnosed with SK, and six people were excluded because the histopathological feature showed dermal nevus. The number of study subjects who met the inclusion criteria was 42 people. The study subjects (n=42) assessed characteristics based on gender, age, duration of exposure to SM, location of lesions, number of lesions, size of lesions, duration of disease, and comorbidities. Of the 42 study subjects, the distribution based on gender was found to be 23 men (54.8%) and 19 women (45.2%)—comparison of men and women 1.2:1. The age range of the study subjects was 33-82 years. The highest age group was 60-69 years as many as 16 people (38%), 26-49 years as many as 12 people (28.6%), 50-59 years as many as ten people (23.8%), and > 70 years as many as four people (9.5%).

Of the 42 study subjects, based on the distribution of occupations sun-exposed, 21 people (50%) had a duration of 3-6 hours per day, 11 people (26.2%) had a duration of > 6 hours per day. Ten people (23.8%) had < 3 hours per day. Based on the distribution of the location of the lesions, 35 people (83.3%) were in the sun-exposed region, and 7 (16.7%) areas were not sun-exposed.

Table 1. Characteristics of study subjects

Characteristics	N(%)	Mean (SD)	Med (Min-Max)
Gender			
• Female	19 (45.2%)		
• Male	23 (54.8%)		
Age			
• 26-49	12 (28.6%)	55.79 (12.09)	56.5 (33 – 82)
• 50-59	10 (23.8%)		
• 60-69	16 (38%)		
• > 70	4 (9.5%)		
Occupation			
• Housewife	10 (23.8%)		
• Civil servant	21 (50.0%)		
• Private	11 (26.2%)		
Duration of sun exposure			
• Less than 3 hours	10 (22.8%)		
• 3-6 hours	21 (50.0%)		
• More than 6 hours	11 (26.2%)		
Location			
• Facialis	21 (50.0%)		
• Trunkus	7 (16.7%)		
• Colli	12 (28.6%)		
• Extremities	2 (4.8%)		
Location of Exposure			
• Exposure (facial, colli)	33 (78.6%)		
• Unexposed (truncus, extremities)	9 (21.4%)		
Number			
• Single	13 (31.0%)		
• Multiple (> 1 lesion)	28 (69.0%)		
Size			
• Less than 1 cm	18 (42.9%)		
• 1 cm	24 (57.1%)		
Comorbidity			
• None	29 (69.0%)		
• Hypertension	11 (26.2%)		
• Diabetes mellitus	2 (4.8%)		
Disease duration			
• Less than 1 year	2 (4.8%)		
• 1-3 years	17 (40.5%)		
• More than 3 years	23 (54.8%)		
Histopathology examination			
• SK acanthotic type	17 (40.5%)		
• SK type keratotic	13 (31.0%)		
• SK reticular type	6 (14.3%)		
• Mixed type SK (irritated and acanthotic, reticular and pigmented SK)	3 (7.1%)		
• SK type irritated	2 (4.8%)		
• SK type pigmented	1 (2.4%)		

Of the 42 study subjects, based on the number of lesions, the most multiple SK were 29 people (69%), and single SK were 13 people (30.1%). Based on the size of the lesions, the most were large lesions (≥1 cm) in 24 people (57.1%), followed by small lesions <1 cm in 18 people (42.9%). Based on the duration of SK disease, the highest number was found > 3 years in 23 people (54.8%), followed by 1-3 years in 17 people (40.5%), and < 1 year in 2 people (4.8%). Based on comorbidities, consecutively 11 people (26.2%) with hypertensive heart disease, two people (4.8%) with DM, and having no comorbidities in 29 people (69%). The most histopathological SK subtypes were acanthotic subtypes as many as 17 people (40.5%), SK keratotic subtypes as many as 13 people (30.1%), SK subtype reticular as many as six people (14.3%), SK mixed subtype as many as three people (7.1%), SK irritated subtype as many as two people (4.8%), and SK pigmented subtype as many as one person (2.4%). Characteristics of study subjects are shown in table 1.

Data were combined in the form of independent variables, namely, the expression of the protein p16 was negative-weak and moderate-strong. Statistical testing between the two groups used the Fisher Exact statistical test because the smallest Expected Count value was below 5%. From the statistics, it is known that multiple lesions dominate the expression of negative-weak p16 protein by 61.8%. This value was lower than the moderate-strong p16 protein expression in multiple lesions by 100% (table 2). There was a significant relationship between p16 protein expression and the number of single and multiple SK lesions (p-value 0.043).

Table 2. p16 protein expression related to single and multiple SK

Variable		Number of lesions				PR	95% Confidence Interval		p-value
		Multiple (n=29)		Single (n=13)					
		N	%	N	%		Lower	Upper	
p16 protein expression	Moderate-strong	8	100	0	0	1,619	1,243	2,109	0.043
	Negative-weak	21	61.8	13	38.2				

*All statistical tests for 2x2 tables use the Fisher Exact statistical test because the expected count value is < 5%

Testing the age variable on p16 protein expression using the independent statistical test T-Test showed that there was no significant relationship between age and the p16 protein expression group with a negative-weak value and the p16 protein expression group with a moderate-strong value (p-value: 0.830) (table 3).

Table 3. Analysis of the relationship between age and p16 protein expression

p16 protein expression	N	Mean ±SD	p-value
Negative-weak	34	55.59 ± 12.74	0.830
Moderate-strong	8	56.63 ± 9.49	

4. Discussion

Seborrheic keratosis is a benign epidermal skin tumor often found in middle age and adulthood with a varied clinical picture, generally in the form of patches, thin plaques, and pale brown to black, well-defined, verrucous surfaces, some smooth, single, multiple, or stalked. There are more men than women, and the number increases with age.[1,8]

Based on gender, the highest frequency of SK in this study was 23 males (54.8%) compared to 19 females (45.2%). Based on Roh (2016), SK demographic data (n=271), in male as many as 144 people (53.1%) more than female as many as 127 people (46.9%).[5] Contrast to the study of Gill et al. (2000), the prevalence and distribution of SK (n=170), the frequency of female was 51.8% more than 48.2% of male.[17] According to the theory, sun exposure plays a role as one of the risk factors that influence the occurrence of SK, it is

suspected that men are more often exposed to the sun than women.[1,12,18]

On study, based on age, the highest ages were 60-69 years as many as 16 people (38%), 26-49 years as many as 12 people (28.6%), 50-59 years as many as ten people (23.8%), and >70 years as many as four people (9.5%). According to an epidemiological study by Taylor (2017), the prevalence of SK in Australia (n=100) showed multiple SK at 100% at age 51 years, single SK at 12% at age 26-50 years, and 15-25 years, respectively. Seborrheic keratosis increases with age.[6]

On study, by occupational associated with the duration of sun exposure, it was found that SK at a duration of 3-6 hours per day was 21 people (50%), a duration of > 6 hours per day were 11 people (26.2%), and duration of < 3 hours per day was ten people (23.8%). In Korea, Kwon et al. (2003) found that patients with cumulative sun exposure more than 6 hours per day were associated with a 2.28-fold more significant risk of SK than sun exposure less than 3 hours per day.[19] Chazal et al. found that p16 protein plays a significant role in regulating the cell cycle of keratinocytes in response to keratinocyte damage due to the influence of UV radiation. Due to UV light, DNA damage in keratinocyte cells can form a genotoxic role in skin carcinogenesis.[9,11] Cumulative sun exposure is affected by latitude, altitude, cloud conditions, and season, measured using dosimetry. According to the theory, the number of SK increases as the cumulative exposure to UV radiation.[20]

On study, based on the location of the lesion, SK was found to be successively more frequent at locations sun-exposed (facial and colli) as many as 35 people (83.3%) compared to locations, not sun-exposed (truncus and extremities) as many as seven people (16,7%). According to a study by Kwon et al. (2003), the distribution of SK (n=2.636) showed that SK was most commonly found in exposed areas (face, neck, chest, upper extremities) as many as 64.2% compared to 35.8% in unexposed areas.[19] Study by Roh et al. (2016), distribution of SK (n=271), showed that SK was most commonly found in exposed areas (head, face, neck, and upper extremities) as many as 63.1% compared to non-exposed areas as many as 36.9%.[5]

Based on the number of lesions, the highest number consecutively in multiple SK was 29 people (69%), and single SK as many as 13 people (30.1%). A study by Balin (2020) stated that SK at the age of 15 years to > 75 years in Australia (n=100), the number of SK is from 5 to 69 per person. The increasing number of SK occurs with increasing age.[18]

On study, based on the size of the lesions, the most were large (≥ 1 cm) as many as 24 people (57.1%), and small lesions (< 1 cm) as many as 18 people (42.9%). Epidemiological studies in Australia, UK, Netherlands, and Korea have shown an increase in the incidence, number, and size of SK with increasing age. However, further study is needed.[8]

On study, based on the duration of SK, the highest number consecutively was > 3 years in 24 people (55.8%), 1-3 years in 17 people (39.5%), < 1 year in 2 people (5.1%). According to a review by Sun et al. (2021), proving that SK thickens with age. The increase in the size of the SK lesion is slow, and the thickening of the lesion occurs gradually.[8,18]

In the study, SK without comorbidities was found in 29 people (69%), SK with hypertensive heart disease in 11 people (26.2%), and SK with DM in 2 people (4.8%). A study by Alexandrova (2016), p16 protein expression in single and multiple SK (n=20), found strong p16 protein expression in multiple SK as many as seven people (70%), and weak values in single SK as many as three people (30 %), strong p16 protein expression relation with comorbid diabetes mellitus (DM).[12]

In the study, the most histopathological subtypes of SK were acanthotic subtypes as many as 17 people (40.5%), SK hyperkeratotic subtypes as many as 13 people (30.1%), reticular SK subtypes as many as 6 people (14.3 people). %), mixed subtype SK (irritated-acanthotic SK and reticular-pigmented SK) as many as 3 people (7.1%), irritated SK subtype as many as 2 people (4.8%), and pigmented subtype SK as many as 1 person (2, 4%). The general histopathological features of SK are hyperkeratosis, acanthosis, papillomatosis, and pseudohorn cyst. Based on the theory, there are six histopathological subtypes of SK, namely acanthotic, keratotic, reticular/adenoid, clonal, irritated, and pigmented.[4] Roh, et al (2016),

histopathological description of SK (n=206), the highest number of acanthotic subtypes was 93 people (45.1%), mixed subtypes were 41 people (19.9%), keratotic subtypes 35 people (17%), reticulated subtype 9 people (4.4%), and irritated subtype 4 people (1.9%).[5] According to Cimpean, et al (2019), if clinical manifestations of SK are found with erythema, crusting, ulcers, and pruritus, accompanied by histopathological features of atypical nuclei and mitotic cells, required an IHC examination.[21]

In the study, negative-weak p16 protein expression in single SK as many as 13 samples (38.2%). Moderate-strong p16 protein expression in multiple SK was 8 samples (100%), negative-weak p16 protein expression was 21 samples (61.8%). Nakamura, et al (2003), increased expression of p16 protein in SK indicates that accumulation in aging epidermal cells during the G1/S phase, in accordance with the theory of keratinocyte aging. Alexandrova, et al (2016), in single and multiple SK (n=20), found strong p16 protein expression in multiple SK as many as 7 people (70%), moderate as many as 3 people (30%), and in single SK with weak p16 protein expression as many as 8 people (80%). There is increasing p16 protein expression in multiple SK, to assess the progression of SK still needs further study because the number of SK samples is small.[12]

Based on the study, the results of the Fisher Exact statistical test showed a significant relationship between p16 protein expression with single and multiple SK (p-value 0.043). Moderate-strong p16 protein expression in multiple SK, with potential for moderate-strong p16 protein expression 1,619 times compared to single SK (PR: 1.619). According to several previous researchers, there is a theoretical relationship between the aging process of keratinocytes and UV exposure with increasing p16 protein expression, although further study is needed with a larger number of SK samples. Based on the hypothesis Chazal, et al (2002) explained that UV radiation is genotoxic to keratinocytes inducing hyperplasia. Azazmeh et al (2020), on cultured mouse keratinocytes after UV exposure, increased the expression of p16 protein that accumulates in aging keratinocyte tissue and induces premalignant lesions in the epidermis/dermis.[9,12]

Statistically, there was no significant relationship between p16 protein expression in single and multiple SK with age (p-value: 0.830). Although several studies have shown p16 protein expression is associated with cell cycle regulatory genes where there is an increase in p16 protein expression with aging. Hafner, et al (2007), assessed risk factors of aging and cumulative UV exposure induces mutations in FGFR3 gene.[22] Based on a review of D'Arcangelo, et al (2017), explained the role of the p16 gene in regulating epidermal homeostasis (stem cell self-renewal), the aging process, and tumor development. The accumulation of p16 protein in aging keratinocyte tissue induces the aging process, dysregulation of several enzymes occurs and the function of the immune system decreases works synergistically to form an unstable genome, resulting in tumorigenesis.[15]

The limitation of this study is the small number of SK samples for examination of p16 protein expression. The SK sample was obtained only based on the population at Dr. Mohammad Hoesin general hospital (hospital base) so that the results obtained could not assess the malignant transformation of SK.

5. Conclusion

There is a significant correlation between p16 protein expression with single and multiple SK. The study has several limitations. The sample size was small. Other supporting examinations are needed in SK, including polymerase chain reaction (PCR).

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