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**HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW**  
**KARYA ILMIAH : JURNAL ILMIAH**

Judul Karya Ilmiah (Artikel) : Profile of p53 Protein Expression in Basal Cell Carcinoma Low – Risk and High-Risk Subtype

Penulis : 5 Orang

Status Pengusul : Alendra Chakramurty, Meira Dewi Kusuma Astuti, Hermawan Istiadi, Awal Prasetyo, Dik Puspasari

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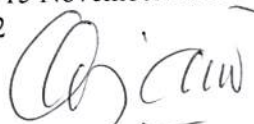
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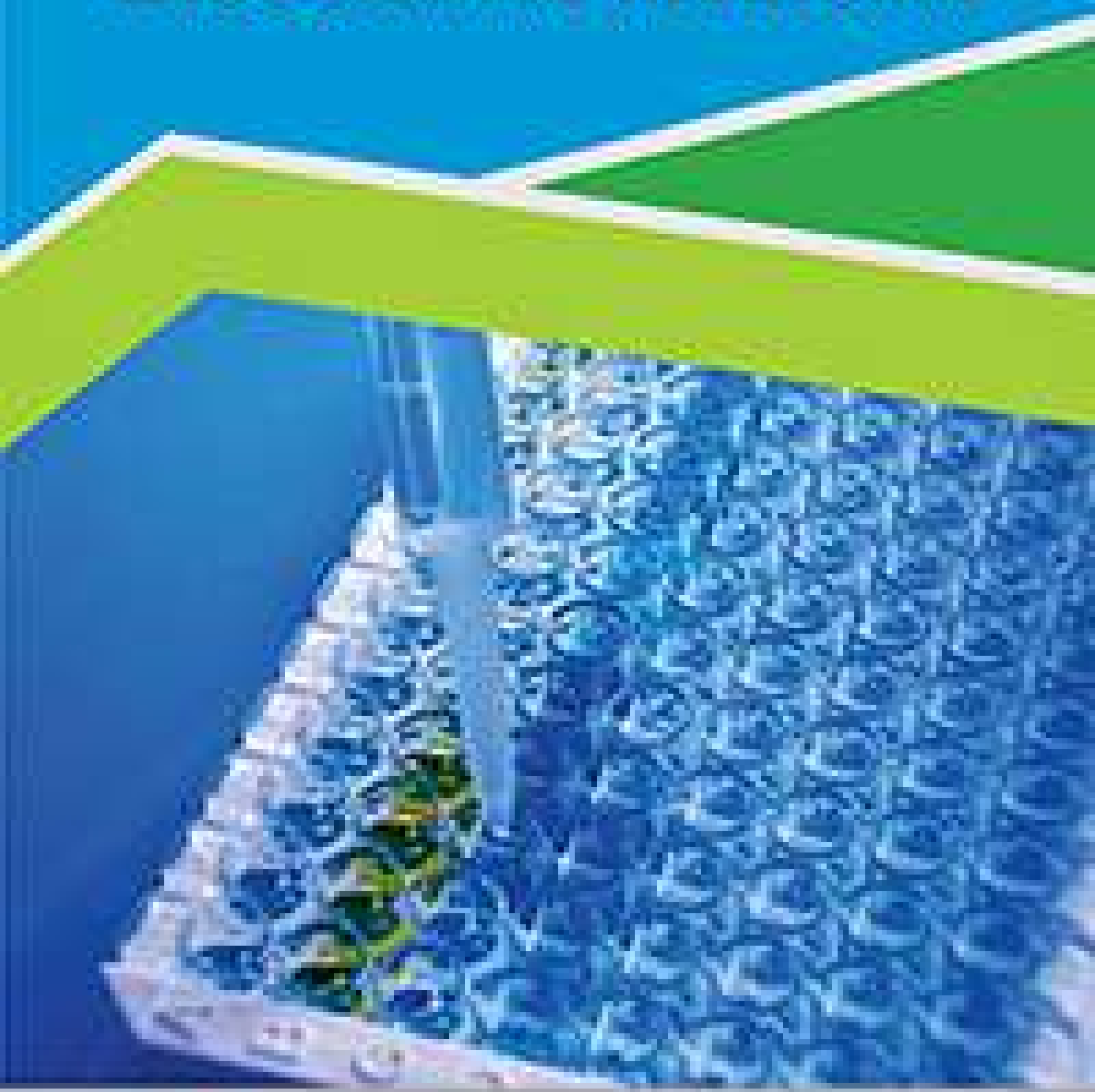
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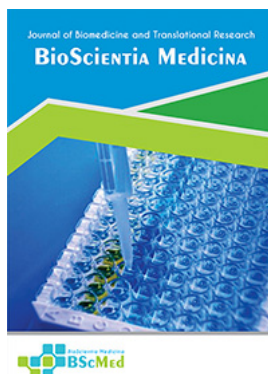
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# Profile of p53 Protein Expression in Basal Cell Carcinoma Low – Risk and High- Risk Subtype

*by* Awal Prasetyo

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### Profile of p53 Protein Expression in Basal Cell Carcinoma Low-Risk and High-Risk Subtype

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#### ABSTRACT

**Background:** Basal cell carcinoma is one of the most common cancers in the world, and its incidence is increasing over time. It is estimated that it accounts for three-quarters of all non-melanoma skin cancers, and the incidence increases exponentially in predominantly white areas. The p53 gene is the gene that most undergoes mutations due to sun exposure and is the cause of skin cancer, especially basal cell carcinoma. This finding explains that p53 expression can be used as marker analysis in cases of basal cell carcinoma. **Methods:** Observational analytic design was carried out with a cross-sectional. The population and samples were paraffin blocks which histopathologically diagnosed Basal Cell Carcinoma in the anatomical pathology laboratory, Dr. Kariadi General Hospital Semarang. Samples were processed by the p53 immunohistochemical staining method. The p53 expression was given a quantitative score to be 0 = negative, 1-2 = low, 3-4 = high. The expression of p53 was considered positive when it was colored in the cell nucleus. The correlation between variables was analyzed by Spearman and Mann Whitney correlation test. **Results:** There is no correlation between p53 expression and the age of p53 patients with  $p = 0.390$  ( $p > 0.05$ ). There is no correlation between p53 expression and lesion location with  $p = 0.817$  ( $p > 0.05$ ). There is no difference significant, between p53 expression and gender with  $p = 0.576$  ( $p > 0.05$ ). **Conclusion:** There is no correlation between high and low-risk Basal Cell Carcinoma subtype associated with age, gender, and lesion location.

#### 1. Introduction

Skin cancer is one of the most common cancers in the world, and its incidence is increasing over time. In general, skin cancer is divided into cancers originating from melanocytes (melanoma) and those originating from the epidermis (non-melanoma skin cancer). These two groups represent the majority of skin cancers (95%), while other skin cancers make up a very small percentage. Non-melanoma skin cancer is divided into 2 broad lines, namely basal cell carcinoma and squamous cell carcinoma. Both types of cancer originate from epidermal cells and have similar characteristics. Basal cell carcinoma accounts for up to 80% of non-melanoma skin cancers.<sup>1,2</sup>

Basal cell carcinoma is estimated to account for three-quarters of all non-melanoma skin cancers, and its incidence increases exponentially in predominantly white areas such as North America, Europe, and Australia. In the US, the incidence rate is about 76 cases per 100,000 person-years. The male-to-female ratio is about 1.5:1. Referring to the 2018 GLOBOCAN cancer data, the highest incidence of skin cancer in the world, both melanoma, and non-melanoma, are in Australia and New Zealand. Non-melanoma skin cancer (NMS), basal cell carcinoma, and squamous cell carcinoma are the most common cancers in Australia. In Germany, 120,000 cases of NMSC were estimated in 2009. In Spain, the incidence is 4 per 100,000. In



African Americans, the incidence is 1 per 100,000, while in Caucasians, it is 25 per 100,000.<sup>3-8</sup>

One of the reasons for this worldwide incidence is that the level of exposure to ultraviolet radiation varies in different parts of the world, although the rate of skin cancer, especially basal cell carcinoma, is very high in Caucasian whites as well as non-whites. They have a risk of basal cell carcinoma.<sup>9</sup> Based on research conducted by Marwali et al, three provinces in Indonesia (East Java, Central Java, and North Sumatra) with a total population of 56,801,000 recorded 467 cases over 5 years. Overall each year, histologically confirmed basal cell carcinoma is 0.2 in women and 0.2 in men per 100,000. There were no significant differences between women and men.<sup>10-14</sup> Data from the Anatomical Pathology Laboratory, Dr. Kariadi General Hospital Semarang, the number of patients with basal cell carcinoma from January 2015 to June 2021 was 96 cases.

Basal cell carcinoma is a tumor with slow growth progression but can be aggressive depending on each subtype. Basal cell carcinoma rarely metastasizes. The main morbidity comes from lesions that are not treated properly and have a recurrence. Basal cell carcinoma most commonly occurs at an average age of 40 years. The National Comprehensive Cancer Network (NCCN) divides basal cell carcinoma into 2 subtypes, namely low risk, and high risk. Included in the low-risk criteria are nodular basal cell carcinoma, superficial basal cell carcinoma, pigmented basal cell carcinoma, infundibulocystic basal cell carcinoma, and fibroepithelial basal cell carcinoma. While those included in the high-risk criteria are basosquamous carcinoma, sclerosing/morphotic basal cell carcinoma, infiltrating basal cell carcinoma, basal cell carcinoma with sarcomatoid differentiation, and micronodular basal cell carcinoma.<sup>15</sup>

Basal cell carcinoma originates from pluripotent epithelial cells with p53 mutations. Some basal cell carcinomas occur due to activation of the “aberrant sonic hedgehog signaling” pathway, resulting in mutations in the PTCH.<sup>16</sup> The p53 gene is the gene that undergoes the most mutations due to sun exposure

and causes skin cancer, especially basal cell carcinoma.<sup>17-20</sup> p53 plays a role in the cell cycle and performs its functions by inhibiting the cell cycle, programmed cell death (apoptosis), and DNA repair. Overexpression of p53 can be stimulated by UV light exposure which causes DNA damage. When there is a mutation or damage to p53, it causes disturbances in the cell cycle, such as uncontrolled cell growth and inhibition of the DNA repair system.<sup>21,22</sup> These findings suggest that p53 expression can be used as marker analysis in cases of basal cell carcinoma.<sup>23,24</sup>

Several studies showed p53 expression in Basal Cell Carcinoma variants with different levels of aggressiveness. It was reported that p53 showed greater expression in the aggressive group. This study was also supported by Aupemkiete et al., who stated that there was a relationship between p53 expression and aggressive Basal Cell Carcinoma. It is also supported by research conducted by Zagrodnik et al. that p53 expression was used to assess recurrence after radiotherapy. This immunoreactivity has been reported to be associated with risk factors for sun exposure and the aging process. However, De Rosa et al. found no correlation between p53 immunoreactivity and patient age or lesion location. De Rosa and Barrett et al. found a significant relationship between clinicopathological aggressiveness and p53 immunoreactivity. On the other hand, Healy et al. reported that there was no significant difference in the percentage of reactivity between primary basal cell carcinomas that recurrence.<sup>25,26,27</sup>

Based on some of these studies, the relationship between the expression of the p53 protein and several factors of basal cell carcinoma is still unclear. Therefore, in this study, researchers wanted to find out the relationship between p53 expression and basal cell carcinoma low and high-risk subtypes associated with age, sex, and location of lesions.

## 2. Methods

The research design was an analytical observational study with a cross-sectional approach to examine the relationship between p53 protein

expression in paraffin block patients with basal cell carcinoma and age, gender, and lesion location profiles. A total of 31 research subjects participated in this study, where the subjects had met the inclusion criteria, namely having a paraffin block preparation for basal cell carcinoma and having complete medical record data. The research was conducted at the Anatomical Pathology section of Dr. Kariadi General Hospital Semarang, Indonesia. This study has been approved by the Health Research Ethics Committee of Dr. Kariadi Semarang General Hospital (No.754/EC/KEPK-RSDK/2021).

The expression of p53 protein was assessed quantitatively with a certain score. Cells were declared positive for p53 expression when the nucleus was stained with brown. Score 0, if only 0-5% positive cells; Score 1, if the cells are positive about 6-25%; Score 2, if the cells are positive about 26-50%; Score 3, if the cells are positive around 51-75%; Score 4, if the positive cell is more than 75%. Scores of 0 are considered negative, scores of 1 and 2 are considered low risk, and scores of 3 and 4 are considered high risk. Examination of p53 expression was carried out by immunohistochemical examination where the slide was rehydrated first with graded alcohol and xylene, then antigen retrieval was performed, and immunohistochemical staining was performed with p53 antibody. Determination of the degree of risk of basal cell carcinoma confirmed histopathological diagnosis by staining hematoxylin-eosin (H&E). Low risks include nodular, superficial, pigmented, infundibulocystic, and fibroepithelial basal cell carcinomas. High risks include basosquamous basal cell carcinoma, sclerosing/morphoeic, infiltrating, differentiated, and micronodular sarcomatoid.

Data analysis was carried out with the help of SPSS 25 software. First, a univariate analysis was carried out to present the frequency distribution of each research variable. Furthermore, the bivariate test with a chi-square test was carried out with a p-value < 0.05.

### 3. Results

Table 1 shows that most of the patients with Basal Cell Carcinoma (Basal Cell Carcinoma) at Dr. Kariadi General Hospital. Basal Cell Carcinoma patients were diagnosed at the age of 21-40 years (7.1%), 41-60 years (47.6%), >60 years (45.2%) with the overall mean age at diagnosis was 56.16 ± year. Data on p53 expression in Basal Cell Carcinoma in this study showed 1 person (2.4%) with negative expression and 31 people (73.8%). Based on these results, it is known that p53 expression in Basal Cell Carcinoma patients in this study was mostly weak positive expression. Data distribution of p53 expression in the high-risk Basal Cell Carcinoma subtype, which amounted to 12 people in this study, was 28.6%. The expression of p53 in low-risk Basal Cell Carcinoma subtypes, which amounted to 30 people in this study, was 71.4%. Patients with high-risk Basal Cell Carcinoma subtype were diagnosed at age 21-40 years 0(0%), 41-60 years 8(40%), >60 years 4(21.1%), while those with low-risk Basal Cell Carcinoma subtype were diagnosed at age 21-40 years 3(100%), age 41-60 years 12(60%), age >60 years 15(78.9%). Gender data obtained in this study were male (52.4%), and female (47.6%), with the most gender being male. Basal Cell Carcinoma subtype high risk in men 8(36,4%), Basal Cell Carcinoma low-risk subtype in men 14(63,5%). Basal cell carcinoma subtype is high risk in women 4(20%), low-risk basal cell carcinoma subtype 16(80%). Most of the Basal Cell Carcinoma patients at Dr. Kariadi General Hospital have nasal (28.6%), labium oris (11.9%), periauricular (7.1%), periorbital (23.8%), facial (28.6%) locations. In Basal Cell Carcinoma high risk subtypes are nasal 2(16,7%), periauricular 1(33,3%), periocular 5(50%), labium oris 1(20%), facial 3(25%). Basal Cell Carcinoma subtypes low nasal risk 10(83.3%), periauricular 2(66.7%), periocular 5(50%), labium oris 4(80%), and facial 9(75%).

Table 1. Characteristics of age, sex, lesion location, and their correlation with p53 expression in low-risk and high-risk basal cell carcinoma subtypes.

Clinical parameters	Amount (%)	Basal cell carcinoma			p53		P Sig.(2-tailed))	
		High-risk subtype	Low-risk subtype	(-) negative (%)	(+) low (%)	(+) high(%)	Low risk	High risk
Age							0.086*	0.121*
21-40 years	3(7.1%)	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0		
41-60 years	20(47.6%)	8 (40%)	12 (60%)	0 (0%)	16 (80%)	4 (20%)		
>60 years	19(45.2%)	4 (21.1%)	15 (78.9%)	1 (5 ,3%)	12 (63.2%)	6 (31.6%)		
Gender							0.158**	0.118**
Male	22(52.4%)	8 (36.4%)	14 (63.6%)	0 (0%)	18 (81.8%)	4 (18.2%)		
Female	20(47.6%)	4 (20%)	16 (80%)	1 (5%)	13 (65%)	6 (30 %)		
Location of lesion							0,776** *	0.988***
Nasal	12(28.6%)	2 (16.7%)	10 (83.3%)	0 (0%)	9 (75%)	3 (25%)		
Periauricular	3(7.1%)	1 (33.3%)	2 (66.7%)	0 (0%)	3 (100%)	0 (0%)		
Periorbital	10(23.8%)	5 (50%)	5 (50%)	0 (0%)	7 (70.7%)	3 (30%)		
Labium oris	5(11.9%)	1 (20%)	4 (80%)	0 (0%)	4 (80%)	1 (20%)		
Facial	12(28.6%)	3 (25%)	9 (75%)	1 (2.4%)	8 (66.7%)	3 (25%)		

\*,\*\*\*: Spearman correlation, \*\*Mann-Whitney test

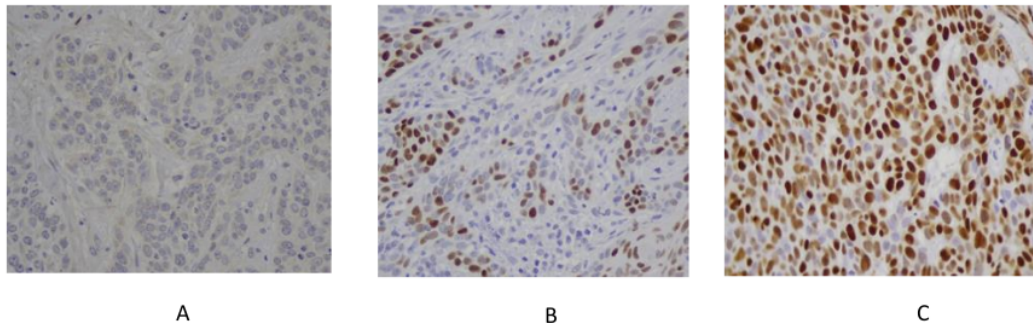


Figure 1. Expression of p53 in high-risk and high-risk BCC is low using a score of 0 = negative, a score of 1-2-low, and a score of 3-4 = high. A. Negative p53 expression in BCC. B. Expression of p53 with a low score on BCC. C. P53 expression with a high score on BCC.

Based on the Spearman's correlation test between the age of the patient and p53 expression in the total sample, the results were  $p = 0.390$ , the high-risk group got  $p = 0.121$ , and in the low-risk group, the results were  $p = 0.086$  because the  $p\text{-value} > 0.05$  so it can be concluded that there is no significant relationship between patient age and p53 expression in high-risk and low-risk Basal Cell Carcinoma. Based on the Mann-Whitney test between the sexes of patients with p53 expression in the total sample, the results were  $p = 0.576$ , the high-risk group got  $p = 0.118$  and the low-risk group got  $p = 0.158$  because the  $p\text{-value} > 0.05$  so it can be concluded that there is no there was a significant difference between the sex of patients with p53 expression in high-risk and low-risk Basal Cell Carcinoma. Based on the Spearman's correlation test between the location of patients with p53 expression in the total sample, the results were  $p = 0.817$ , the high-risk group obtained  $p = 0.877$ , and in the low-risk group, the results were  $p = 0.776$  because the  $p\text{-value} > 0.05$  so it can be concluded that there is no there is a significant relationship between patient location and p53 expression in high-risk and low-risk Basal Cell Carcinoma.

#### 4. Discussion

Our results are inconsistent with other studies,

which state that excessive exposure to ultraviolet light and age are associated with p53 expression in low-risk and high-risk Basal Cell Carcinoma subtypes. Another study stated that there was a correlation between age and p53 expression. Other studies contradict this study which stated that there was no correlation between age and p53 expression<sup>28,29</sup>. Our results are in line with studies that state that there is no significant correlation or relationship between p53 expression and age<sup>30</sup>. However, other studies have concluded that chronic sun-exposed skin areas cause keratinocytes to overexpress p53, also known as the epidermal p53 clone. The size and number of epidermal p53 clones are allegedly related to the age of the patient and the cumulative amount of exposure to ultraviolet light.<sup>31</sup>

Another study showed that there was no significant correlation between p53 expression in low-risk and high-risk Basal Cell Carcinoma subtypes with gender.<sup>32</sup> Similar studies on the expression of p53 and cyclin D1 in Basal Cell Carcinoma did not find a significant correlation between p53 and Cyclin D1 with sex.<sup>33</sup> Similar research to other studies which stated that there was no significant correlation between p53 expression and gender, as well as other studies that compared p53 expression in Basal Cell Carcinoma and Squamous Cell Carcinoma, from 28 samples, there was no significant correlation between p53 expression and gender.<sup>34</sup>



Although it has been known from several studies that excessive exposure to ultraviolet light is one of the etiological factors for the occurrence of Basal Cell Carcinoma, many research authors do not specifically mention whether there is a relationship between p53 expression and Basal Cell Carcinoma at certain locations of the body and whether there is a relationship as well. Exposed to or protected from ultraviolet light. This study focused more on whether locations in areas chronically exposed to ultraviolet light were associated with p53 expression. The results of this study showed that there was no significant correlation between p53 expression and certain locations on the body (nasal, periorbital, periauricular, labium oris, and facial). This study contradicts other studies, from 50 samples with lesion sites that were averagely exposed to ultraviolet light showed a significant correlation between tumor lesion sites chronically exposed to ultraviolet light and p53 expression in low-risk and high-risk Basal Cell Carcinoma subtypes.<sup>35</sup> However, this study is consistent with other studies, which state that there is no significant correlation between p53 expression and the location of tumor lesions. Another study also showed similar results that there was no significant correlation between p53 expression and the location of tumor lesions both exposed and protected from sunlight.<sup>36</sup>

## 5. Conclusion

There is no relationship between high and low-risk basal cell carcinoma subtype associated with age, gender, and lesion location.

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