Frequency of P53 immunohistochemical expression in all histopathological types of basal cell carcinoma and its correlation with clinicopathological features

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Abstract: Background: Basal cell carcinoma is the most common malignant tumor in humans. Some immunohistochemical markers such as P53 gene mutation have been known in the pathogenesis. This study was done to investigate the **frequency** of immunohistochemical expression and intensity of P53 as a tumor marker and its correlation with other data such as age, sex and anatomical site of the tumor in all histopathological types. **Methods:** This cross sectional study was done on one hundred basal cell carcinoma specimens. At first the specimens were fixed by formalin, and stained by hematoxylin-eosin. Histopathological types of the tumor were determined. Immunohistochemical expression and intensity of P53 was investigated. Other data such as age, sex and anatomical site of the tumor were collected. **Results:** The specimens comprised 62 men (62%) and 38 women (38%), ages 22-107 years. Prevalence of P53 expression was 76%. The intensity of the P53 was strong in 70% and weak in 30% of cases. There was not a significant correlation between this marker and other variables (P> 0.05). **Conclusions:** In total we indicated that P53 mutation may possibly play a role in basal cell carcinoma. Our study does not support a correlation between expression of this marker with gender and anatomical lesion sites. In addition, there was not a significance association between the histopathological types based on the P53 expression and staining intensity of it.

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Key words: basal cell carcinoma; P53; expression; staining intensity

1. Introduction

Skin cancers are the most frequent malignancies in Europe and the USA. Non-melanoma skin cancers incidence appear to be increasing day by day. Basal cell carcinoma (BCC) is the most common skin cancer and considered about 80% of non-melanoma skin cancers (1,2). Increased rates of non-melanoma skin cancers are associated with sun exposed regions of fair-skinned persons, longevity, genetic factors, etcetera (3). Even though the potential for metastasis is low, but its local invasion, destruction and recurrence are well known (4,5). Based on histopathological features, BCC can be divided into two groups, undifferentiated and differentiated. Further classification according to growth patterns of the tumor has been recommended more than differentiation (6,7).

Genetic mutation can be change cells behavior and lead to tumoral cell and these changes usually occur in suppressor genes such as P53 (8). It is realized that the chronic exposure to sunlight can be responsible for P53 mutation in cutaneous carcinogenesis and plays an important role in the development of basal cell carcinoma (9,10). Various studies have reported that overexpression of the P53 protein in BCC (11-13). The first this immunoreactivity was attributed to be associated with sun exposure and an age-dependent process. However, in several studies did not find relationship between P53 immunoreactivity and age of patients or sites of the lesion (14,15). So the real relationship between P53 protein expression and the behavior of the BCC is not clearly yet. In this study, we determine frequency of P53 expression in all histopathological types of basal cell carcinoma and its correlation with clinicopathological features such as gender, age and histopathological variants.

2. Materials and Methods

2.1. Cases studied

This cross sectional study was done on one hundred archived skin specimens from BCC patients that referred to dermatology clinic of Farshchian hospital in Hamadan, west of Iran, from 2008 to 2009. Clinical features of the patients such as age, gender, lesion site were collected from their files. Hematoxylin-eosin stained sections from all

specimens prepared from formalin-fixed paraffin embedded blocks of each case were re-evaluated and confirmed by two expert pathologists through light microscope. Meanwhile types of the BCC were determined.

2.2. Immunohistochemistry

Detection of P53 protein expression was performed by immunohistochemical staining of BCC specimens as follows (14). Paraffin-embedded tissue was cut and a 3 mm thick section was deparaffinized and dehydrated. Antigen retrieval was done by boiling the sections in 0.01 M sodium citrate buffer pH 6.0 for 5 min in a microwave oven. The activity of endogenous peroxidase was blocked with 0.5% H2O2 for 10 min. Then the normal goat serum was used to suppress the binding of non-specific protein. Sequentially, the sections were incubated with Primary and secondary mouse monoclonal antibody P53 protein (Novocastra, Newcastle, UK, DO -7) and avidin biotin complex-horseradish peroxidase (ABC-HPR). Immunoreactivity was illustrated by 3,3 - Diaminobenzidine tetrahydrochloride (DAB) and the slides were counterstained with haematoxylin. In the experiment, a P53 strong positive control slide in every run of the staining procedure for reference was used.

2.3. Interpretation

Nuclear staining, presenting a positive result so the percentage of positively stained cells was recorded. The grade of expression of P53 protein was scored as 0: no staining, 1plus sign : staining in 1-25% of the cells, 2 plus sign : staining in 26-50% of the cells, 3 plus sign : staining in 51-75% of the cells and 4plus sign: 76-100% of the cells. Staining intensity was evaluated as 0: no staining, 1+: weak staining and 2+: strong staining.

2.4. Statistical Analysis

Data collected analyzed by SPSS software. Two types of statistics were done:

- Descriptive statistics: number, percentage and mean ± standard deviation (SD).
- Analytical statistics: determinant of difference in proportion applying by t-test, chi-square or fisher exact test when appropriate. The P-value was considered significant when it was less than 0.05.

3. Results

3.1. Clinical and pathologic data

One hundred archived skin specimens from BCC patients were used in this study. The clinicopathological characteristics of basal cell carcinoma are shown in Table 1. The specimens comprised 62 men (62%) and 38 women (38%), ages 22-107 years (mean ± SD: 63.97 ± 14.36).

Table 1. Clinicopathological characteristics of the specimens

Variables	Cases
Age (mean ± SD, range)	63.97 ± 14.36 (22-107) years
Sex	
Male	62
Female	38
Sites	
Sun-exposed	
Face	40
Scalp	15
Neck	2
Nose	27
Ears	7
Sun-protected	
Trunk	7
Extremities	2

Most of the lesions were located on the areas that exposure to the sun, especially the face. Cases studied in terms of histopathological types were classified in one of the groups defined by 10-fold (16). Based on this nodular and micronodular were the most types. Also according to the pathologic types behavior (recurrence or aggressive), the specimens were divided in two groups : high risk (morphoeic, micronodular, superficial and metatypical) and low risk (other types) (16) (Table 2). Results showed that 44% and 56% of cases are in the high risk and low risk groups, respectively. On the other side there was not a significant association between the tumors with high and low risk groups and exposure to the sun (P = 0.34). So that among the 44 cases in the high risk group 40 cases were in sun exposed and out of 56 of low risk group 51 cases were exposure to the sun. Distribution of histopathological types based on the site lesions summarized in Table 3.

Table 2. Histopathological types of the tumor

Histopathological types	Cases
High risk	
Micronodular	16
Morphoeic	6
Superficial	6
Metatypical	1
Low risk	
Nodular	19
Nodular & Micronodular	15
Pigmented	15
Cystic	14
Keratotic	5
Adenoid	3

Table 3. Distribution of histopathological types based on the site lesions

Site of lesion Pathologic type	Face	Scalp	Neck	Nose	Ears	Trunk	Extremities
Nodular	8	0	0	10	0	1	0
Micronodular	10	1	0	2	1	1	1
Morphoeic	2	0	0	4	0	0	0
Nodular & Micronodular	6	3	0	4	1	1	0
Superficial	1	4	0	0	0	1	0
Metatypical	0	0	0	1	0	0	0
Pigmented	6	1	1	3	2	2	0
Cystic	4	5	1	2	1	1	0
Keratotic	3	1	0	0	1	0	0
Adenoid	0	0	0	1	1	0	1
Total	40	15	2	27	7	7	2

3.2. Immunohistochemistry Data

P53 expression was present in 76 (76%) of BCC cases. Immunohistochemical findings are summarized in Table 4. Statistical analyze with chi-square test did not show a significance association between high or low risk groups with expression of P53 and staining intensity (P = 0.66, P = 0.44) (Figure 1 and 2).

Table 4. Percentage expression and staining intensity of P53 expression in the specimens

Immunohistochemical	Cases	Percent
P53 expression grade		
Negative (0%)	24	24%
1+ (1-25%)	9	9%
2+ (26-50%)	7	7%
3+ (51-75%)	19	19%
4+ (76-100%)	41	41%
P53 staining intensity grade		
1+ (weak)	23	30%
2+ (strong)	53	70%

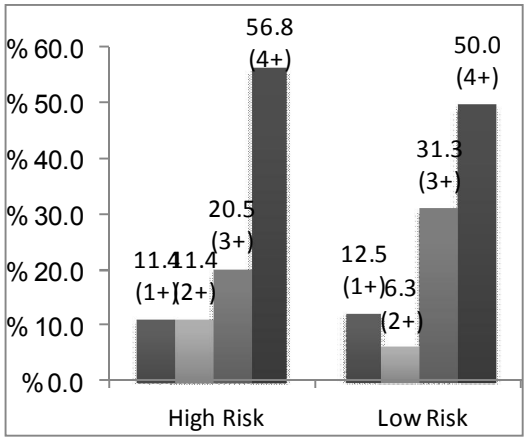

Figure 1. Frequency of P53 expression based on the high and low risk groups.

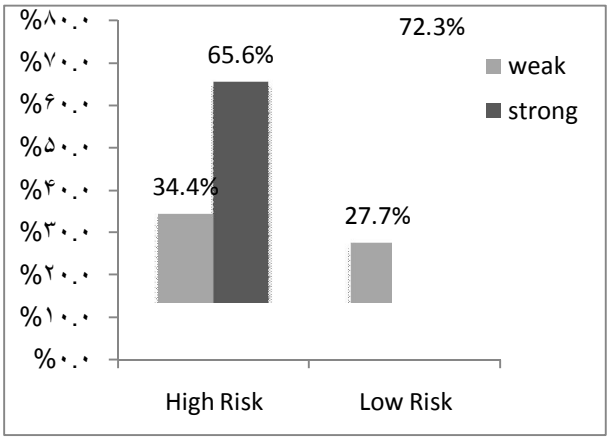

Figure 2. Frequency of P53 staining intensity based on the high and low risk groups.

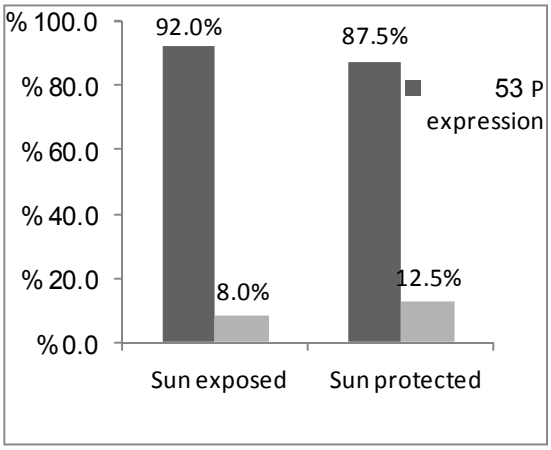

Figure.3 Frequency of P53 expression based on the sun exposed or sun protected anatomical sites.

The relationship between P53 expression and staining intensity with tumor covered or exposed to the sun was examined and did not indicate a significance correlation (P = 1.00 , P = 0.66) (Figure 3 and 4). About the association between marker

expression and age the mean age of the specimens that expressed P53 were 63.99 ± 13.61 and the specimens that did not express this marker were 63.92 ± 13.83 (P = 0.98). Also this correlation was evaluated about staining intensity and the mean age of specimens that expressed strong and weak were 65.26 ± 13.97 and 61.04 ± 12.54 respectively(P = 0.21).

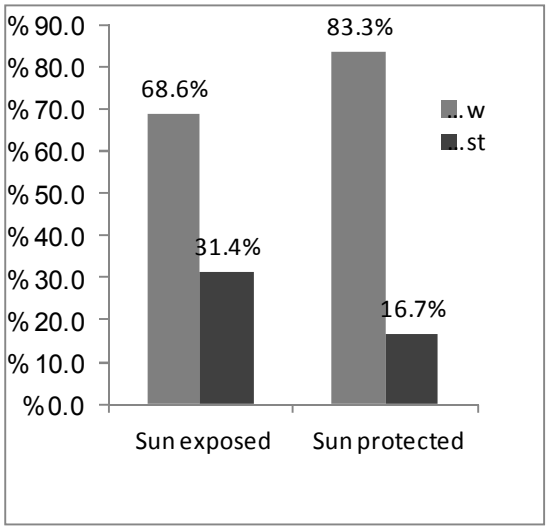

Figure.4 Frequency of P53 staining intensity based on the sun exposed or sun protected anatomical sites.

4. Discussion

BCC is a disease that multiple factors are involved in appearing it. As far as both environmental and host genetic factors are implicated in carcinogenesis. Oncogenic potential may increase by mutation of the P53 gene in a loss of tumor suppressor activity. So according to the importance of BCC and mutation of P53 in skin tumors and no similar studies in Iran we investigated the immunohistochemical expression and intensity of P53 as a tumor marker and its correlation with other data such as age, gender and anatomical site of the tumor in all histopathological types of basal cell carcinoma.

In this cross sectional study one hundred archived skin specimens from BCC patients were used. The specimens comprised 62 men (62%) and 38 women (38%), ages 22-107 years (mean ± SD: 63.97 ± 14.36). This can be indicate that the men more than the women are in risk of this tumor. It can due that the men are more exposure to the sun than the women for job reasons. Different studies confirm this finding (13,17,18) but not approved by all studies (19). However this increasing of the likelihood is not enough that the gender introduce a risk factor for BCC. Basal cell carcinoma occurs after the fourth decade typically (20). Increased incidence of BCC rises with increasing age. Concerning anatomical of site lesions, face (40%), nose (27%) and scalp (15%) were the most conflict sites that are exposed to the sun more than all. Previous studies emphasized the role of UV and increased the risk of the sites that are exposed to the sunlight (8,19,21,22).

In this study P53 protein expressed in 76 (76%) of BCC cases. Out of these 76 cases, 9 cases (11.8%) were expressed 1plus sign, 7 cases (9.2%) 2 plus sign, 19 cases (25%) 3 plus sign and 41 cases (54%) 4 plus sign. Among these 76 cases, 23 cases (30%) had weak staining intensity and 53 cases (70%) were with strong staining intensity. In different studies P53 protein overexpressed in the rang 42-90 % (23,24). Karagece Yalçin et al in a study in 2012 observed that P53 expressed in 98% of BCC specimens (25). Also Mateoiu et al reported strong expressed of P53 in the studied tumors (26). Our results are consistent with different studies about P53 expression.

In the present study we evaluated the frequency of P53 expression in all histopathological types of BCC based on the high and low risk groups. Our results reveal that among the 44 cases in high risk group, 32 cases (72%) and out of 56 cases with low risk histopathological types, 44 cases (78%) expressed P53. In high risk group, 65.6% had a strong expression and in low risk group there was 72.3% with strong expression intensity of P53. According to our results was not found significance association between P53 expression and staining intensity with histopathological types. Examination these correlations were done in few studies. Several studies emphasized our results (27). Unlike our findings Ansarin, De Rosa and Auepemkiate reported that P53 expression is related to the histopathological feature (13,14,28). These conflicts in results can be caused by different used technique, sample size, case selection and multi-factorial pathogenesis of BCC. Two important factors that surveyed were the association of P53 expression and its intensity with exposure to the sun and age. In none of the above there was not a significant correlation. In the studies that were done by De Rosa, Auepemkiate and Bolshakov were obtained the similar results (14,28,29). Despite the mentioned studies in several researches there was a strong linear correlation between P53 expression and exposure to the sun (30,31). A reason for these various results can be that P53 gene do not affect just by UV. In fact other factors such as smoking, exposure to free radicals and arsenic are the other effective factors. Some limitations of the study should be noted. Our study was done on one hundred BCC patients. If this study be performed in various skin tumors and with a large sample size to comparison different ranges of age can

be achieved to more accurate results. Furthermore, using an immunohistochemical technique in evaluating P53 expression may not reveal the actual molecular event. Therefore more molecular analysis is required. Meanwhile other contributing factors that involve in the pathogenesis of BCC can be useful. In conclusion, our findings indicate that P53 mutation may possibly play a role in basal cell carcinoma. Our study does not support a correlation between expression of this marker with gender, age and anatomical lesion sites. In addition, there was not a significance difference between the high and low risk groups based on the P53 expression and staining intensity of it.

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Conflict of interest
We declare that we have no conflict of interest.

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Running title: Immunohistochemical expression of P53 in basal cell carcinoma

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