

Overexpression and prognostic significance of p53 in Basal Cell Carcinoma, Squamous cell Carcinoma and Malignant Melanoma of the Skin

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ABSTRACT

Objective: To evaluate the immunohistochemical overexpression of p53 in basal cell carcinoma squamous cell carcinoma, collectively known as non-melanoma skin cancer and malignant melanoma of skin in the local population and determine its prognostic significance.

Methods: It was a descriptive cross-sectional study conducted in the Pathology Department of Pakistan Institute of Medical Sciences, Islamabad. All skin biopsies received in the pathology department and diagnosed as basal cell carcinoma, squamous cell carcinoma and malignant melanoma were included in the study. 55 skin cancers were included which comprises of 61.8% basal cell carcinoma, 25.5% squamous cell carcinoma and 12.7% malignant melanoma. p53 overexpression was evaluated by immunohistochemistry and the results were analyzed by using SPSS version 21. A p-value <0.05 was considered significant.

Results: p53 over expression was seen in 82.3% of basal cell carcinoma, 50% of squamous cell carcinoma while none was seen in malignant melanoma. (p value <0.001). 55.8% cases of basal cell carcinoma showed strong staining, 20.5% had moderate staining and 5.8% had weak staining pattern. Squamous cell carcinoma showed strong (57.1%) and moderate (42.8%) staining pattern. A significant correlation between p53 over expression and the tumor size of both basal and squamous cell carcinoma was observed (p value 0.001)

Conclusion: Majority of the squamous and basal cell carcinomas expressed p53 over expression with an important prognostic implication. Complete absence of p53 in malignant melanoma suggests another carcinogenic pathway not involving gene p53 mutation.

Key words: p53 over expression, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, skin

Introduction

Malignant cutaneous neoplasms incorporate a vast majority of histological types however the most common types being reported include malignant melanoma (MM) and basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).¹

A high incidence of these cancers is seen primarily in areas with an intense sunlight exposure particularly the regions at low altitude since its pathogenesis commonly involves ultraviolet rays induced damage which leads to cancer initiation and progression². These malignant neoplasms of the skin are fairly common in Caucasians due to their lighter skin tone.^{2,3} Pakistan belongs to the region of world with a high solar ultraviolet radiation exposure however the detrimental effect of these rays are lessened due to darker skin characteristics which provide protective effects against these radiations.³ Unfortunately, due to the absence of a nationwide cancer registry the incidence of skin cancers is lacking in Pakistan and has been reported at a variable rate in different regions of Pakistan. Meta-analysis done by Idrees et al found

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skin cancers to be amongst the 5 most common cancers in different studies of Pakistan.⁴ It is the 3rd most common cancer reported in *Khyber Pakhtunkhwa* province (previously known as N.W.F.P) of Pakistan with a rising trend.⁵ The rising trend of skin cancers is alarming and relates to the ozone layer depletion which has increased the ultraviolet radiation exposure. BCC is the most frequent morphological form of cutaneous malignancy reported in various studies. It arises from the basal layer of epidermis and is characterized by a slow growing nodular lesion usually on the sun exposed parts of the body.⁶ BCC is generally characterized by an indolent growth; however aggressive behavior may lead to cosmetic disfigurement and functional disabilities to the locally involved tissues. Although the associated risk of metastasis is low, but a high recurrence rate has been associated with the aggressive variants of BCC thereby leading to an increase financial burden on the patients and hospital.⁷

SCC arises from the malignant transformation of the basal cells but retaining its control to differentiate into epidermal keratinocytes. Since solar ultraviolet radiation (UVR) is a major risk factor involved in its initiation; this cancer type is also seen mostly on sun-exposed parts of the body, like face, dorsal side of hands and ears. Not only do they behave more aggressively when compared to BCCs, but their common anatomic location (face, ear, hands, face etc.) also portends them into the high-risk category for metastasis.⁸

Consistent with national cancer institute, MM is amongst the top five cancers with the highest incidence rates in United States and is increasing at a rate of 2.3%.⁹ They originate from the epidermal melanocytes and are commonly observed on the dorsal and ventral surface of palms and soles. Unlike BCC and SCC, MM is generally associated with intermittent sun exposure especially in skin areas with severe sun burns. UVR along with other risk factors as melanocytic nevi, family history and genetic susceptibility are responsible for the development of melanomas.¹⁰

With progressive depletion of stratospheric ozone layer, the exposure to UVR has substantially increased particularly the UVB rays. This has led to an increase risk of the skin cancer⁸. These radiations along with various factors including ionizing radiation, environmental carcinogens cause mutations in the tumor suppressor gene TP53, thereby leading to escape of the mutant cells from apoptosis. A high incidence of TP53 mutation has been reported to be

involved in the pathogenesis of BCC and SCC; melanoma however, have been found to have an aberrant MAPK pathway signaling, which in majority of the cases is due to BRAF mutation³.

Though, numerous studies have proclaimed p53 as a predictor of progression of premalignant conditions to malignant, limited information is available regarding the role of p53 as an aggressive behavior of tumor. We evaluated the expression and prognostic significance of p53 overexpression in BCC, SCC and MM in our local Pakistani population at tertiary care hospital. To date no study in Pakistan has reported these findings to the best of our knowledge.

Methods

This cross-sectional study was conducted in the Pathology department in collaboration with Dermatology and Plastic surgery department of Pakistan Institute of Medical Sciences (PIMS) Hospital, Islamabad, Pakistan from March 2016 to February 2017. After approval from hospital ethics committee, informed written consent was taken from all patients. Cases were selected by non-probability consecutive sampling technique. Skin biopsies (in 10% buffered formalin) received in the Pathology department of PIMS, diagnosed as BCC, SCC and MM on routine hematoxylin and eosin stain were included in the study. Patient's data along with hospital registration number, tumor size and site were entered in the Performa. Immunohistochemistry was used to evaluate the overexpression of p53 in the skin tumors. P53 overexpression was evaluated by examining the largest tumor area on the slide and the percentage of positive staining was calculated in 200 tumor cells nuclei.

A positive as well as negative control was used in each staining run. The nuclear reactivity was classified into 4 scores;

1. Score 1 is when p53 stained less than 1 % of the tumor cells
2. Score 2: more than 1% but less than 5% positive tumor cells
3. Score 3: 5-50% tumor cells positive for p53
4. Score 4: more than 50% cells positive.

Score 1 was taken as negative while score 2, 3 and 4 were positive with different staining intensity.

Using the statistical package for social sciences IBM SPSS software, (SPSS Version 21) statistical analysis was performed. The numerical variable like age was analyzed as mean and standard deviation. The

categorical variable like sex, site of specimen, H&E diagnosis, IHC score and p53 findings were calculated as frequency and percentages. Comparison between different variables was made by using chi-square test. A p-value <0.05 was considered significant statistically.

Results

A total number of 55 cases of skin cancers were received in the pathology department which comprises of 61.8% (n= 34) of BCC, 25.5% (n= 14) SCC and 12.7% (n= 7) MM. The mean age of patients was 61.6±16.5 years with an age range of 26-98 years. The mean age of the patients of BCC was 58.1±15.2 years; SCC was 66.3±19.3 years while MM was 69.1±13.8 years. There were only 5 cases of BCC which were less than 40 years of age while all the remaining patients (90.9%, n= 55) were more than 40 years old.

A slightly higher frequency was noted in male (56.4%, n= 31) than females (43.6%, n= 24) with a male to female ratio of 1.2:1, with a similar reflection in the individual cancer categories. The p value, although not significant was noted to be 0.26.

The most common site of involvement was nose (32.7%, n= 18), followed by cheek (18.2%, n= 10), lip (9.1%, n= 5) and scalp (7.3%, n= 4). BCC was predominantly seen in nose with a p value of 0.002 while SCC was mostly seen on cheek, forehead and lip. Melanoma had a very wide distribution and was observed in foot, forearm, scalp, and scapula. Distribution of cases according to site is shown in table 1.

Table 1: Distribution of cases according to site of tumor

Tumor Site	Histological diagnosis			Total
	BCC	SCC	MM	
Chest wall	0	1	0	1
Cheek	5	4	1	10
Ear	0	1	0	1
Eyelid	3	0	0	3
Foot	1	0	2	3
Forearm	1	0	1	2
Forehead	1	2	0	3
Leg	0	1	0	1
Lip	2	2	1	5
Nasolabial fold	1	0	0	1
Nose	17	1	0	18
Scalp	2	1	1	4
Scapula	0	0	1	1
Temple	1	1	0	2
Total	34	14	7	55

BCC (Basal cell carcinoma), SCC (Squamous cell carcinoma), MM (Malignant melanoma) p value was 0.002

P53 over expression was seen in 82.3% (n=28) cases of BCC, 50% (n=7) cases of SCC while no expression was seen in 100% cases of MM with a p value of 0.000 (fisher’s exact) as shown in figure 1.

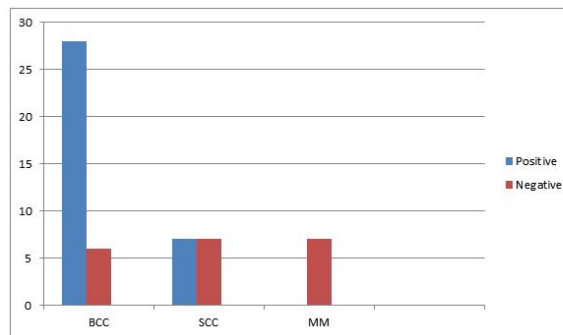


Figure 1: p53 over expression in BCC (Basal cell carcinoma), SCC (Squamous cell carcinoma), MM (Malignant melanoma) (p value 0.0001)

55.8% cases of BCC showed strong staining pattern of p53 while 20.5% had moderate staining and 5.8% had weak staining pattern. There were 6 cases of an aggressive variant of BCC however no difference in staining pattern was observed between aggressive and non-aggressive variants of BCC (p value 0.13). All positive cases of SCC showed strong (57.1%) and moderate (42.8%) staining pattern of p53 (figure 2).

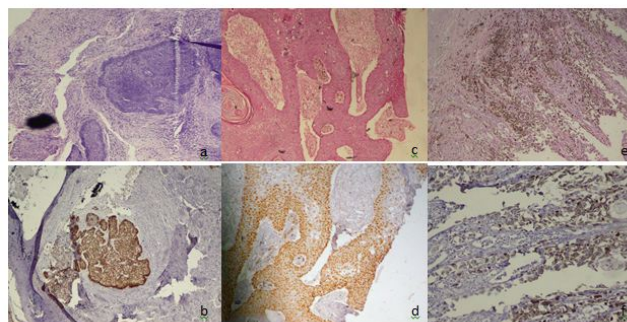


Figure 2: Basal cell carcinoma: H & E (a) and p53 immunohistochemical staining (b), Squamous cell carcinoma: H & E (c) and p53 immunohistochemical staining (d), Malignant Melanoma: H & E (e) and p53 immunohistochemical staining (f)

p53 over expression was correlated with the size of the tumor (NMSC) and a significant correlation was found between the two. Majority of the cases (76.9%) negative for p53 immunostain were less than 0.5 cm in size while the expression and intensity of p53 increased with tumors size (p value 0.001) as shown in figure 3.

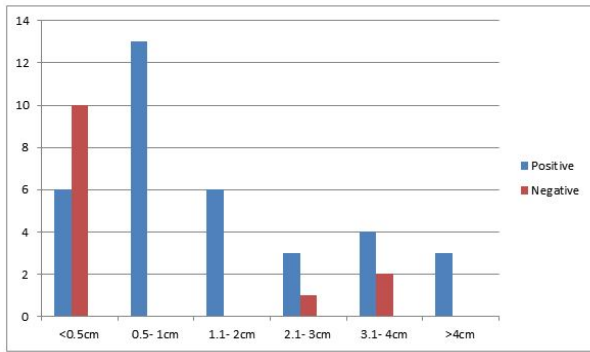


Figure 3: p53 over expression according to tumor size (p value 0.001)

Discussion

The geographical location of Pakistan near the equator is an important factor of a high sunlight exposure. With the progressive depletion of the ozone layer, the quantitative exposure to ultraviolet radiation has increased substantially especially the ultraviolet ray B and C, which has led to an increase in the genetic mutations thereby steering the precancerous skin damages towards skin cancers.³

We observed BCC as the most frequent skin cancers followed by SCC and MM in our study. Similar findings have been reported in multiple studies of Pakistan and also various studies in Asia and United States^{11, 12}.

The mean age of patients with skin cancer was 61.6±16.5 years however notable differences were seen in the mean age of presentation in different histological categories. BCC was frequently observed in the 6th decade while SCC and MM were in the 7th decade. Chinem and Miot have also reported that BCC has a younger age at presentation when compared with SCC.¹³ However, MM has been reported in a much younger age in contrast to our study.¹⁰ This might be due to the increase content of melanin in the Asian population which has photo protective and antioxidant effects against UVR.¹⁴

A slight male predominance in NMSC was found in the recent study which is attributed to a high sunlight exposure than females due to their occupation. The females generally in our population are housewives. Soomro FR et al studied cutaneous malignant tumors at Larkana and also reported a predominance of male involvement in NMSC. He found 47% of male patients were farmers and had more sun exposure which is a common factor in pathogenesis of NMSC.¹⁵ Malignant melanoma however showed a female predominance in the present study. Rastrelli et al studied the incidence

of melanoma in relation to the age and found a higher incidence in female as compared to males in patients younger than 40 years of age however the incidence raised by 3 times in males by 75 years of age.¹⁰ This is in contrast to our findings. Not only is there female dominance in the MM category but they were also commonly observed at an older age group. However since the cases in the present study are very few they might not be representative of whole population. More studies with large sample size need to be conducted to determine the gender distribution of MM in our population.

In present study, face was found to be the most common site of NMSC. This is comparable with results concluded by Jina A et al who also found face the most common site of NMSC¹⁶. Face is mostly involved because of direct exposure to sun lights either due to occupational purposes or lack of awareness of use of sun blocks while going out. While in case of malignant melanoma majority of cases were found at non sun-exposed sites. These results are similar to that provided by Bradford PT, who found that melanomas in Asians are more common at non-sun-exposed sites¹⁷. This is because in case of melanoma other risk factors like genetics, family history, skin, hair and eye color, and number of moles are factors that also play a role in development of melanoma.

Immunohistochemical analysis of p53 overexpression has been evaluated by various studies to demonstrate the aggressiveness of NMSC.¹⁸ Although the results are not consistent but its role as an early predictor of progression of premalignant conditions like actinic keratosis to invasive squamous cell carcinoma has been substantiated by multiple studies.¹⁹

P53 over expression was most frequently observed in BCC (p value <0.0001). This is in accordance with the findings observed by Adamkovet al who found 90.3% BCC overexpressed p53 whereas Khodaeiani et al established this percentage as 67.7%.^{18, 20} SCC showed a much lower p53 over expression (50%) than BCC. However, these results are comparable to other studies. Stratigos et al observed p53 protein immunoreactivity in 64.3% SCCs.²¹ Similarly Khodaeiani et al also found p53 over expression in 50.2% of SCCs.¹⁸ The observation of mutant p53 gene over expressed in the Pakistani population substantiates the role of UV radiations in the pathogenesis of NMSC.

P53 overexpression in MM has been reported in the range of 0-30% in various studies which is comparable to the present study where 0% cases of MM were

positive for p53 immunoreactivity.²² This confirms an alternate pathway involved in the initiation and progression of MM carcinogenesis.

A strong correlation was found between p53 positivity and the size of the tumor with a p value of 0.001; also intensity of p53 increased with the tumor size (p value 0.001). Bukhari et al studied the expression of Ki-67 and p53 mutated protein in the cutaneous squamous cell carcinoma and found significant correlation between the p53 staining and stage of the tumor.²³ Lima et al studied the p53 expression in different subtypes of basal cell carcinoma and found a significant expression in the infiltrative type of BCC which had a high relapse rate.²⁴ Although there is a low metastatic potential associated with NMSC but local aggressiveness and recurrence not only increases the morbidity rate but also financial burden on the patient and hospital. Tumor size and central facial location of BCC are important determinants of recurrence. p53 over expression leads to an increase tumor size which might be responsible for recurrences²⁵.

p53 overexpression is an important risk factor for assessment of not only tumor progression of cutaneous malignancies but also for tumor recurrence and aggressiveness. More studies are required with a large sample size in our population to further validate the above findings since Pakistan being in high sunlight exposure zone is expected a high incidence in future.

Conclusion

A large majority of basal and squamous cell carcinomas overexpressed p53 in contrast to MM. Also, p53 reactivity in of basal and squamous cell carcinomas identified aggressive nature of these cancers and can be used as a prognostic factor for recurrence of these lesions.

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