Comparison of COX2 expression in radiation induced basal cell carcinoma and non-radiation induced basal cell carcinoma

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INTRODUCTION

Basal cell carcinoma (BCC) has attracted considerable attention as the most common skin malignancy in human 1. The natural course of the majority of BCC varieties is slow and does not cause mortality, but it is remarkable due to chronicity and incapacitation of the patient. A history of radiotherapy in childhood is one of the known risk factors for the occurrence of BCC 2. Radiation-induced BCC is often multiple, large, and recurrent that often requires extensive excision and graft, leaving deformity or chronic ulcer in some cases 3. On the other hand, radiodermatitis due to a previous radiotherapy reduces the successful regeneration rate of the surgery site with graft and flap; therefore, the search for non-surgical treatments to prevent relapse or occurrence of BCC in those with a history of radiotherapy seems to be logical. COX2 is considered as a factor in the...
reurrence of BCC. El-Khalawany et al. concluded that COX$_2$ overexpression is a risk factor for BCC relapse. Tjiu et al. showed that in human BCC samples, high levels of COX$_2$ were not only associated with neovascularization but also with the depth of tumor invasion and they stated that the tumor-associated macrophages might activate COX$_2$ in BCC cells and thus enhance the invasion and angiogenesis. It was shown that the inhibition of PGE2 production by COX$_2$ inhibitors and NSAIDs somewhat inhibits UV-associated carcinogenesis. Vogel stated that COX$_2$ expression affects the risk of BCC development. Karahan showed that COX$_2$ expression might be associated with local invasion and recurrence of BCC. The reduced induction of skin carcinoma or papilloma by UVB has been demonstrated following feeding the mice with Celexib or Indomethacin. Tang et al. have shown that oral Celexib reduces carcinogenesis in PTCH-/+ mice and it has also a considerable impact against BCC in human subjects with nevoid basal cell carcinoma syndrome. Considering the fact that COX$_2$ expression in radiation-induced basal cell carcinoma has not been investigated so far, we tried to evaluate and compare the expression of COX$_2$ by immunohistochemistry in radiation-induced basal cell carcinoma with BCC due to other factors.

MATERIAL AND METHODS

In this cross-sectional study, 86 paraffin-embedded basal cell carcinoma samples (43 blocks from patients with a history of radiation therapy and 43 blocks from those without radiotherapy history) were extracted from the archives of Department of Pathology, Imam Reza Hospital, Mashhad University of Medical Sciences, and their histopathology was re-examined by a dermatopathologist. Then, demographic characteristics of patients, including age, sex, clinical type, relapse, and radiotherapy history were registered in the questionnaires and all the patients were contacted by the phone call to ensure their history of radiotherapy. Inclusion criterion was a definite diagnosis of basal cell carcinoma in pathological examination and exclusion criteria were incomplete patient records, lack of or insufficient tissue in paraffin-embedded blocks. Finally, COX-2 expression was evaluated by immunohistochemistry using anti-COX$_2$ antibodies on paraffin blocks and COX$_2$ was semi-quantitatively analyzed. In terms of staining percentage of cells (P), the samples were divided to five groups (including less than 1%, 1-25%, 25-50%, 51-75%, 76-100%) and were divided into four groups (negative, weak, moderate, severe) according to the staining intensity (I). The score ranges of 0-4 and 0-3 were attributed to percentage of staining and staining intensity groups, respectively. Then, for each sample, the scores of percentage and intensity of staining were summed up and the resulting figure represented the COX$_2$ expression score. The samples with a score above 2 are considered positive and those with a score above 4 are considered strongly positive. Accordingly, based on COX$_2$ score, the samples were divided into three groups: group 1 with a score of (0-2), group 2 with a score of (3-4), and group 3 with a score of (5-7). Evaluation of the stained slides was conducted by two pathologists and in cases of disagreement between them, the slides were simultaneously assessed by them using a binocular microscope to resolve the problem. COX$_2$ immunohistochemical staining kit (Novocastra, England) was used to detect COX$_2$ marker. The stained slides were assessed under a light microscope (Nikon, Japan) with 100× and 400× magnification. The accuracy of staining was ensured via comparison with positive and negative control samples, COX$_2$ intensity and percentage was assessed in 10 fields with 100× and 400× magnification, respectively, and the average staining level was assessed and expressed as percentage of staining. To describe the data, diagrams, and statistical tables, SPSS statistical software version 16 was used and chi-square test, t-test or its non-parametric equivalent, as well as Mann-Whitney and Kruskal-Wallis tests were used for statistical analysis.

RESULTS

Eighty-six patients with basal cell carcinoma were enrolled in this study that were divided into two groups of 43 patients with and without a history of radiotherapy. Fourteen patients (32.6%) with a history of radiotherapy and 19 patients (44.2%) without a history of radiotherapy were women and 29 patients (67.4%) with a history of radiotherapy
and 24 patients (8.55%) without a history of radiotherapy were men. Chi-square test showed no statistically significant difference between the history of radiotherapy and gender (P=0.3).

Majority of the patients under study were within the age group of 60-69 years, including 32 patients (37.2%) and the age group of 40-49 years had the lowest frequency with 11 patients (12.8%). Majority of the patients with a history of radiotherapy were within the age group of 60-69 years and the lowest number of these patients was within the age group of 40-49 years. Mean age of patients was 61.5 years with SD of 1.006 and median of 60. Maximum and minimum age of the patients was 40 and 91 years, respectively. Statistical analysis by t-test showed no significant correlation between age and history of radiotherapy (P=0.3). There were six cases of relapse among which five patients (11.6%) had a history of radiotherapy and 88.4% of patients with a history of radiotherapy did not mention their history of recurrent lesions. Chi-square test indicated no relationship between relapse and history of radiotherapy (P=0.2).

In patients without a history of radiotherapy, most of lesions were on face (52.3%) and the least on the neck (4.7%). In total, 37 lesions (43%) were on the head, 45 (52.3%) on face, and 4 (4.7%) on the neck. In cases with a history of radiotherapy, 30 patients (69.8%) had lesions on head, 10 (23.3%) on face, and 3 (7%) on the neck. Statistical analysis by chi-square test indicated a significant relationship between location of lesion with a history of radiotherapy (P<0.001) and scalp was a common site for radiation-induced BCC.

Out of 86 samples under study, 38 cases (44.2%) were solid, 16 (18.6%) infiltrative, 24 (27.9%) mix (solid+ pigmented/solid+ adenoid/infiltrative+ adenoid) pathology subtypes and 8 (9.3%) were related to other subtypes (superficial /micronodular /morphemic /adenoids). In total, there was a higher frequency of solid pathology subtype.

In 43 samples of patients with a history of radiotherapy, 20 cases (46.5%) were of solid type, 6 cases (14%) of the infiltrative type, 10 cases (23.3%) of mix type and 7 cases (16.3%) of other types.

The frequency of solid pathology subtype was higher among the samples of patients with and without a history of radiotherapy. Statistical analysis by chi-square test showed no significant relationship between pathology subtype and a history of radiotherapy (P=0.09).

In assessment of COX2 expression score in the two groups with and without a history of radiotherapy, based on the results of Table 1 and using Mann-Whitney test, score intensity of COX2 expression in radiation-induced BCC was considerably higher than the group without such history (P<0.001).

There was no correlation between COX2 expression intensity in basal cell carcinoma samples and gender of patients in Mann-Whitney test (P=0.68). In addition, Kruskal-Wallis test showed no correlation between the intensity of COX2 expression score in BCC samples, age of patients (P=0.22), pathology subtypes (P=0.7), and tumor location (P=0.18).

**DISCUSSION**

BCC is the most common skin cancer in human and the chronicity and incapacitation of patients with BCC causes significant morbidity, but the normal course of the majority of its forms is slow with no mortality. History of childhood radiotherapy is among the most well known risk factors for BCC. The first reports on a possible role of ionizing radiation in the development of non-melanoma skin cancers (NMSC) were related to the incidence of these cancers on the hands of radiology technicians working without protection. Increased NMSC has been observed among the **Table 1. Distribution of subjects based on the staining score of tumor cells (considering the score intensity) and a history of radiotherapy.**

<table>
<thead>
<tr>
<th>COX2 expression score</th>
<th>History of radiotherapy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Percent</td>
</tr>
<tr>
<td>(0-2)*</td>
<td>8</td>
<td>18.6%</td>
</tr>
<tr>
<td>(3-4)*</td>
<td>12</td>
<td>27.9%</td>
</tr>
<tr>
<td>(5-7)**</td>
<td>23</td>
<td>53.5%</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Mann-Whitney test result: z score= 3.91         P-value=0.001
workers in uranium mines, radiologists, and those with a history of radiotherapy in childhood for treatment of Tinea capitis. There was also a significant increase in this type of cancer after atomic bombing of Hiroshima and Nagasaki. The number of BCC lesions in patients with a history of radiotherapy was higher compared to the group without a radiotherapy history in the study of Meibodi et al. In the prospective study by Karagas et al. for comparison of two groups with and without a history of radiotherapy (not necessarily because of Tinea capitis), BCC incidence was significantly higher in the group with a history of radiotherapy. In the study of Maalej and colleagues on 98 patients with a history of radiotherapy in childhood who had tumors in the irradiated area, it was concluded that BCC was the most common tumor that occurred in radiodermatitis sites. Radiation-induced BCC is often multiple and recurrent and due to its large size often requires extensive excision and graft, leaving deformity or chronic ulcer for patient in some cases. On the other hand, radiodermatitis induced by a previous radiotherapy reduces the successful repairing of surgery site with graft and flap; therefore, it appears logical to find non-surgical treatments to prevent relapse or BCC occurrence in those with a history of radiotherapy. COX2 is a factor considered involved in the recurrence of BCC.

Cyclooxygenase (COX) is an enzyme responsible for biosynthesis of prostaglandins (including prostaglandins, prostacyclin and thromboxane) that are among the most important chemical mediators in the body. At present, three isoenzymes of COX, including COX1, COX2, COX3, have been identified. COX1 is expressed in many tissues and plays different physiological roles whereas the overexpression of COX2 occurs in several types of epithelial tumors. COX2 is a rate-limiting enzyme in the biosynthesis of prostaglandins from arachidonic acid and the expression of its gene is increased by various stimuli like mitogens, cytokines, growth factors, and tumor promoters. It has been implicated in the development of several types of tumors.

Recent studies have indicated the relationship between COX2 with invasion induction, apoptosis suppression, cellular immune response suppression, and tumor angiogenesis. COX2 production after UV exposure contributes to epidermal hyperplasia, edema, and inflammation and inhibits UV-induced apoptosis. Inhibition of COX2 activity or reduced expression of it in mice with deleted genes leads to a significant reduction in UV-dependent carcinogenicity; while leading to COX2 overexpression in transgenic mice increases the UV-dependent tumor growth. COX2 expression level in some tumors corresponds with tumor aggressiveness and prognosis, suggesting an important role of COX2 in tumor development and progression. COX2 can be found in normal skin, benign proliferations, and malignant cutaneous neoplasms. UVB radiation affects keratinocytes and increases prostaglandin E production and COX2 expression in them. Studies showed that BCC is positive in a small percentage of biopsies studied for COX2, the expression of which was consistent with angiogenesis in BCC.

In our study, COX2 expression score was significantly higher in tumor cells of patients with a history of radiotherapy than those without a history of radiotherapy (P<0.001). There was no correlation between COX2 expression score with gender and age of patients, site of lesion, relapse history, and tumor pathology subtype. In the study of El-Khalawany et al. in 2013, to evaluate the predictive markers for recurrence of BCC, COX2 expression was significantly different in 20 out of 22 samples of recurrent BCC (90.9%) compared to 14 cases (59.1%) out of 22 BCC cases without relapse (P=0.04). Moderate to high intensity was observed in 13 cases of recurrence and 2 cases without tumor recurrence and it was concluded that the overexpression of COX2 can be used as a risk factor of relapse in addition to other clinical and histological factors of BCC.

According to the study of El-Khalawany, this biomarker has a promising role in prognosis assessment of BCC and early detection of recurrence, as well as a high expression level of COX2 is a risk factor for BCC relapse. In the study of Karahan, COX2 expression in primary BCC group of infiltrative type was significantly higher than superficial and nodular types and in the recurrent BCC type, COX2 expression was significantly higher than primary BCCs (P=0.013). It was stated that COX2 expression may be associated with local invasion and recurrence in BCC and COX2 inhibition can be an adjunctive therapy, especially in recurrent tumors with a high COX2 expression.
However, in our study, no relationship was found between COX\textsubscript{2} expressions with recurrence of lesions, which may be due to low number of relapse samples in this experiment. There was no correlation between COX\textsubscript{2} expressions with pathology subtypes of tumors.

Reduction in UVB-induced skin papilloma or carcinoma has been observed following feeding of mice with Celecoxib or indomethacin. Topical use of Celecoxib also inhibits chronic inflammation and UVB-induced carcinoma in mice\textsuperscript{11}. More importantly, interrupting the COX\textsubscript{2} signaling is an effective strategy for preventive treatment of non-melanocytic skin cancers, especially in people with a high risk of developing these cancers. However, any potential benefit of these drugs should be contrasted with their known adverse events (e.g. cardiovascular and gastrointestinal complications) for each patient. Topical NSAIDs are effective to prevent sunburn reactions such as redness of the skin. In five out of six studies on the use of topical Diclofenac, as a non-specific inhibitor of COX having a more prominent effect on COX\textsubscript{2} relative to COX\textsubscript{1}, there has been significant impact with respect to the improvement of precancerous lesions (actinic keratosis) due to apoptosis. Currently, Diclofenac gel has been approved for topical treatment of actinic keratosis in USA and Europe. In contrast, the use of oral Celecoxib (a specific inhibitor of COX\textsubscript{2}) is effective to prevent SCC and BCC but it has no effect on actinic keratosis\textsuperscript{24}.

Preventive topical treatment by green tea extract (1mg/cm\textsuperscript{2}) widely inhibits acute COX\textsubscript{2} response to UVB in mice and humans\textsuperscript{15}. Tang et al. showed the effects of oral Celecoxib in PTC\textsubscript{H1}+/− mice, as well as its effect against BCC in patients with nevus basal cell carcinoma syndrome\textsuperscript{12}.

CONCLUSION

Radiation-induced BCC is often multiple and recurrent and given the overexpression of COX\textsubscript{2} in BCC lesions caused by radiotherapy, COX\textsubscript{2} inhibitor drugs such as Celecoxib may play a role in the prevention of BCC or its recurrence in patients with a history of radiotherapy, which requires a clinical trial. We also proposed another study on role of COX\textsubscript{2} in the pathogenesis of radiation induced basal cell carcinoma.

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Conflict of Interest: None declared.

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