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Evaluation of The Best Surgical Margin for Basal Cell Carcinoma Excision: A Clinicopathological Study

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ABSTRACT

Background: Basal cell carcinoma (BCC) is one of the non-melanocytic malignancies of the skin, originating from the epidermal basal cells. It is considered the most common skin malignancy in humans. The treatment should remove the lesion with preserving the maximum level of healthy surrounding tissues. So, treatment is controversial between adequate safety margin and leaving satisfactory cosmetic results.

Objective: In this study, we aim to evaluate the intact safety margin for BCC post-operative, being regarded as 3 mm or more before surgery *(pathologically by routine and immunohistochemical staining using BerEP4).*

Patients and Methods: The study included 40 patients presented with basal cell carcinoma (BCC). All were assessed for the duration, size, type, and site of the lesion. The treatment of the primary lesion and recurrence time was reported for all cases. Different reconstructive types of local fascio-cutaneous flaps were performed. The excised specimens were referred to pathological verification of the diagnosis and evaluation of the boundaries, either free or infiltrated.

Results: There was a statistically significant association between recurrence and tumor size (p=0.001), deep facial invasion (p<0.001), muscle invasion (p=0.001), ill-defined tumor borders (p<0.001), positive surgical margins, and safety margins size (p<0.001).

Conclusion: Excision of BCC with 3 mm or more as a safety margin is enough to preserve healthy tissues and avoid the requirement for difficult procedures of reconstruction. The use of BerEP4 is a highly specific marker for detecting BCC cells that, can be missed by routine H&E staining.

Keywords: Basal cell carcinoma, safety margin, Immunohistochemical, BerEP4, Fascio-cutaneous flaps.

INTRODUCTION

Basal cell carcinoma (BCC) is one of the non-melanocytic skin malignancies that, originate from the epidermal basal cells. Its first discovery was by Jacob et al. in 1827, also named rodent ulcer. It represents about 80% of the non-melanocytic malignancy of the skin [(1)].

The BCC prevalence increases by 3–10% annually. It was found that 8/10 cancer skin cases are BCC as reported by the American Cancer Society (2012) and more than 2 million newly diagnosed cases are recorded every year [(2)].

Generally, BCC is best treated by surgical excision. A 95% 5-year cure rate is accepted and considered a reasonable aim to accomplish [(3)]. The tumor must be excised completely in the first surgical interference, as primary BCC shows higher recovery rates more than recurrent tumors. Moreover, recurrent BCC is usually more aggressive. Therefore, the suitable excision margins for recurrent tumors should be nearly two times bigger than those for primary BCC removal [(4)].

The treatment should remove the lesion and preserve the maximum amount of healthy surrounding tissues. So, treatment is controversial between adequate safe boundary and leaving satisfactory cosmetic results.

Currently, histopathological examination by Hematoxylin and Eosin (H&E) has the major role in verifying the dermoscopic, clinical diagnosis, and invasion of BCC. Yet, it is not always having the ability to detect and assess margins infiltration of some types of BCC, having the same morphologic appearance of other carcinoma types [(5)]. Immunohistochemistry is a procedure used to detect cell antigens, amino acids, infectious agents, and proteins on specific cells. Currently, immunohistochemistry is an essential method in medical research that can assess cells that, cannot be discovered by ordinary H&E staining [(6)].

EpCAM is an epithelial adhesion molecule that has a vital role in cell signaling, migration, proliferation, differentiation, and adhesion. It was reported to act as an oncogenic signaling molecule via the Wnt signaling passageway. Now, anti-EpCAM antibodies are available for histopathological diagnosis and therapy as for breast and colonic carcinoma in humans [(7)].

BerEP4 is an anti-EpCAM antibody and is regarded as a sensitive marker for BCC. It is a monoclonal antibody that, is extracted from adenocarcinoma cell line of the human breast (MCF-7). This monoclonal antibody identifies 34 kDa and 39 kDa anon covalently united

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glycopeptides present commonly on the epithelial cells of humans [8].

In this study, our aim was to assess the intact safe boundary of BCC post-operative, being regarded as 3 mm or more before surgery *(pathologically by routine and immunohistochemical staining using BerEP4).* Is it a free safety margin or not??

PATIENTS AND METHODS

The present our prospective study was executed at the departments of surgical oncology, Ismailia teaching oncology hospital, general surgery department and pathology departments, Zagazig University Hospital, Egypt. It included 40 cases of basal cell carcinoma (BCC), during the interval from February 2019 to February 2021. Patients included in this study, were presented by skin BCC, proved by pathological confirmation. Exclusion criteria were synchronous skin diseases or other neoplastic lesions.

Ethical approval:

Our study has an approval by ethical committee of Ismailia Teaching Oncology Hospital.

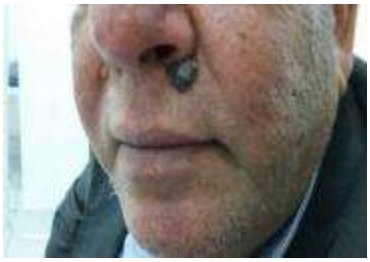
All patients were consented to be included in this study and for medical photography. We toke history from all our patients, adequate clinical examination moreover, necessary laboratory investigations and punched tissue biopsy. All clinical data were reported including sex and age at first time diagnosis, duration, size, type, and position of the lesion. The treatment of the primary lesion was reported for all cases and recurrence time was estimated for each case.

Surgical procedure:

Patients had different presentation sites of the tumor, BCC of the scalp **(9)**, cheeks (11), nose (8), lips (6), and peri-orbital (6) cases. The size, site, and availability of tissue around the tumor can predict the surgical techniques to be performed as follows: clinically free margins were detected all around the lesion by sterile marker, a 3 mm safe margins were designed (Figure 1).

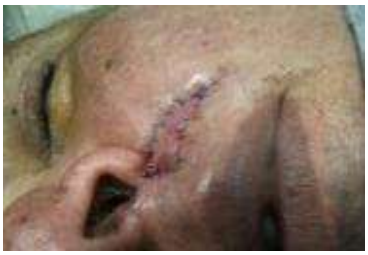
Anesthesia was by infiltration of 0.1% lignocaine and adrenaline (one in 100,000), injected under and around the site designed to be excised and reconstructed, and the excised sample was marked with a suture for orientation. The excised tumor was referred to an expert pathologist for confirmation of the diagnosis and comment on the boundaries, either free or infiltrated. Reconstructive procedures were carried by many types of local fascio-cutaneous flaps (rhomboid, rotational, island, and advancement flap).

We closed the wound in layers by Vicryl 4/0 and proline 5/0. Removal of the sutures was in the fifth to seventh postoperative day and patients were followed up weekly after suture removal for one month, monthly for one year, and then every six months for 36 months.

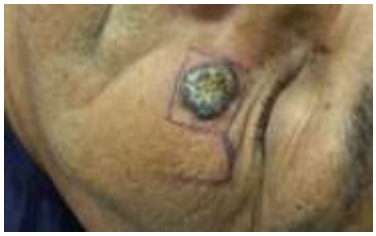


(A) Lip BCC

(B) Advancement flap design (3mm safety margin)

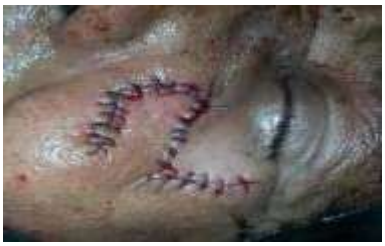


(C) After 1 week

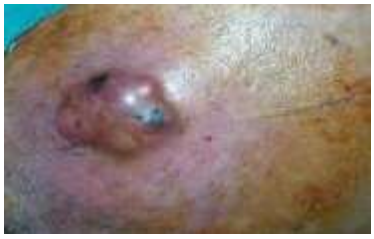


(A) Cheek BCC

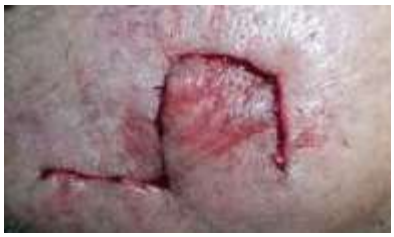
(B) Rhomboid flap design (3mm safety margin)



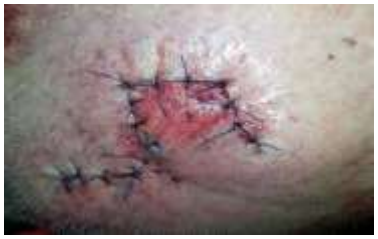
(C) After 1 week



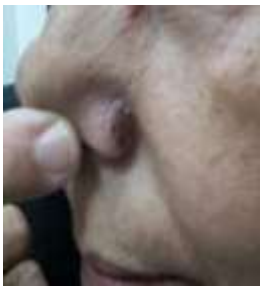
(A) Scalp BCC



(B) Rhomboid flap design (3mm safety margin)

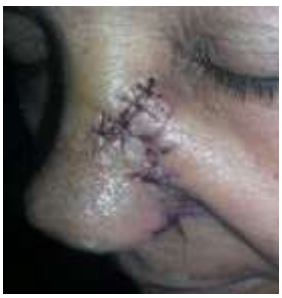


(C) After 1 week



(A) Nasal BCC

(B) Bilobed flap design (3mm safety margin)



(C) After 1 week

Figure (1): BCC of the lip, cheek, scalp, and nose with 3 mm safety margin and reconstruction flaps.

Pathological procedures:

Histopathological examination:

All surgically excised tumor samples from the 40 patients included in our study have been referred to an expert pathologist. Every sample was fixed immediately in buffered formalin (10%), prepared in variant degrees of alcohols, and lastly prepared into blocks of paraffin wax. Paraffin serially sectioned to 4µm thick and stained by ordinary Hematoxylin & Eosin (H&E).

Pathological subtyping was as follows: The solid (nodular) type is formed of islands of cells showing palisading at the periphery with the central cells arranged haphazardly. The superficial (multifocal) type consists of multiple small basaloid cell islands attached to the bottom of the epidermis and usually restricted to the dermal papillae. The pigmented type showing colonization of tumor cells with melanocytes. The fibrosing type showing strands and nests of basaloid cells with limited palisading and thick sclerotic stroma.

Immunohistochemical examination:

The immunohistochemical staining procedure for BerEP4 was achieved by using the streptavidin-biotin immunoperoxidase approach (Dako-Cytomation, Glostrup, Denmark). Sections were cut at 3–5 µm thickness from blocks on slides positively charged then we used xylene to remove the paraffine followed by rehydration using graded alcohol. After this, sections were heated in buffered citrate (pH 6.0) for 20 minutes and washed in PBS (pH 7.3).

6% H2O2 in methanol was used for blocking the activity of endogenous peroxidase. The immunohistochemical staining for BerEp4 was prepared with a mouse monoclonal antibody (Clone Ber-EP4, 1:20; Dako, Carpinteria, California). Slides then were incubated for 2 hours with the primary antibody at room temperature, washed by PBS, finally immersed with a biotin-conjoined secondary antibody (Lab Vision Corporation, Fermont, USA). DAB was used as chromogen and Mayer's hematoxylin as a counterstain. Then, washed the slides by water and PBS. Stain positive and negative controls at the same setting with studied cases. Omission of the primary antibody gives result of negative controls. Breast and colon carcinoma tissue was used as a positive control. Only membranous staining was accepted as positive and specific [(9)].

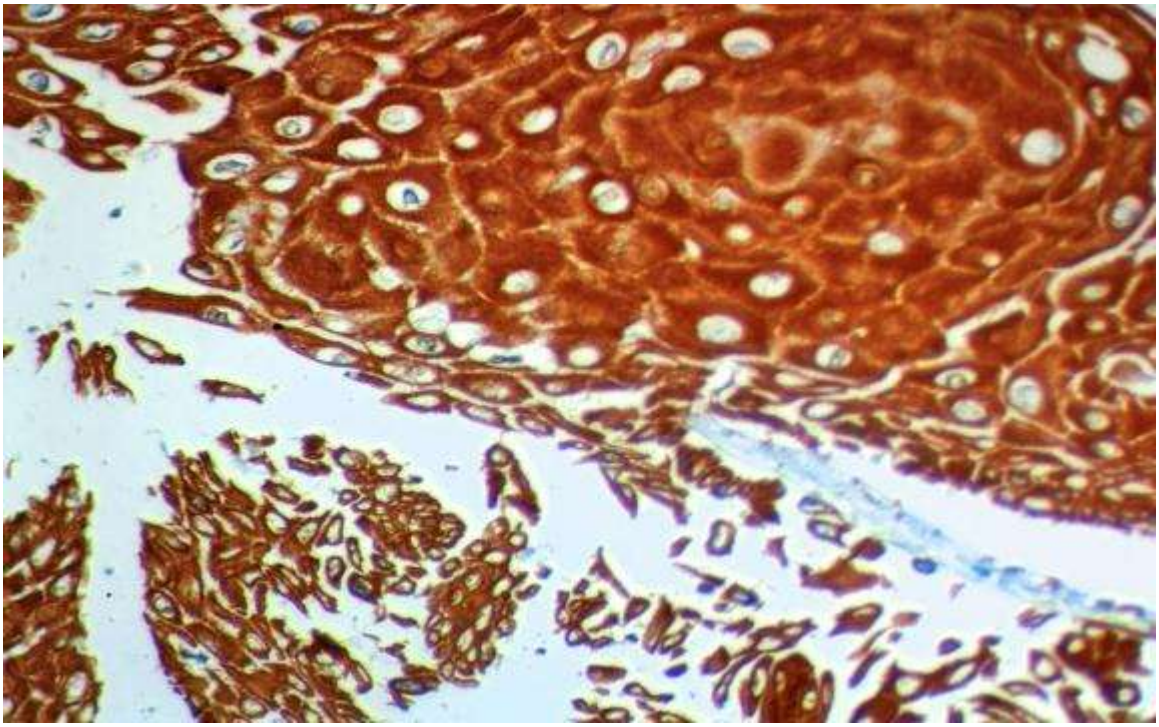


Figure (2): A case of nodular basal cell carcinoma stained by BerEP4 showing malignant infiltrating cells (IHC X 400)

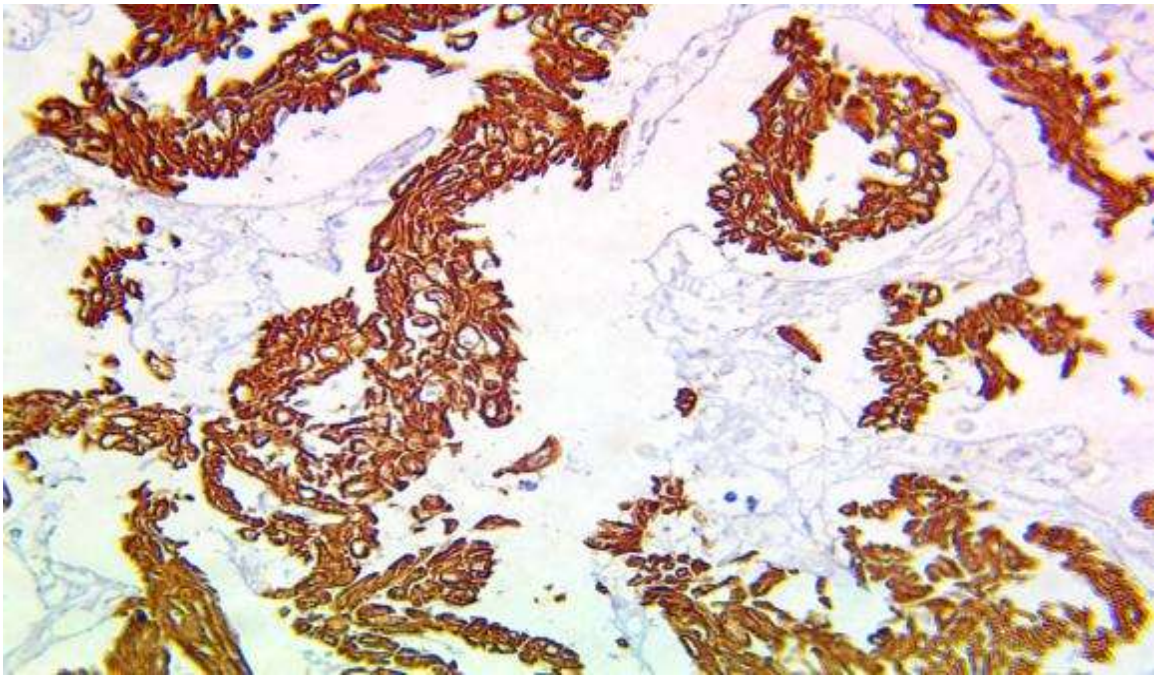


Figure (3): A case of nodular basal cell carcinoma stained by BerEP4 showing malignant cellular infiltrating in the dermis (IHC X 200)

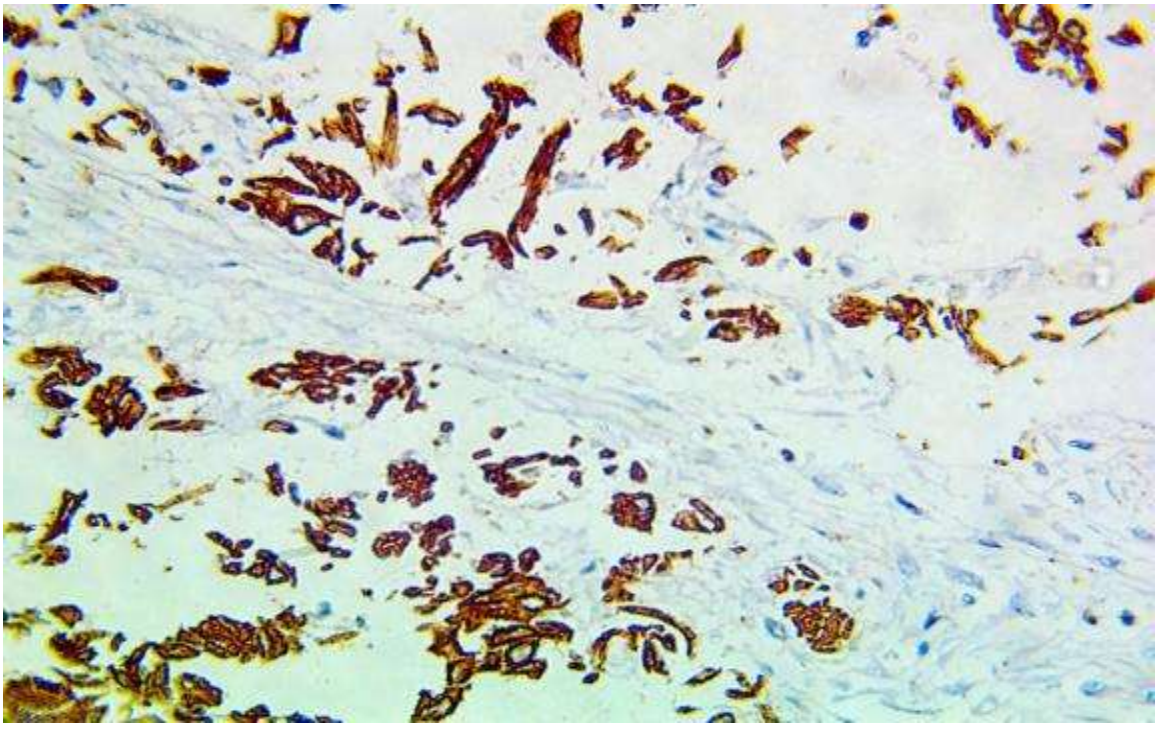


Figure (4): A case of basal cell carcinoma with positive infiltrating margins, the malignant cells are highlighted by BerEP4 stain (IHC X 200)

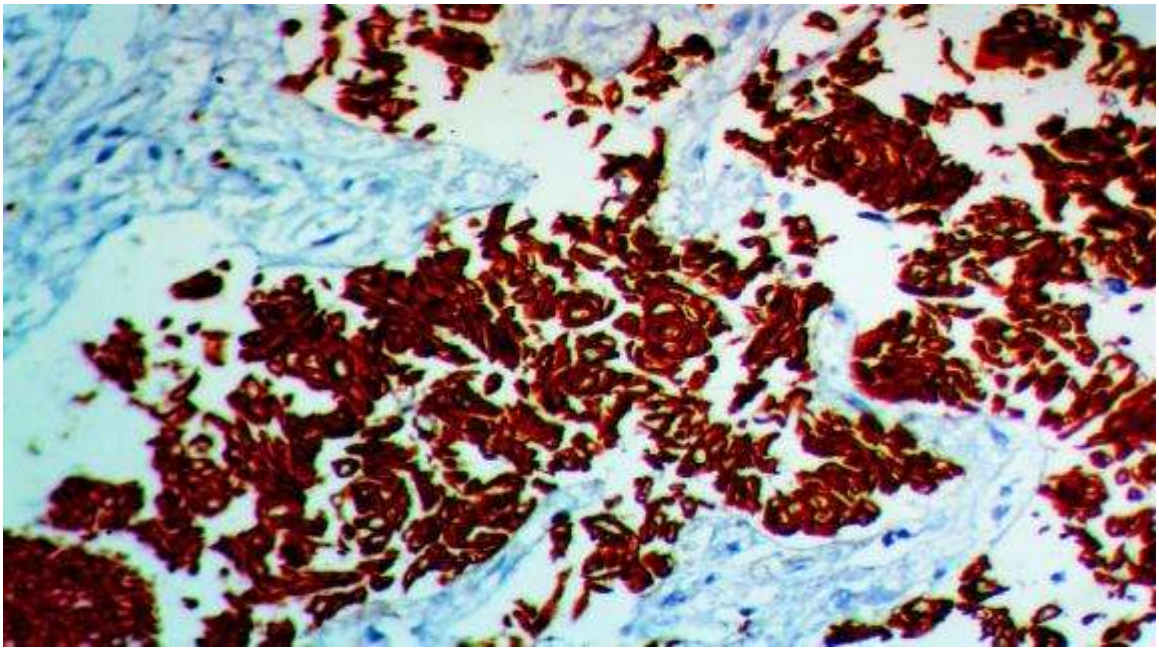


Figure (5): A recurrent case of basal cell carcinoma, showed by BerEP4 stain (IHC X 200)

Statistical Analysis:

The continuous variables were represented as a mean ± SD & median (range), and the categorical variables were represented as a number (percentage). Continuous variables were checked for normality by Shapiro-Wilk test. We used Mann-Whitney U test for comparing two groups of non-normally distributed data. The percentage of categorical variables were compared by Pearson's Chi-square test or Fisher's exact test when was appropriate. The change of distribution for relative frequencies and ordinal data was compared by using Chi-square test for trend. Recurrence Free Survival (RFS) was calculated as the time from the start of treatment to the date of relapse or the most recent follow-up contact that patient was known as recurrence-free. Stratification of RFS was done according to study parameters. The method of Kaplan-Meier plot used to estimate time-to-event distributions and compared using the two-sided exact log-rank test. All tests were two-sided. A p-value <0.05 was regarded significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

RESULTS

The study included 40 patients with BCC, 13 (32.5%) women and 27 (67.5%) men. The patient's age ranged from 38 – 79 years, with a mean of 56.42±8.8 years. Lesions were mostly located on the cheeks (27.5%), followed by the scalp (22.5%). Other sites included nose, lips, and peri-orbital regions (20%, 15%, and 15%, respectively). The lesions size ranged between 3 and 30 mm and the mean was 15.37±8.02mm. Recurrence time ranged from 2 to 12 months, and its mean was 6 months.

Histological assessment of primary lesions showed that, nodular type represented 60% of cases, superficial type 17.5%, fibrosing type 12.5%, and pigmented type in 10%.

All cases underwent surgical excision, with safety margins ranging from 1-8 mm, 6 cases <3 mm (15%) and 34 cases were > 3 mm (85%).

Involved safety margins, were seen in 9 cases (22.5%), 4 in lateral margins, and 5 in-depth (Table 1).

Table (1): Clinicopathological parameters and recurrence among 40 patients with Basal Cell Carcinoma of skin.

Parameters	All patients (N=40)	
	No.	%
Age (years)		
Mean±SD	56.42	±8.80
Median (Range)	57	(38 – 79)
<40 years	2	5%
40-60 years	25	62.5%
>60 years	13	32.5%
Sex		
Male	27	67.5%
Female	13	32.5%
Site		
Scalp	9	22.5%
Cheeks	11	27.5%
Periorbital	6	15%
Nose	8	20%
Lips	6	15%
Location		
Area M	19	47.5%
Area H	21	52.5%
Size		
Mean ±SD	15.37	±8.02
Median (Range)	15	(3 – 30)
<10 mm	12	30%
10-<20 mm	16	40%
>20 mm	12	30%
Deep fascia invasion		
Absent	31	77.5%
Present	9	22.5%
Muscle invasion		
Absent	36	90%
Present	4	10%
Bone/cartilage invasion		
Absent	39	97.5%
Present	1	2.5%

Parameters	All patients (N=40)	
	No.	%
Borders		
Well defined	31	77.5%
Poorly defined	9	22.5%
Type		
Primary	35	87.5%
Recurrent	5	12.5%
Subtype		
Nodular	24	60%
Superficial	7	17.5%
Pigmented	4	10%
Fibrosing	5	12.5%
Risk group		
Low risk	2	5%
High risk	38	95%
Surgical Margin		
Negative	31	77.5%
Positive	9	22.5%
Planned margin size		
Mean±SD	4.90	±2.12
Median (Range)	5	(1 – 8)
<3mm	6	15%
≥3mm	34	85%
Site of margin infiltration		
Free margin	31	77.5%
Lateral margin	4	10%
Deep margin	5	12.5%
Reconstruction		
Advancement	13	32.5%
Skin graft	1	2.5%
Flap	26	65%

There was a statistically significant association between recurrence and tumor size (p=0.001), deep facial invasion (p<0.001), muscle invasion (p=0.001), ill-defined tumor borders (p<0.001), positive surgical margins, and safety margins size (p<0.001) (Table 2).

Table (2): Relationship between clinic-pathological parameters and recurrence among 40 patients with Basal Cell Carcinoma of skin.

Parameters	All patients (N=40)		Recurrence				p-value
			Absent (N=31)		Present (N=9)		
	No.	%	No.	%	No.	%	
Age (years)							
Mean±SD	56.42	±8.80	57.32	±9.40	53.33	±5.70	0.195•
Median (Range)	57	(38 – 79)	58	(38 – 79)	53	(45 – 61)	
<40 years	2	5%	2	100%	0	0%	0.499§
40-60 years	25	62.5%	18	72%	7	28%	
>60 years	13	32.5%	11	84.6%	2	15.4%	
Sex							
Male	27	67.5%	20	74.1%	7	25.9%	0.690‡
Female	13	32.5%	11	84.6%	2	15.4%	
Site							
Scalp	9	22.5%	8	88.9%	1	11.1%	0.215‡
Cheeks	11	27.5%	10	90.9%	1	9.1%	
Periorbital	6	15%	4	66.7%	2	33.%	
Nose	8	20%	4	50%	4	50%	
Lips	6	15%	5	83.3%	1	16.7%	
Location							
Area M	19	47.5%	17	89.5%	2	10.5%	0.133‡
Area H	21	52.5%	14	66.7%	7	33.3%	
Size							
Mean ±SD	15.37	±8.02	17.64	±7.44	7.55	±4.18	0.001•
Median (Range)	15	(3 – 30)	18	(5 – 30)	6	(3 – 15)	
<10 mm	12	30%	5	41.7%	7	58.3%	0.001§
10-<20 mm	16	40%	14	87.5%	2	12.5%	
>20 mm	12	30%	12	100%	0	0%	
Deep fascia invasion							
Absent	31	77.5%	31	100%	0	0%	<0.001‡
Present	9	22.5%	0	0%	9	100%	
Muscle invasion							
Absent	36	90%	31	86.1%	5	13.9%	0.001‡
Present	4	10%	0	0%	4	100%	
Bone/cartilage invasion							
Absent	39	97.5%	31	79.5%	8	20.5%	0.225‡
Present	1	2.5%	0	0%	1	100%	
Borders							
Well defined	31	77.5%	31	100%	0	0	<0.001‡
Poorly defined	9	22.5%	0	0%	9	100%	
Type							
Primary	35	87.5%	28	80%	7	20%	0.311‡
Recurrent	5	12.5%	3	60%	2	40%	
Subtype							
Nodular	24	60%	18	75%	6	25%	0.702‡
Superficial	7	17.5%	5	71.4%	2	28.6%	
Pigmented	4	10%	4	100%	0	0%	
Fibrosing	5	12.5%	4	80%	1	20%	
Risk group							
Low risk	2	5%	0	0%	2	100%	0.046‡
High risk	38	95%	31	81.6%	7	18.4%	
Surgical Margin							
Negative	31	77.5%	31	100%	0	0%	<0.001‡
Positive	9	22.5%	0	0%	9	100%	
Planned margin size							

Mean±SD	4.90	±2.12	5.70	±1.63	2.11	±0.78	<0.001•
Median (Range)	5	(1 – 8)	5	(3 – 8)	2	(1 – 3)	
<3mm	6	15%	0	0%	6	100%	<0.001‡
≥3mm	34	85%	31	91.2%	3	8.8%	
Site of margin infiltration							
Free margin	31	77.5%	31	100%	0	0%	<0.001‡
Lateral margin	4	10%	0	0%	4	100%	
Deep margin	5	12.5%	0	0%	5	100%	
Reconstruction							
Advancement	13	32.5%	11	84.6%	2	15.4%	0.619‡
Skin graft	1	2.5%	1	100%	0	0%	
Flap	26	65%	19	73.1%	7	26.9%	

Table (3): Relationship between clinicopathological parameters and recurrence among 40 patients with Basal Cell Carcinoma of skin.

Parameters	**N**	**Recurrence Free Survival**					**p-value†**
		Mean	**(95%CI)**	**12month**	**24month**	**36month**	
All patients	40	37.74 months	(30.02 – 44.70)	87.1%	68.3%	68.3%	------
Size							
<10 mm	12	23.92 months	(10.39 – 37.44)	66.7%	28.6%	0%	0.011
10-<20 mm	16	22.93 months	(20.26 – 25.61)	93.3%	85.6%	------	
>20 mm	12	24 months		100%	100%	100%	
Deep fascia invasion							
Absent	31	25 months		100%	100%	------	<0.001
Present	9	14.56 months	(5.37 – 23.74)	44.4%	11.1%	11.1%	
Muscle invasion							
Absent	36	22.72 months	(20.76 – 24.76)	91.5%	72.4%	------	0.006
Present	4	19 months	(0 – 38.15)	50%	25%	25%	
Borders							
Well defined	31	25 months		100%	100%	------	<0.001
Poorly defined	9	14.56 months	(5.37 – 23.74)	44.4%	11.1%	11.1%	
Risk group							
Low risk	2	5.50 months	(4.52 – 6.48)	0%	------	------	<0.001
High risk	38	39.04 months	(31.60 – 46.47)	0%	71.9%	71.9%	
Surgical margin							
Negative	31	25 months		100%	100%	------	<0.001
Positive	9	14.56 months	(5.37 – 23.74)	44.4%	11.1%	11.1%	
Planned margin size							
<3mm	6	15.50 months	(2.27 – 28.72)	50%	16.7%	16.7%	<0.001
≥3mm	34	23.73 months	(22.23 – 25.23)	93.8%	78.2%	------	
Site of margin infiltration							
Free margin	31	25 months		100%	100%	------	<0.001
Lateral margin	4	13.75 months	(5.31 – 22.20)	75%	0%	------	
Deep margin	5	15.20 months	(0 – 31.33)	20%	20%	20%	

Continuous variables were expressed as mean (95%CI); categorical variables were expressed as number (percentage); † Log-rank test; $p<0.05$ is significant.

DISCUSSION

In this study we aim to clear basal cell carcinoma with a least adequate surgically free border, in order to gain a negative pathologic boundary with the least surgical margin [10].

The range of our patient's age was from 38–79 years, with a mean of 56.42±8.8 years, this matches with **Godoy *et al.*** [10], who suggested that the incidence of 55-75 years, is nearly 100 times higher in patients younger than 20 years.

In our study, there was a statistically higher relation between surgical margin and safety margin ($p<0.001$). A negative surgical margin was seen in 77.5% (31 cases) and infiltrated or positive surgical margin was in 22.5% (9 cases). This falls in the same range reported by **Codazzi *et al.*** [11], who reported that the involvement of the boundaries varied between 7% and 25%.

In our study, the most histologically famous subtype was, the nodular (60%), then superficial (17.5%), fibrosing (12.5%), and pigmented (10%). This matches with **Godoy *et al.*** [10] who found that nodular type of BCC is the predominant one, it accounts for 60%.

As regards to the anatomical site of the tumor, we found that most cases were seen in the head and neck regions and this was a usual finding, since that BCC is common at photo-exposed areas. This matches with **Ocanha *et al.*** [12] who found that 70% of the tumor was located at the face.

Bisson *et al.* [13] have found that, less than 3 mm margins, augment the risk of recurrence, even if histopathologic examination of the margin is free of tumor.

According to our study, we recommend 3 mm or more as a safety margin of BCC, this matches with the guidelines of European Dermatology Forum (EDF) on BCC safety boundaries, recommends 3-4 mm edge peripherally for low-risk patients with BCC, and 5-10 mm for BCC with high-risk [14].

According to **Nahhas *et al.*** [14], the international guidelines recommend a safe margin of 3 mm for BCC with low risk to perform total excision in 85% of cases, and the incomplete excision rate was found to be about 15%.

Bisson *et al.* [13] reported that, the performance of narrow safe margins of excision (3 mm), will diminish the defect size.

Lin *et al.* [15] followed-up their 143 patients, for 5-years to detect whether a 3 mm margin of excision was enough for bcc cure, and they used recurrence as a result measure. They found that 3 mm safe boundaries are enough for pigmented BCC excision, however, non-pigmented BCC had a higher recurrence rate and so, it needs strict follow-up.

According to **Univerdi *et al.*** [16] study, they found that a margin of 3 mm is more than enough for BCC complete excision.

Gulleth *et al.* [3] used different surgical margins with adequate excision for a comparison and found that for cure rate of 95%, 3-mm surgical margin for 2 cm or less basal cell carcinoma is enough.

Thomas *et al.* [17] suggested that a four mm surgical margin was enough for aggressive types of BCCs and a 3 mm for BCCs with well outlined margin.

In contrast with our study **Bichakjian *et al.*** [4], recommend a four mm at the periphery to reach to adequate excision rate of 95 % however, a 4-6 mm free margin is adequate for high-risk tumors.

Luz *et al.* [18] found that boundaries less than 3 mm, increased the risk of recurrence, even though these margins are histopathologically free. They also reported that micrographic margins more than 6 mm, are enough for recurrent tumors.

Qazi *et al.* [19] found that, a four mm margin is enough for reaching a clear one, for low-risk BCC (2 cm or morphea form or infiltrative).

Adequate management of BCC is considered, when reaching to a free margin. The tumor can extend microscopically beyond the clinically detected margins. So, the goal of surgical management is to excise both the clinically detected lesion and it's widen microscopically into the healthy appeared skin around. It can be reached by excision of the lesion with clinically free surrounding skin margin. Although subdermal adipose tissue has a resistance to spread yet, it is essential to remove BCC till the tissue at subdermal level [20].

In the current study, assessment of safety margins was proved not only by routine staining but also immunohistochemically using BerEp4 which is a highly specific marker for detecting BCC cells that can be missed by routine H&E staining.

Beer *et al.* [21] study was one of the early studies that used BerEP4 in detecting BCC cases, who detected all 39 samples of BCC included in his study. The same results were observed by **Ishida *et al.*** [22] on 20 samples of BCCand **Krahl and Sellheyer**[23] on 28 infiltrative and sclerosing types of BCCs.

A previous study on the use of BerEP4 was carried out by **Tan and Sunjaya** [24] on 23 samples with 394 micro lesions. They observed 100% positivity, sensitivity and specificity. In their study, BerEP4 could give positive results even in the early BCC stages.

Patients with BCC have a risk for recurrence even with complete excised margin that, grossly appeared free and by staining with H&E, as many types of BCCs showed micro lesions that spread upon the surface of skin. There is a difficulty in detection of these micro lesions

with ordinary staining as they are still a little group of malignant cells [25]. So, in this study, the margins were assessed by routine and BerEP4 immunostaining.

CONCLUSION

Excision of BCC with 3 mm or more as a safety margin is enough to preserve healthy tissues and not to be in need for difficult procedures for reconstruction. The use of BerEP4 as a highly specific marker for detecting BCC cells that, can be missed by routine H&E staining. Moreover, it can detect the presence of BCC cells accurately after treatment and therefor, decrease the rate of recurrence.

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