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Recurrence of Basal Cell Carcinoma After Mohs Micrographic Surgery: A 4-Year Retrospective Analysis

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Abstract

Background: Among non-melanoma skin cancers (NMSC), Basal cell carcinoma (BCC) is one of the most common.
Objectives: We endeavored to assess the recurrence rate of BCC after surgery and compare tumors recurrence based on different aspects.
Methods: This was a retrospective and observational study which was analyzed medical records of 154 patients who had Mohs micrographic surgery (MMS) from March 2013 to February 2017 in two clinical centers. For finding if the clinical characteristics of the patients have related to tumoral recurrence, we gathered them, including gender and age of the patient, tumor size, site, and its histological type plus the existence of ulcer in malignancy. Data were analyzed using SPSS 22 statistical software. Statistical difference between proportions was determined by χ^2 analysis.
Results: The majority of patients (74%) were female (57%) older than 60 years old. Most tumors, based on the histopathological diagnosis, were Infiltrating (43.5%) and nodular and infiltrating (40.3%), respectively. The mean tumor size was 1.81 cm and most of them (74%) were larger than 1 cm. The tumor site was mostly in the nasal (56.5%) followed by the cheek (11%). Most patients (96.8%) had one lesion. The recurrence rate was 1.9%. The mean interval between surgeries to recurrence was 13 months. There was no significant difference between recurrence rate and age, sex, tumor type in terms of clinical diagnosis, tumor location, tumor size, number of lesions, and type of tumor ($P < 0.05$).
Conclusions: the rate of recurrence of BCC in patients treated with MMS is low (1.9%). We recommend the utilization of the MMS technique for the treatment of BCC.

Keywords: Basal Cell Carcinoma, Moh's Surgery, Recurrence

1. Background

Among non-melanoma skin cancers (NMSC), Basal cell carcinoma (BCC) is one of the most common (1). BCC is not life-threatening but can affect the quality of life. Apart from the financial cost of the disease, it also has psychological, physiological, and physical effects on the patient, the most important of which is the loss of sensory perception and disfigurement. According to some reports, the incidence of skin cancer is increasing worldwide, with up to 10% annual increase in incidence (2). The annual incidence of BCC is estimated to be 2.75 million new cases worldwide (3). The method of treatment for basal cell carcinoma with high risk is surgery which includes cryosurgery, excision surgery, cautery, curettage, and Mohs micrographic surgery (MMS). Among these methods, the MMS has been proposed as the gold standard treatment method (3). It suggested that in a patient with an aggressive tumor, MMS is the best treatment way (2, 4) if the surgery fails or cannot perform, the other modalities, such as radiotherapy, cryotherapy, and topical chemotherapy may be used (4).

BCC recurrence is more difficult to treat than primary tumors (5).

Most relapses (two-thirds) occur during the first three years (2). However, they may occur between 6 months to 10 years after treatment. According to previous studies, the recurrence rates after 5-year is 15.4% (6). Positive excision margins and high-risk histological types are the best predictors for tumor recurrence (7).

2. Objectives

The aim of the present study was to evaluate the recurrence rate and possible factors affecting the recurrence of BCC after MMS based on gathered data from two clinical centers.

3. Methods

The current study was a retrospective cohort of all patients with BCC diagnosed during 2013 - 2017 and their treatment method was MMS. Clinical patients data was gathered from two clinical centers of Kermanshah Province, Iran. Procedures adopted in this study have approved by the Ethics Committee of the Kermanshah University of Medical Sciences and are according to the Declaration of Helsinki principles. Tumor subtypes were classified according to the World Health Organization (WHO) classification. Eligible patients were all those who were diagnosed with BCC by biopsy attending dermatologists, confirmed by a pathologist, and treated with MMS. This method briefly is done as follows, the four directions of the desired texture layer are marked with a marker at 3, 6, 9, and 12 o'clock. After the local anesthesia, the visible tumor removes with a curette then a thin margin of tissue around that deeply removed. The tissue layer cut into halves or quadrants and then marked with various colors to facilitate precise mapping of the tumor. The tissue is then pressed flat, so the epidermal edge occupies the same tissue plane as the deep margin. The tissue is then cut and processed in a horizontal direction. after that, a pathologist examines the tissue under a microscope. If a residual tumor found, the Mohs map would mark and the tissue will precisely be removed in that portion that was found. This process is repeated until the tumor is negative according to the result of pathology. For closing the site of surgery, depending on the site of surgery, a variety of methods such as primary closure, flaps, grafts, and the like are used (8).

The main source for obtaining data on the tumor recurrence was the medical record with an average of four years after treatment. To supplement this review, patients were examined by a dermatologist after this period of treatment. Any irregularity near the treatment site was recorded. Patients who had scaling, papule, erythema, erosion, induration, or cyst-like lesion near their treatment site were referred to a dermatologist for discernment. In cases of tumor recurrence, the entire case was re-examined by a dermatologist to confirm the result. If the type of tumor and its location were similar to the original tumor, that tumor was considered as a tumor recurrent. The lesion had to be reported by a clinician as recurrent or previously treated. Recurrence time is when a biopsy is taken from the recurrent lesion.

Data was analyzed using SPSS 22 statistical software. For comparing recurrence rates between groups, a chi-square test was used. P value ≤ 0.05 was considered as statistically significant.

4. Results

The mean $\pm$ standard division (SD) age of the samples was 68 ± 15, with 57% female and 43% male; 94.8% of tumor type were primary and 5.2% were recurrence. The most pathologic type was infiltrating (43.5%) and nodular and infiltrating (40.3%). The size of the tumor in 75.4% of the patients was less than 2 cm and the number of lesions in 96.8% of the patients was one lesion (Table 1). The most common tumor location was on the nose (56.5%) and cheek (11%) (Table 2).

Table 1. Frequency of Demographic and Clinical Characteristics of The Patients with BCC[a]

	Patients (N)	P-Value
Age, y		
< 40	6	3.8
40 - 50	4	2.6
51 - 60	30	19.5
61 - 70	43	28
71 - 80	43	28
> 80	28	18
Gender		
Male	66	43
Female	88	57
Number of lesions		
Single	149	96.8
Multiple	5	3.2
Size of tumor, cm		
< 2	116	75.4
> 2	38	24.6
Type of tumor		
Primary	146	94.8
recurrence	8	5.2
Tumor clinical form		
Nodular	67	10.4
Infiltrating	62	43.5
Nodular and infiltrating	16	40.3
Micro nodular	6	3.9
Adenoid	1	0.6
Nodular and pigmented	1	0.6

Abbreviation: BCC, basal cell carcinoma.
[a]Values are expressed as No. (%).

The recurrence time in 66.7% of the patients was less than 15 months and in 33.3% of them was more than 15 months. The recurrence rate was 1.9%. The most frequent

Table 2. Frequency of Tumor Location of The Patients with BCC[a]

Tumor location	Patients (N)	The Event
Nose	87	56.5
Canthus	7	4.5
Lip	6	3.9
Forehead	11	7.1
Temporal	9	5.8
Cheek	17	11
Scalp	2	1.3
Ears	8	5.2
Abdomen	1	0.6
Head	1	0.6
Eyelid	3	1.9
Lateral eye	1	0.6
Neck	1	0.6

Abbreviation: BCC, basal cell carcinoma.
[a]Values are expressed as No. (%).

age of recurrence was in less than 70-year and in females (66.6%). Nose in the type of infiltrating was associated with a greater likelihood of local recurrence (66.6%).

There was no significant difference between recurrence rate and age, sex, tumor type in terms of clinical diagnosis, tumor location, tumor size, number of lesions, and type of tumor ($P < 0.05$) (Table 3).

5. Discussion

This study was performed for the first time on the recurrence rate of BCC after MMS with a follow-up of four years in Kermanshah, west of Iran. According to the result of the recurrence rate of BCC after MMS (1.9%), MMS is the best way for avoiding the early recurrence of BCC among the current treatment methods. Despite hopes of achieving the effectiveness of new targeted molecular therapies, surgical excision and MMS remain as standard therapies for BCC (9). In the comparison of excision and photodynamic therapy for nodular BCC at five years, the recurrence rate for Photodynamic therapy and excision surgery was 14% and 4%, respectively (10). The rate of recurrence after four years in surgical incision with a margin of 2 mm compared to brachytherapy, superficial x-ray therapy, or conventional radiotherapy was reported to be 0.7% versus 7.5% (11). Depending on the physician's skill, the recurrence rate of BCC 5 years after treatment with Electrodesiccation and curettage was 5.7% to 18.1%, respectively (12). Van Loo et al. (13) showed that MMS is more effective in preventing recurrences for both high-risk primary BCC and recurrence BCC

Table 3. Four-Year Rates of Recurrence Following MMS for BCC Patients Based on Demographic and Clinical Characteristics[a]

	Patients (N)	The Event	P Value
Age, y			0.654
< 70	83	2 (2.4)	
> 70	71	1 (1.4)	
Gender			0.736
Male	66	1 (1.5)	
Female	88	2 (2.3)	
Number of lesions			0.749
Single	149	3 (100)	
Multiple	5	0	
Size of tumor, cm			0.515
< 1.5	74	1 (2.7)	
> 1.5	80	1 (1.3)	
Type of tumor			0.682
Primary	143	3 (100)	
Recurrence	8	0	
Tumor clinical form			0.982
Nodular	67	1 (1.5)	
Infiltrating	62	2 (3.2)	
Nodular and infiltrating	16	0	
Micro nodular	6	0	
Adenoid	1	0	
Nodular and pigmented	1	0	
Pigmented	1	0	
Tumor location recurrence			0.869
Nose	87		
Canthus	7		
Lip	6		
Forehead	11		
Temporal	9		
Cheek	17		
Scalp	2		
Ears	8		
Abdomen	1		
Head	1		
Eyelid	3		
Lateral eye	1		
Neck	1		

Abbreviations: BCC, basal cell carcinoma; MMS, Mohs micrographic surgery.
[a]Values are expressed as No. (%).

in the face compared to Surgical excision.

In different studies, the discrepancy in the result of the recurrence rate was mainly due to the differences in the study methods. In another study, Nassiripour et al. (14) showed that the recurrence rate of patients who had the same history in Isfahan, is 9.5% after 4 years of follow-up, which was higher than the present study and the previous studies in Iran. They mentioned that these differences might be due to the investigation of BCC in all parts of the body while others studied the recurrence rate for face BCC. Taheri et al. (15) reported the recurrence rate of scalp BCC 2.26% among 495 cases. According to a retrospective study conducted by Paoli et al. in Sweden, the recurrence rate of BCC after MMS was 3.3% (16). In a randomized clinical trial 30-month follow-up in 408 cases of BCC in the face, the recurrence rate after Mohs micrographic surgery was 1.47% (17). The recurrence rate in Mohs micrographic surgery in a similar study suggested 2.5% (18). The recurrence rate of BCC after 5 years of treatment with the MMS method was approximately 1.4% to 3.2% for primary BCCs and 2.4% to 6.7% for recurrent BCCs (18-20).

The mean age of BCC patients in the present study was 68 years. It suggested that age is one of the risk factors for BCC. The risk of developing BCC increases with age owing to attenuate the ability for repairing damaged deoxyribonucleic acid (DNA) due to ultra violate (UV) radiation, which leads to the accumulation of carcinogens (21). Most of the patients were female although we could not find any significant difference between recurrence rat, age, and sex. In the previous studies, the incidence rate of BCC was reported to be more prevalent in females older than 60 years old which is in accordance whit the results of this study (15, 19, 22) while some investigation reported otherwise (14). This contradiction is probably due to the location of the study and the different ways it is done.

The recurrence time of BCC is important and commonly depends on the treatment method. Rowe et al. reported that most BCC recurrence occurs less than 3 years after treatment (66%) and a small percentage of recurrences occur between 6 and 10 years after primary surgery (23). In the report of Nassiripour et al. (14) regarding the rate of recurrence after surgery, it is stated that a large percentage of recurrences (85%) occur less than three years after surgery. In this study, the recurrence time in 66.7% was less than 15 months and 33.3% more than 15 months.

The most common locations for recurrence in our report were nose and cheek. Although our results were consistent with previous studies (16, 22, 24) the type of research conducted has influenced this conclusion. The results of a study on the whole body showed that the most common lesion was in the scalp (50%), nose (15%), and around the eyes (15%) (14). Leibovitch et al. in 3370 patients in a 5-year follow-up period after MMS, reported that the most common anatomic site was the nose followed by the cheek and maxilla (19).

The most common recurrence sites corresponded to the most common locations of BBC. Previous studies have suggested the same results (16, 19, 22, 24).

The recurrence won't happen in all types of tumors. The more common pathologic type in the samples of this study was infiltrating and nodular-infiltrating. The infiltrating type in the nose was the most form of BCC which had a recurrence. According to previous literature, some forms of BCC like aggressive forms, flat lesions, lesions that are not well limited and perineal invasion, infiltrating, and micro-nodular subtypes of BCC, especially if located on the face, have a higher risk of recurrence (25, 26). According to the BCC form, Zagrodnik et al. (27) reported a recurrence rate of 8.2%, 26.1%, and 27.7% for the nodular, superficial, and sclerosing forms of BCC after 5 years, respectively. In a study of the recurrence rate of BBC in different parts of the body by Anvari et al. (24) after examining 420 patients with NMSC, they reported scalp lesions compared to other locations of the body have a significant recurrence. They explained that this happened probably because of the hardness of removing enough marginal tissue, mainly in large and deep lesions (24). Despite the benefits reported for invasive treatments such as surgery in the treatment of BCC, research has pointed to the weakness of this method, especially in the periocular region, which includes a lack of control of the tumor margin and its possible side effects (28, 29).

Like the other investigation, we found that most of the tumoral size was limited less than 2 cm (19) but the recurrence rate in tumors larger than 2 cm was more than those less than 2 cm. Wolf and Zietlli (30) reported that 95% of lesions which are less than 2 cm were cleared with standard excision (30).

According to the present study, no statistically significant association between tumor location, age, gender, tumor type in terms of clinical diagnosis, tumor size, number of lesions, and the recurrence rate was found.

5.1. Conclusion

The recurrence rate of BCC after MMS (1.9%) is low. For avoiding the early recurrence of BCC among the current treatment methods, MMS is recommended.

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Footnotes

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References

1. Rogers HW, Weinstock MA, Harris AR, Hinckley MR, Feldman SR, Fleischer AB, et al. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. *Arch Dermatol*. 2010;**146**(3):283–7. doi: 10.1001/archdermatol.2010.19. [PubMed: 20231499].
2. Bath-Hextall F, Leonardi-Bee J, Smith C, Meal A, Hubbard R. Trends in incidence of skin basal cell carcinoma. Additional evidence from a UK primary care database study. *Int J Cancer*. 2007;**121**(9):2105–8. doi: 10.1002/ijc.22952. [PubMed: 17640064].
3. Ghafouri-Fard S, Abbasi A, Moslehi H, Faramarzi N, Taba Taba Vakili S, Mobasheri MB, et al. Elevated expression levels of testis-specific genes TEX101 and SPATA19 in basal cell carcinoma and their correlation with clinical and pathological features. *Br J Dermatol*. 2010;**162**(4):772–9. doi: 10.1111/j.1365-2133.2009.09568.x. [PubMed: 19886887].
4. Neville JA, Welch E, Leffell DJ. Management of nonmelanoma skin cancer in 2007. *Nat Clin Pract Oncol*. 2007;**4**(8):462–9. doi: 10.1038/ncponc0883. [PubMed: 17657251].
5. Hamilton JR, Parvataneni R, Stuart SE, Chren MM. Rerecurrence 5 years after treatment of recurrent cutaneous basal cell and squamous cell carcinoma. *JAMA Dermatol*. 2013;**149**(5):616–8. doi: 10.1001/jamadermatol.2013.3339. [PubMed: 23677098]. [PubMed Central: PMC3733327].
6. Silverman MK, Kopf AW, Grin CM, Bart RS, Levenstein MJ. Recurrence rates of treated basal cell carcinomas. Part 1: Overview. *J Dermatol Surg Oncol*. 1991;**17**(9):713–8. doi: 10.1111/j.1524-4725.1991.tb03424.x. [PubMed: 1890243].
7. Kyrgidis A, Vahtsevanos K, Tzellos TG, Xirou P, Kitikidou K, Antoniades K, et al. Clinical, histological and demographic predictors for recurrence and second primary tumours of head and neck basal cell carcinoma. A 1062 patient-cohort study from a tertiary cancer referral hospital. *Eur J Dermatol*. 2010;**20**(3):276–82. doi: 10.1684/ejd.2010.0903. [PubMed: 20406722].
8. Prickett KA, Ramsey ML. *Mohs micrographic surgery. StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing; 2020, [cited Jan].
9. Totonchy M, Leffell D. Emerging concepts and recent advances in basal cell carcinoma. *F1000Res*. 2017;**6**:2085. doi: 10.12688/f1000research.11314.1. [PubMed: 29259776]. [PubMed Central: PMC5717469].
10. Rhodes LE, de Rie MA, Leifsdottir R, Yu RC, Bachmann I, Goulden V, et al. Five-year follow-up of a randomized, prospective trial of topical methyl aminolevulinate photodynamic therapy vs surgery for nodular basal cell carcinoma. *Arch Dermatol*. 2007;**143**(9):1131–6. doi: 10.1001/archderm.143.9.1131. [PubMed: 17875873].
11. Avril MF, Auperin A, Margulis A, Gerbaulet A, Duvillard P, Benhamou E, et al. Basal cell carcinoma of the face: surgery or radiotherapy? Results of a randomized study. *Br J Cancer*. 1997;**76**(1):100–6. doi: 10.1038/bjc.1997.343. [PubMed: 9218740]. [PubMed Central: PMC2223779].
12. Kopf AW, Bart RS, Schrager D, Lazar M, Popkin GL. Curettage-electrodesiccation treatment of basal cell carcinomas. *Arch Dermatol*. 1977;**113**(4):439–43. [PubMed: 848972].
13. Van Loo E, Mosterd K, Krekels GA, Roozeboom MH, Ostertag JU, Dirksen CD, et al. Surgical excision versus Mohs' micrographic surgery for basal cell carcinoma of the face: A randomised clinical trial with 10 year follow-up. *Eur J Cancer*. 2014;**50**(17):3011–20. doi: 10.1016/j.ejca.2014.08.018. [PubMed: 25262378].
14. Nassiripour L, Amirsadri M, Tabatabaeian M, Maracy MR. Factors affecting recurrence and costs of basal cell carcinoma undergoing mohs micrographic surgery: A hospital-based retrospective cohort study. *Int J Cancer Manag*. 2019;**12**(8). en. e84695. doi: 10.5812/ijcm.84695.
15. Taheri A, Khoshnevisan A, Alipour A, Khorasani G, Molaei H. Survival and recurrence in non-melanoma skin cancers of scalp. *MCI J*. 2018;**2**(4):25–9.
16. Paoli J, Daryoni S, Wennberg AM, Molne L, Gillstedt M, Miocic M, et al. 5-year recurrence rates of Mohs micrographic surgery for aggressive and recurrent facial basal cell carcinoma. *Acta Derm Venereol*. 2011;**91**(6):689–93. doi: 10.2340/00015555-1134. [PubMed: 21681360].
17. Essers BA, Dirksen CD, Nieman FH, Smeets NW, Krekels GA, Prins MH, et al. Cost-effectiveness of Mohs micrographic surgery vs surgical excision for basal cell carcinoma of the face. *Arch Dermatol*. 2006;**142**(2):187–94. doi: 10.1001/archderm.142.2.187. [PubMed: 16490846].
18. Mosterd K, Krekels GA, Nieman FH, Ostertag JU, Essers BA, Dirksen CD, et al. Surgical excision versus Mohs' micrographic surgery for primary and recurrent basal-cell carcinoma of the face: a prospective randomised controlled trial with 5-years' follow-up. *Lancet Oncol*. 2008;**9**(12):1149–56. doi: 10.1016/S1470-2045(08)70260-2. [PubMed: 19010733].
19. Leibovitch I, Huilgol SC, Selva D, Richards S, Paver R. Basal cell carcinoma treated with Mohs surgery in Australia II. Outcome at 5-year follow-up. *J Am Acad Dermatol*. 2005;**53**(3):452–7. doi: 10.1016/j.jaad.2005.04.087. [PubMed: 16112352].
20. Smeets NW, Kuijpers DI, Nelemans P, Ostertag JU, Verhaegh ME, Krekels GA, et al. Mohs' micrographic surgery for treatment of basal cell carcinoma of the face–results of a retrospective study and review of the literature. *Br J Dermatol*. 2004;**151**(1):141–7. doi: 10.1111/j.1365-2133.2004.06047.x. [PubMed: 15270883].
21. Moriwaki S, Ray S, Tarone RE, Kraemer KH, Grossman L. The effect of donor age on the processing of UV-damaged DNA by cultured human cells: reduced DNA repair capacity and increased DNA mutability. *Mutat Res*. 1996;**364**(2):117–23. doi: 10.1016/0921-8777(96)00029-8. [PubMed: 8879277].
22. Kuiper EM, van den Berge BA, Spoo JR, Kuiper J, Terra JB. Low recurrence rate of head and neck basal cell carcinoma treated with Mohs micrographic surgery: A retrospective study of 1021 cases. *Clin Otolaryngol*. 2018;**43**(5):1321–7. doi: 10.1111/coa.13176. [PubMed: 29953746].
23. Rowe DE, Carroll RJ, Day CJ. Mohs surgery is the treatment of choice for recurrent (previously treated) basal cell carcinoma. *J Dermatol Surg Oncol*. 1989;**15**(4):424–31. doi: 10.1111/j.1524-4725.1989.tb03249.x. [PubMed: 2925988].
24. Anvari K, Hosseini S, Toussi MS, Afifi S. Non melanoma skin cancers: a retrospective study in department of radiation oncology, Mashhad, Iran. *Iranian Journal of Dermatology*. 2014;**17**(1):27–30.
25. Brown CI, Perry AE. Incidence of perineural invasion in histologically aggressive types of basal cell carcinoma. *Am J Dermatopathol*.

2000;**22**(2):123–5. doi: 10.1097/00000372-200004000-00006. [PubMed: 10770431].
26. Raasch BA, Buettner PG, Garbe C. Basal cell carcinoma: histological classification and body-site distribution. *Br J Dermatol*. 2006;**155**(2):401–7. doi: 10.1111/j.1365-2133.2006.07234.x. [PubMed: 16882181].
27. Zagrodnik B, Kempf W, Seifert B, Muller B, Burg G, Urosevic M, et al. Superficial radiotherapy for patients with basal cell carcinoma: recurrence rates, histologic subtypes, and expression of p53 and Bcl-2. *Cancer*. 2003;**98**(12):2708–14. doi: 10.1002/cncr.11798. [PubMed: 14669293].
28. Netscher DT, Spira M. Basal cell carcinoma: An overview of tumor biology and treatment. *Plast Reconstr Surg*. 2004;**113**(5):74–94. doi: 10.1097/01.prs.0000113025.69154.d1.
29. Telfer NR, Colver GB, Bowers PW. Guidelines for the management of basal cell carcinoma. *Br J Dermatol*. 1999;**141**(3):415–23. doi: 10.1046/j.1365-2133.1999.03033.x. [PubMed: 10583044].
30. Wolf DJ, Zitelli JA. Surgical margins for basal cell carcinoma. *Arch Dermatol*. 1987;**123**(3):340–4. [PubMed: 3813602].