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RESEARCH ARTICLE

COMPARISON OF FREE LATERAL AND DEEP MARGINS OF BASAL CELL CARCINOMA (BCC) BY THE MEANS OF TWO STAINING METHODS: HEMATOXYLIN AND EOSIN STAIN (H&E) AND IMMUNOHISTOCHEMISTRY (IHC)

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ABSTRACT

Background: BCC is one the common skin cancers. There are controversies regarding the best margin. The goal of this study was to determine margins with H&E and cytokeratin 17.
Method: medical records of 96 patients reviewed. Pathologic specimens were reviewed and cytokeratin 17 was applied. Lateral and deep free margins were measured and compared by H&E and IHC in different subtypes of BCC. The extent and intensity of CK17 staining were examined too.
Results: mean age of all patients was 66.3 years. 61 were male and 35 were female. Nose was the most common site of involvement. The most common type of the disease was infiltrative. Mean free lateral margin in H&E and IHC were significantly different in infiltrative and micronodular types ($p<0.001$ and 0.01, respectively). Mean free deep margin in H&E and IHC were significantly different in infiltrative, micronodular and nodular types ($p =0.002$, 0.04 and 0.04, respectively). The mean intensity and extent of CK 17 staining were not significantly different between various subtypes. ($p=0.2$ and 0.9, respectively)
Conclusion: mean lateral and deep margins were not similar in two methods.

INTRODUCTION

Basal cell carcinomas (BCCs) is the most common non-melanoma skin cancer which its incidence is increasing worldwide (Mohan and Chang, 2014). It is indolent, locally invasive neoplasm which affects fair-skinned people (Telfer *et al.,* 1999). Increasing age, sun exposure, immigration is among predisposing factors. Every year 2.8 new cases occur in United States which leads to 3000deaths every year (Gould and Missailidis, 2011). These tumors are low-growing and rarely metastasize (Lo *et al.,* 1991). They usually grow locally but some tumors infiltrate tissues in a three-dimensional way (Gulleth *et al.,* 2010). The tumor could remain small for years or could progress rapidly (Gulleth *et al.,* 2010). The optimal choice for BCC is surgical excision with different approaches: curettage, surgical excision, excision under frozen section control, and Mohs' micrographic surgery (Gulleth *et al.,* 2010). Based on oncologic safety and less resection of normal tissues, the tumor margin should be determined. In the literature, 4-mm margins have been recommended for BCCs (Wolf and Zitelli, 1987; Thomas *et al.,* 2003). It is important to apply the best method for establishing the optimal margin for the tumor. Cytokeratin 17 is one of the intermediate filaments of the keratin which is expressed in palmoplantar keratinocytes and in the nail bed, hair follicle, and sebaceous and sweat glands (Tong and Coulombe, 2006). In previous studies, expression of cytokeratin 17 in BCC lesions have been noted (Markey *et al.,* 1992; Moll *et al.,* 1982; Yoshikawa *et al.,* 1995). There is limit studies regarding cytokeratin 17 expression in BCCs. The goal of this study was to compare free margins of BCC lesions with two staining methods: H&E and cytokeratin 17.

MATERIALS AND METHODS

Patients

In this study which conducted in Razi hospital (affiliated hospital of Tehran university of medical sciences) between 2013-2014, medical records of 100 patients with BCC diagnosis who treated with surgical excision and were free margin, randomly selected (25 from each sub type).

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Methods

Demographic characteristics (age, sex), location of the tumor, subtype, lateral and deep free margins of H& E were recorded. The archived specimens were reviewed by an expert pathologist. For CK-17 immunostaining, sections were cut at 4 mm and fixed in cold acetone for 10 minutes. Then the specimens were washed in TBS, and the sections were incubated in 3% hydrogen peroxide for 5 minutes to block the endogenous peroxidase activity. Nonspecific reactivity blocking was accomplished by incubating the sections in goat normal serum for 20 minutes. Then, the sections were incubated with the primary antibody (mouse antihuman CK17 from Dako) for 1 hour. Sections were then raised to remove excess antibody and subsequently incubated for 30 minutes with the secondary antibody (goat antimouse polymer-horse radish peroxidase, EnVision system, DAKO) (Anderson-Dockter *et al.,* 2012).

The samples were evaluated again by IHC .Lateral and deep free margins were measured by pathologist. Intensity of staining with CK17 was scored as; 0(absence), 1(mild).2(moderate) and 3(severe) and extent of staining was scored as 0(absence), 1(focal:<10%), 2(patchy:11-75%) and 3(diffuse:> 75%).

Statistical analysis

We used SPSS 20 statistical software to analyze the patients' data. Descriptive values (including patients' age and sex) are presented as Mean ± SD and frequencies. The paired sample t-test applied for comparison between two groups. P-value less than 0.05 was considered statistically significant.

RESULTS

Finally, 96 specimens evaluated. The balance of 25 for each subtype deviated due to pathologists comments, 32 (33/3 %) infiltrative, 14 (14/6 %) micro nodular, 26 (27/1 %) nodular and 24 (25 %) superficial. Four cases were excluded from study because there was no tumor when more sectioning for IHC was done. Mean age of cases was 66/3±11/6 years. Sixty one (63/5%) were male and 35 (36/5%) were female. The most common location of the lesions were the nose (28.1%) followed by scalp (21.9%) (Table 1 and Figure 1). The most common subtype was infiltrative (Table 2, Figure 2) mean intensity was 2/4±0/87. Median extent was 3. The frequency of different extents is shown in Table 3.

Mean free IHC Intensity and median IHC Extent were not significantly different between four different subtypes (p= 0.2, p=0.9, respectively). (Table 4).

Mean free lateral margins by means of two methods (IHC& H&E) were significantly different in Infiltrative and micro-nodular subtypes (p<0.001, p=0.01, respectively). (Table 5)

Mean deep margins by means of two methods (IHC& H&E) were significantly different in Infiltrative, micro-nodular and nodular subtypes (p=0.002,p=0.04 and p= 0.04, respectively) (Table 6).

Mean difference of lateral margins between two groups was 0/29±0/55 and mean difference of deep margins between two groups was 0/1±0/34.

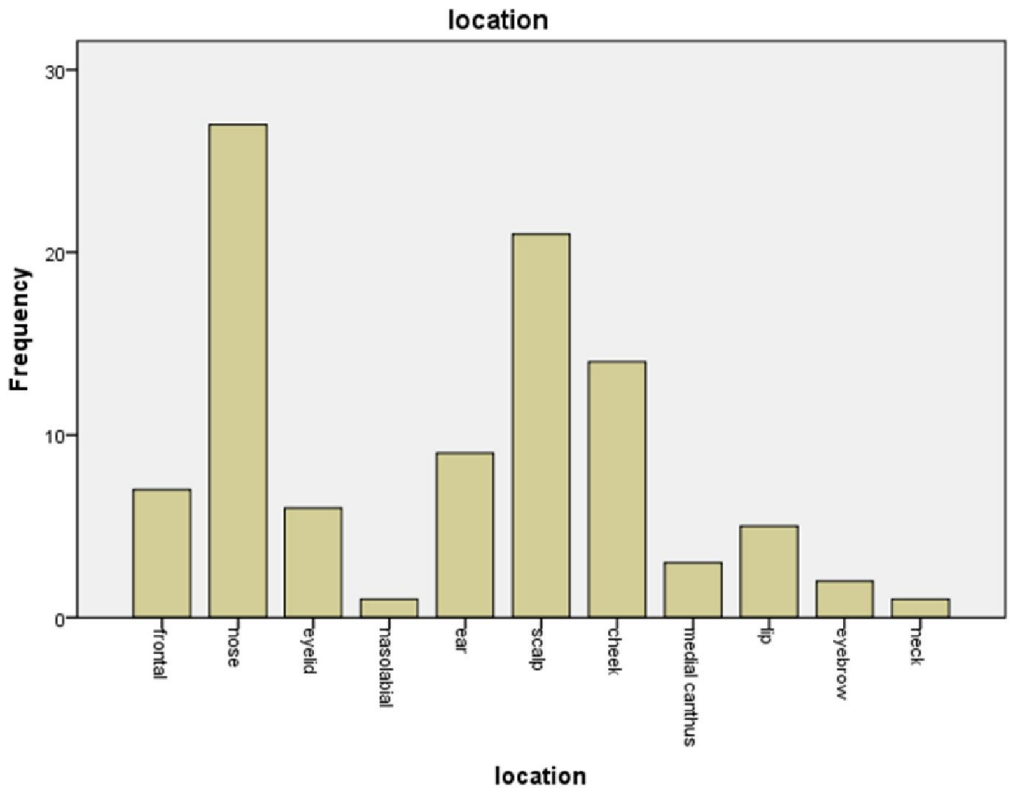

Figure 1. frequency of BCCs locations among the study group

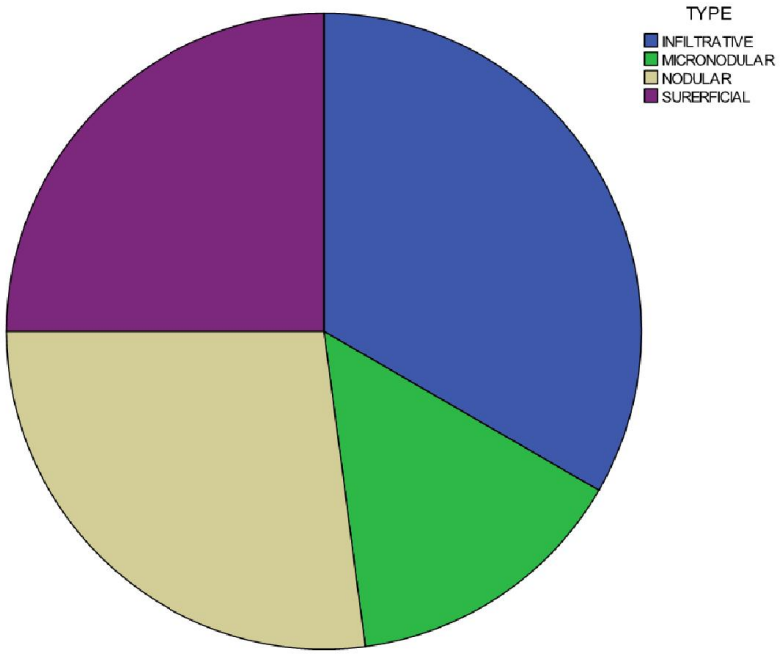

Figure 2. Frequency of BCC subtypes among patients

Table 1. The frequency of BCCs among study group

location	frequency	percent%
frontal	7	7.3
nose	27	28.1
eyelid	6	6.3
nasolabial	1	1
ear	9	9.4
scalp	21	21.9
cheek	14	14.6
medial canthus	3	3.1
lip	5	5.2
eyebrow	2	2.1
neck	1	1

Table 2. The frequency of different BCC subtypes among study group

Subtype	Frequency	Percent%
Infiltrative	32	33.3
Micronodular	14	14.6
nodular	26	27.1
superficial	24	25

Table 3. the frequency of extent of CK17 staining among study group

Extent	Frequency	Percent%
0	7	7.3
1	1	1
2	14	14.6
3	74	77.1

0(no staining), 1(less than 10%), 2(11-75&) , 3(more than 75%)

Table 4. Comparison of intensity and extent of CK17 staining between different BCC subtypes

	Infiltrative	micronodular	nodular	superficial	P value
Intensity(mean)	2.6 ± 0.6	2.6 ± 0.9	2.2 ± 1	2.4 ± 0.9	0.2
Extent(median)	3	3	3	3	0.9

- Intensity, 0(no staining), 1(mild) ,2(moderate), 3(severe)
- Extent,0(no staining),1(less than 10%),2(11-75%),3(more than 75%)

Table 5. Comparison of free lateral margin between H&E and IHC among different BCC subtypes

Subtype	Lateral margin(H&E)	Lateral margin(IHC)	P value
Infiltrative	4.2 ± 2.4	3.7 ± 2.2	< 0.001
Micronodular	4.9 ± 3.3	4.6 ± 3.3	0.01
Nodular	2.8 ± 1.8	2.7 ± 1.9	0.09
superficial	3.3 ± 1.8	3.1 ± 1.9	0.06

- Numbers are in milimeteres(mm)

Table 6. Comparison of free deep margin between H&E and IHC among different BCC subtypes

Subtype	Deep margin(H&E)	Deep margin (IHC)	P value
Infiltrative	2.6 ± 1.3	2.4 ± 1.4	0.002
Micronodular	2.5 ± 1.1	2.4 ± 1.1	0.04
Nodular	2.4 ± 1.4	2.3 ± 1.3	0.04
superficial	4.4 ± 1.5	3.9 ± 1.2	0.08

DISCUSSION

The result of current study showed that lateral and deep margins of BCC tumors are not exactly compatible by IHC and H&E methods. Mean lateral and deep margins were significantly different in two methods ($p<0.001$). In most centers, margin of 4 mm was introduced as the best margin for BCCs. As some BCCs locate on nose or eyelid, determining the best margin is an issue of matter.

Gulleth *et al.* (2010) showed in one large meta analysis on cases of BCC that there is a similar negative pathologic margins between 2,3,4 and 5mm surgical margin while they have significantly different relative risk of recurrence. They concluded although a larger surgical margin lowers the relative risk of recurrence, achieving a clear surgical margin does not correlate with lower recurrence rate. We can postulate that this is explained by the presence of tumoral cells at margin while they are not detected in routine H&E.

There are different studies for comparison of H&E vs IHC staining for evaluation of various tumors. In a previous study conducted by Behdadipour *et al*. determined that diagnostic value of IHC staining is higher than H&E staining in breast cancer tumors. (Behdadipour *et al*., 2004) Blide *et al.* worked on oral SCCs and showed that IHC with P53, P16 was positive at the margin while H&E was normal. (Blide *et al*., 2009) The exact margin of normal tissue should be based on oncologic safety and precise resection of normal tissues (Gulleth *et al*., 2010). The standard surgical margins for BCCs have been recommended as 4 mm (Wolf and Zitelli, 1987; Thomas *et al*., 2003). As the most tumors locate on the eyelid or nose like the findings of our study, taking standard 4-mm surgical margins may not be desirable or necessary (Gulleth *et al*., 2010).

To date, there is no study which compares H&E and IHC in BCCs. In this study we evaluated the free lateral and deep margin of four different subtypes of BCCs by H&E and IHC staining in order to compare the results. We chose cytokeratin 17 for our study because its positivity in BCC was shown in previous studies. (Markey *et al*., 1992; Moll *et al*., 1982; Yoshikawa *et al*., 1995) Keratin intermediate filaments are among structure of epithelial cells. Along with structural role, these filaments could modulate cell signaling (Anderson-Dockter *et al*., 2012). Cytokeratin 17 is expressed in palmoplantar keratinocytes, in the nail bed, hair follicle, and sebaceous and sweat glands (Tong and Coulombe, 2006; Langbein *et al*., 2001). It is also expressed in human epidermal Merkel cell–rich sensory organs (Moll *et al*., 1982). In abnormal keratinization, such as pachyonychia congenital, and palmoplantarkeratoderma mutation in Cytokeratin 17 gene occurs (Terrinoni *et al*., 2001). In previous studies, expression of cytokeratin 17 in BCC lesions have been noted (Markey *et al*., 1992; Moll *et al*., 1982; Yoshikawa *et al*., 1995). As cell activation and differentiation could regulate keratin expression, so some studies by analyzing keratin introduced hair follicle as the potential cells leading to BCC (Ghadially, 1961; Lavker *et al.,* 2003). It could be used as marker for determining the tumor margin. H&E staining is one the most common staining methods which has been considered to be less sensitive than IHC (O'Leary, 2001). But there are little studies evaluating IHC and H&E staining for margin evaluation in BCCs.

The result of current study showed that lateral and deep margins of BCC tumors are not exactly compatible by IHC and H&E methods.

Conclusion

We found that K17 is strongly expressed in BCC lesions and the expression was throughout lesion tissue regardless of tumor subtype. In current study, mean intensity of K17 was not significantly different between subtypes. Mean free lateral margin in IHC was lower in all four subtypes of BCCc (infiltrative, micronodular, nodular and superficial) in comparison with H&E. This difference was statistically significant in infiltrative and micro nodular subtypes only (p value< 0.05).

Mean free deep margin in IHC was lower in all four subtypes of BCCs in comparison with H&E. This difference was statistically significant in infiltrative, micronodular and nodular subtypes (p value< 0.05). Mean lateral and deep margins were significantly different in two methods ($p<0.05$). So it seems that IHC may detect more tumoral cells in comparison with H&E in our study. More studies with larger sample sizes are recommended.

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