Comparison of COX_2 expression in radiation induced basal cell carcinoma and non-radiation induced basal cell carcinoma

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Background: Radiation-induced basal cell carcinoma (BCC) can be multiple, large, and recurring, which complicates its treatment in some cases. According to reports on the role of cyclooxygenase 2 (COX_2) inhibitors in the treatment or prevention of non-melanoma skin cancers and considering the fact that COX_2 expression has not been evaluated in radiation-induced basal cell carcinoma, we set out to assess the expression of COX_2 in these lesions.

Methods: In this study, COX_2 expression was assessed by immunohistochemistry using anti-COX_2 antibody on paraffin-embedded blocks of 86 patients referred to Emam Reza Hospital in Mashhad with BCC diagnosis by pathological examination (43 patients with and 43 without a history of radiotherapy) followed by semi-quantitative evaluation of COX_2.

Results: In our study, COX_2 expression score was significantly higher in patients with a history of radiotherapy than those without radiotherapy ($P<0.001$). No correlation was found between the intensity and percentage of staining with sex, age, site of lesion, recurrence, and pathology of the tumor.

Conclusion: Given the higher expression level of COX_2 in the radiation-induced BCC patients, the use of COX_2 inhibitors in these individuals may be effective in the incidence, recurrence, or treatment of BCC.

Keywords: basal cell carcinoma, cyclooxygenase 2, immunohistochemistry, radiotherapy

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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. COX_2 is considered as a factor in the

recurrence of BCC. El-Khalawany *et al.* concluded that COX_2 overexpression is a risk factor for BCC relapse [4]. 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 [5,6]. It was shown that the inhibition of PGE2 production by COX_2 inhibitors and NSAIDs somewhat inhibits UV-associated carcinogenesis [7,8]. Vogel stated that COX_2 expression affects the risk of BCC development [9]. Karahan showed that COX_2 expression might be associated with local invasion and recurrence of BCC [10]. The reduced induction of skin carcinoma or papilloma by UVB has been demonstrated following feeding the mice with Celexib or Indomethacin [11]. 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 [12]. 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 (Nickon, 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 COX_2 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 COX_2 expression in radiation-induced BCC was considerably higher than the group without such history (P<0.001).

There was no correlation between COX_2 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 COX_2 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.

COX_2 expression score	History of radiotherapy				Total	
	Positive		Negative			
	Number	Percent	Number	Percent	Number	Percent
$(0\text{-}2)^-$	8	18.6	21	48.8	29	33.7
$(3\text{-}4)^+$	12	27.9	16	37.2	28	32.6
$(5\text{-}7)^{++}$	23	53.5	6	14.0	29	33.7
Total	43	100.0	43	100.0	86	100.0

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 [2]. 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.* [3]. 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 [2]. 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 [13]. Radiation-induced BCC is often multiple and recurrent and due to its large size 3 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. COX_2 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 COX_1, COX_2, COX_3, have been identified [14]. COX_1 is expressed in many tissues and plays different physiological roles whereas the overexpression of COX_2 occurs in several types of epithelial tumors [15]. COX_2 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 [16].

Recent studies have indicated the relationship between COX_2 with invasion induction [17], apoptosis suppression [18], cellular immune response suppression, and tumor angiogenesis [19]. COX_2 production after UV exposure contributes to epidermal hyperplasia, edema, and inflammation and inhibits UV-induced apoptosis. Inhibition of COX_2 activity or reduced expression of it in mice with deleted genes leads to a significant reduction in UV-dependent carcinogenicity; while leading to COX_2 overexpression in transgenic mice increases the UV-dependent tumor growth [20]. COX_2 expression level in some tumors corresponds with tumor aggressiveness and prognosis, suggesting an important role of COX_2 in tumor development and progression [16]. COX_2 can be found in normal skin, benign proliferations, and malignant cutaneous neoplasms. UVB radiation affects keratinocytes and increases prostaglandin E production and COX_2 expression in them [21]. Studies showed that BCC is positive in a small percentage of biopsies studied for COX_2, the expression of which was consistent with angiogenesis in BCC [22,23].

In our study, COX_2 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 COX_2 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, COX_2 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 COX_2 can be used as a risk factor of relapse in addition to other clinical and histological factors of BCC [4].

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 COX_2 is a risk factor for BCC relapse [4]. In the study of Karahan, COX_2 expression in primary BCC group of infiltrative type was significantly higher than superficial and nodular types and in the recurrent BCC type, COX_2 expression was significantly higher than primary BCCs ($P=0.013$). It was stated that COX_2 expression may be associated with local invasion and recurrence in BCC and COX_2 inhibition can be an adjunctive therapy, especially in recurrent tumors with a high COX_2 expression [10].

However, in our study, no relationship was found between COX_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_2 expressions with pathology subtypes of tumors.

Reduction in UVB-induced skin papilloma or carcinoma has been observed following feeding of mice with Celexib or indomethacin. Topical use of Celexib also inhibits chronic inflammation and UVB-induced carcinoma in mice [11]. More importantly, interrupting the COX_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_2 relative to COX_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 Celexib (a specific inhibitor of COX_2) is effective to prevent SCC and BCC but it has no effect on actinic keratosis [24].

Preventive topical treatment by green tea extract ($1mg/cm^2$) widely inhibits acute COX_2 response to UVB in mice and humans [15]. Tang *et al*. showed the effects of oral Celexib in PTCH1+/- mice, as well as its effect against BCC in patients with nevoid basal cell carcinoma syndrome [12].

CONCLUSION

Radiation-induced BCC is often multiple and recurrent and given the overexpression of COX_2 in BCC lesions caused by radiotherapy, COX_2 inhibitor drugs such as Celexib 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_2 in the pathogenesis of radiation induced basal cell carcinoma.

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REFERENCES

1. Roasch BA, Buettner PG, Garbe C. Basal cell carcinoma: histological classification and body site distribution. Br J Dermatol. 2006;155:401-7.
2. Karagas MR, Mc Donald JA, Greenberg ER, et al. Risk of basal cell and squamous cell skin cancers after ionizing radiation therapy. J Natt Inst. 1996;88:1848-53.
3. Meibodi NT, Maleki M, Javidi Z, et al. Clinicopathologic evaluation of radiation induced basal cell carcinoma. Indian J Dermatol. 2008;53:137–139.
4. El-Khalawany MA, Abou-Bakr AA. Role of cyclooxygenase-2, Ezrin and matrix metalloproteinase-9 as predictive markers for recurrence of basal cell carcinoma. J Cancer Res Ther. 2013;9:613-7.
5. Tjiu JW, Liao YH, Lin SJ, et al. Cyclooxygenase-2 overexpression in human basal cell carcinoma cell line increases antiapoptosis, angiogenesis, and tumorigenesis. J Invest Dermatol. 2006;126:1143-51.
6. Tjiu JW, Chen JS, Shun CT, et al. Tumor-associated macrophage-induced invasion and angiogenesis of human basal cell carcinoma cells by cyclooxygenase-2 induction. J Invest Dermatol. 2009;129:1016-25.
7. Muller-Decker K, Neufang G, Berger I, et al. Transgenic cyclooxygenase-2 overexpression sensitizes mouse skin for carcinogenesis. Proc Natl Acad Sci USA. 2002;99:12483-8
8. Fischer SM. Is cyclooxygenase-2 important in skin carcinogenesis? J Environ Pathol Toxicol Oncol. 2002;21:183-91.
9. Vogel U, Christensen J, Wallin H, et al. Polymorphisms in COX_2, NSAID use and risk of basal cell carcinoma in a prospective study of Danes. Mutat Res. 2007;617:138-46.
10. Karahan N, Baspinar S, Bozkurt KK, et al. Increased expression of COX_2 in recurrent basal cell carcinoma of the skin: a pilot study. Indian J Pathol Microbiol. 2011;54:526-31.
11. Wilgus TA, Koki AT, Zweifel BS, et al. Inhibition of cutaneous ultraviolet light B-mediated inflammation and tumor formation with topical celecoxib treatment. Mol Carcinog. 2003;38:49-58
12. Tang JY, Aszterbaum M, Athar M, et al. Basal cell carcinoma chemoprevention with nonsteroidal anti-inflammatory drugs in genetically predisposed PTCH1+/- humans and mice. Cancer Prev Res. 2010;3:25-34.
13. Maalej M, Frikha H, Kochbati L, et al. Radio induced malignancies of the scalp about 98 patients with 150 lesions and literature review. Cancer Radiother. 2004;8:81-7.

14. Vane JR, Bakhle YS, Botting RM. Cyclooxygenase 1 and 2. Ann Rev Pharmacol Toxicol. 1998;38:97-120.
15. An KP, Athar M, Tang X, et al. Cyclooxygenase-2 expression in murine and human nonmelanoma skin cancers: implications for therapeutic approaches. Photochem Photobiol. 2002;76:73-80.
16. Dixon DA. Regulation of COX_2 expression in human cancers. Prog Exp Tumor Res. 2003;37:52-71.
17. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA. 1997;94:3336-40.
18. Sheng H, Shao J, Morrow JD, et al. Modulation of apoptosis and Bcl-2 expression by prostaglandin E_2 in human colon cancer cells. Cancer Res. 1998;58:362-6.
19. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer. 2001;85:473-83.
20. Rudhaug JE, Fischer SM. Cyclo-oxygenase-2 plays a critical role in UV-induced skin carcinogenesis. Photochem Photobiol. 2008;84:322-9.
21. Buckman SY, Gresham A, Hale P, et al. COX- 2 expression is induced by UVB exposure in human skin: Implications for the development of skin cancer. Carcinogenesis. 1998;19:723-9.
22. Akita Y, Kozaki K, Nakagawa A, et al. Cyclooxygenase-2 is a possible target of treatment approach in conjunction with photodynamic therapy for various disorders in skin and oral cavity. Br J Dermatol. 2004;151:472-80.
23. O'Grady A, O'Kelly P, Murphy GM, et al. COX_2 expression correlates with microvessel density in non-melanoma skin cancer from renal transplant recipients and immunocompetent individuals. Hum Pathol. 2004;35:1549-55.
24. Müller-Decker K. Cyclooxygenase-dependent signaling is causally linked to non-melanoma skin carcinogenesis: pharmacological, genetic, and clinical evidence. Cancer Metastasis Rev 2011;30:343-61.

