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Can basal cell carcinoma lateral border be determined by fluorescence diagnosis? Verification by Mohs micrographic surgery

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ABSTRACT

Background: The preferential accumulation of 5-aminolaevulinic acid (ALA)-induced protoporphyrin IX (PpIX) in neoplastic cells supports its potential use in the photodetection of epithelial tumours through porphyrin fluorescence.
Objective: To assess the validity of fluorescence diagnosis (FD) as an efficient pre-surgical *in vivo* imaging tool for defining the lateral boundaries of various types of basal cell carcinomas (BCCs).
Methods: The BCC tumour area was determined for 27 patients using FD digitalized imaging system, where the accumulation of PpIX in tumour tissue in relation to normal tissue was measured. Subsequently, BCCs were excised according to the complete area defined by FD using Mohs micrographic surgery (MMS).
Results: Of the 27 BCCs, the FD margin of the lesion coincided with the histopathological picture in 12 BCCs (44.44%). The mean value of accumulation factor (AF) was 2.7. Although 17 pigmented BCCs showed attenuated or absent fluorescence in the center, fluorescence at their periphery was used as a guide for excision, and statistically, the pigmentation of the BCCs showed no effect on the results of the FD efficacy ($p = 1.0$).
Conclusion: Fluorescence diagnosis of BCC may be beneficial as a guide to the safety margin needed before MMS. The safety margin is decided according to the FD tumour diameter in relation to the clinical tumour diameter.

1. Background

The standard management of basal cell carcinoma (BCC) is surgical excision with a safety margin varying between 4 and 10 mm [1,2], followed by postoperative histological examination. A significant proportion of excised BCCs demonstrate histologically positive surgical margins varying between 16.6% [3] and 20% [4]. This is mainly due to a subclinical spread, apparently caused by the histological pattern and irregular infiltration of these tumors [2,5]. Therefore, recurrence rates after simple surgical excision may vary from 1.3 to 10% for low risk BCCs and exceed 17% for high risk BCCs [6–11].

Mohs micrographic surgery is known to be the most precise and accurate method to completely remove a BCC. In the setting of standard surgical management, or MMS, of BCC, it is crucial to find a diagnostic preoperative procedure that would help predict the precise *in vivo* tumor size and lateral borders. Its most important feature must be how well this technique corresponds to the histological tumor boundary. Such a diagnostic tool would lead to better preoperative planning and better cosmetic outcomes.

The fluorescence diagnosis (FD) technique entails the application of 5-aminolevulinic acid (ALA) resulting in increased synthesis of porphyrins, leading to a higher concentration ratio of protoporphyrin IX (PpIX) in tumor tissue, because of its increasing metabolic demands [12].

2. Aim of the study

To assess the validity of FD as an efficient pre-surgical *in vivo* imaging tool for defining the lateral boundaries of various types of BCCs.

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3. Patients

This study was designed as a prospective investigational study that included 27 patients with lesions that were clinically and histologically diagnosed as BCC. BCCs that were less than 1.5 cm in diameter were chosen to facilitate accurate mapping of FD.

4. Methods

Approval was obtained from the Kasr El Aini Hospital research ethics committee, as well as written informed consent from patients before study-related procedures were performed.

4.1. Clinical and Histopathological examination

A full history was obtained from patients followed by the lesion examination. A 3 mm punch biopsy was obtained from lesions and stained by hematoxylin and eosin to document the diagnosis of BCC and determine the histological subtype.

4.2. Fluorescence Diagnosis (FD) procedure

A cream containing 20% 5-aminolevuelinic acid HCl was applied to the lesion and 1 cm of surrounding normal-appearing skin and covered by an occlusive dressing. After 3 h of incubation, fluorescence intensity on the skin was recorded using a digital fluorescence imaging system (Dyaderm, Biocam GmbH, Regensburg, Germany). This system consists of a flash light (xenon light source with a custom band pass filter 370–440 nm) and a 12-bit charged coupled device (CCD) camera combined in one adjustable arm paired with a computer system equipped with custom image capturing software. The resulting image is referred to as "PpIX filtered". PpIX-filtered false-color images automatically display (image segmentation) the highest fluorescence value in red and the lowest in blue [13]. Images of the tumors were taken at a fixed distance of 8 cm. Side effects of FD, if present, were documented. After imaging, two demarcation lines were drawn using the pen in the digital system on the normal colored red–green–blue (RGB) image generated by the system in concordance with the border of the clinically visible tumor margin (line A) and at a further 1 mm distance (line B) (Fig. 1A).

4.2.1. Accumulation factor calculation

On the basis of unfiltered PpIX images (Fig. 1B) the accumulation factor (AF) was calculated, which is the ratio of PpIX fluorescence of tumor to that of surrounding normal skin, and is measured as a unit. This measures the selective accumulation of PpIX in tumor tissue compared to the surrounding tissue.

4.3. Mohs micrographic surgery (MMS) procedure

MMS was performed at a later date in the same week. To verify the histologic lateral spread of the tumor at the margins of BCC, MMS was performed at 1 mm stages to compare its results to that of the FD. If the FD tumor area selected was coinciding with or smaller than the clinical tumor margin demarcation (line A), the MMS excision was done at the 1 mm demarcation line (line B) (Figs.1B, 3B). In case the FD of the tumor area was larger than the demarcation line B (Fig. 2B), the MMS excision was done at an additional 1 mm beyond that demarcation line. The area of excision was anaesthetized using lidocaine 2% with 1:200,000 epinephrine, and the lesion was excised as one saucer-shaped piece and put on gauze while preserving the orientation using a blood dot at 12 o′clock. The lesion was then divided in 2 or 4 pieces and color-coding was performed using red, green and yellow colors at the cut edges of the specimen. An MMS map was then drawn on paper to document the orientation and color-coding of the specimen. The tumor specimen was 1st fresh frozen in OCT then sliced to 4–6 μm sections using a cryostat (SLEE MEV Semi-Automatic Cryostat-Germany). Sections were then put on glass slides and stained with Toluidine blue. If residual tumor was found, its location was marked on the MMS map and excised, mapped, sectioned, and re-examined under the microscope. The procedure was repeated until clear margins were obtained.

Table 1
Summary of demographic and clinical data of patients ($n = 27$).

Item		Values
Age (years)	Range	38–76
	Mean ± SD	62.3 ± 10.6
Sex (no, %)	Males	19 (70.4%)
	Females	8 (29.6%)
Disease Duration (years)	Range	1-15
	Mean ± SD	3.6 ± 3.5
Diameter of BCCs (cm)	Mean ± SD	1.05 ± 0.35
Subtypes of BCC (no, %)	Nodular	15 (55.5%)
	Infiltrative	6 (22.2%)
	Mixed	4(14.8%)
	Superficial	1 (3.7%)
	Adenoid	1(3.7%)
BCC Primary vs Recurrent (no, %)	Primary	23 (85.1%)
	Recurrent	4 (14.8%)

SD: standard deviation, vs: versus.

4.4. Statistical analysis

Data was statistically described in terms of mean and standard deviation (SD), median and range, and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann Whitney *U* test for independent samples when comparing 2 groups and Kruskal Wallis test when comparing more than 2 groups. For comparing categorical data, Chi square test was performed. Exact test was used instead when the expected frequency is less than 5. *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using the computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

5. Results

Twenty-seven BCC patients were the subjects of the present study. Their demographic and clinical data are summarized in Table 1.

5.1. Relation of FD tumor margin in comparison to the clinical tumor

The FD tumor margin coincided with the clinical tumor margin in 9 patients (Fig. 1). The excision margin selected according to the FD results was histologically negative for BCC in 2 patients and positive for BCC (*i.e.*, failed to show the subclinical extent of the tumor) in 7 patients. The FD tumor margin either extended beyond the clinical tumor margin (Fig. 2) or was smaller than that of the clinical tumor margin (Fig. 3) in the remaining 18 cases. Statistical analysis of the FD image diameter compared to the clinical tumor diameter in relation to the FD results was found to be statistically insignificant ($p = 0.247$) (Table 2).

5.2. Efficacy of FD in delineating the lateral margin of BCC evaluated by MMS

Of the 27 BCCs, the FD margin of the lesion coincided with the histopathological picture in 12 BCCs (44.44%) and did not

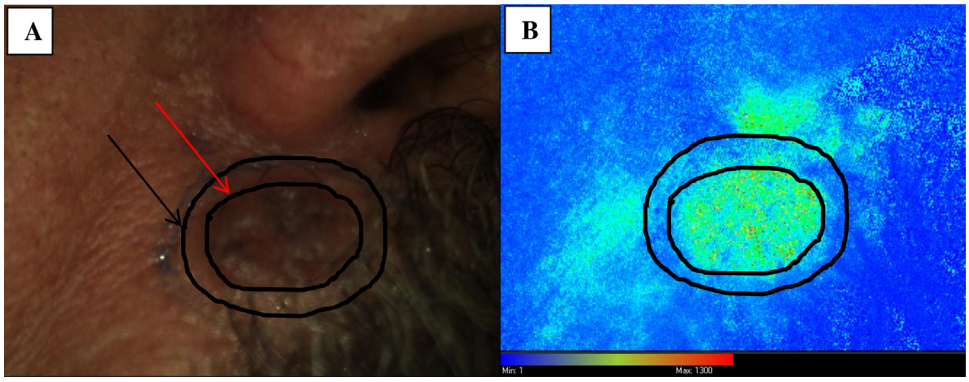

Fig. 1. (A) Coloured image, red arrow: line A, black arrow: line B. (B) PpIX filtered image in pseudocolor. The FD image diameter of the tumour is exactly the same size as the clinical tumour diameter in patient number 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

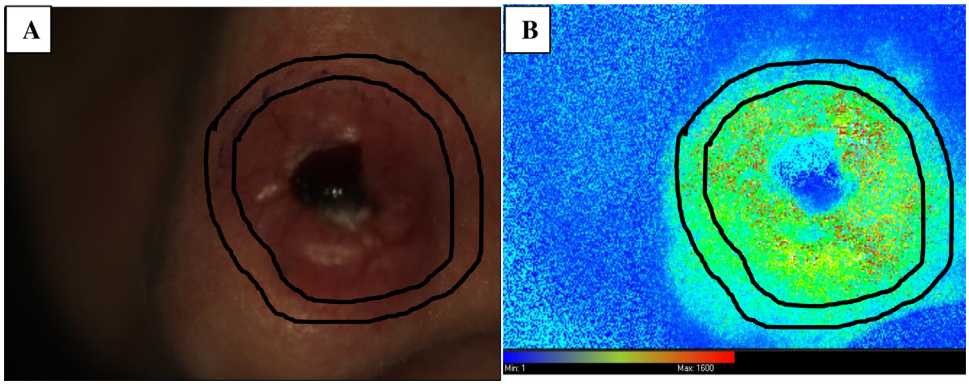

Fig. 2. (A) Coloured image. (B) PpIX filtered image in pseudocolor. The FD tumour diameter image is bigger than the clinical tumour diameter in patient number 6.

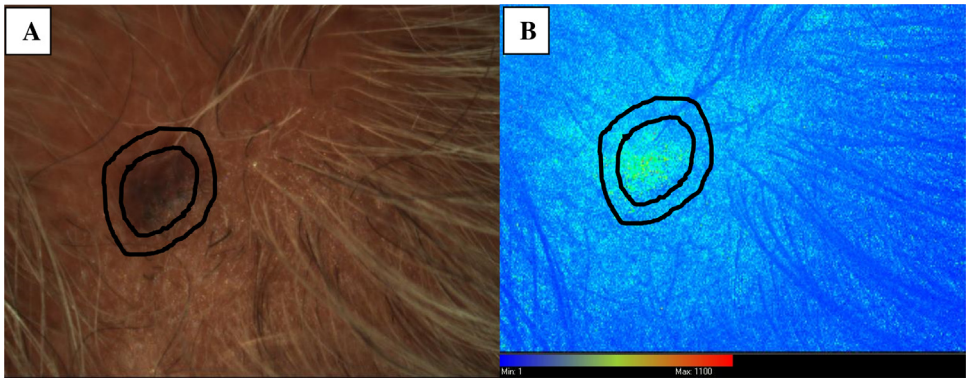

Fig. 3. (A) Coloured image. (B) PpIX filtered image in pseudocolor. The clinical tumour diameter is bigger than the FD tumour diameter in patient number 8.

Table 2
The relation of the FD tumour image diameter compared to the clinical tumour diameter in association with the FD results ($n = 27$).

FD tumour diameter vs clinical tumour diameter	Total number of patients	Excision margin at 1 mm distance −ve for BCC	Excision margin at 1 mm distance +ve for BCC	*P* value
Exact	9	2	7	0.247
Bigger	15	8	7	
Smaller	3	2	1	

vs: versus

coincide in 15 BCCs (55.55%). Of the latter, the number of MMS stages required to clear the lateral margin, each at a further 1 mm excision, was an additional one stage in 14 BCCs (93.3%) and an additional two stages in one BCC (6.6%).

5.2.1. FD results in relation to BCCs diameter

It was found that twelve BCCs had their fluorescence coinciding with the histopathological extent, with a mean diameter of 0.94 ± 0.31 cm. The other 15 lesions, where FD did not coincide with the histopathological extent, had a mean diameter of

1.2 ± 0.39 cm. Subsequently, patients were sub-grouped according to the diameter of their BCC into 2 groups: patients with BCC > 1 cm, and BCC ≤ 1 cm. Thirteen patients had BCC > 1 cm, only two of which showed FD results coinciding with the histopathological picture, whereas 14 patients had BCC ≤ 1 cm, 10 of which showed FD results coinciding with the histopathological picture, ($p = 0.03$). This indicates that FD is better in detecting tumor margin extent in BCC lesions less than 1 cm.

Pigmentation of BCC did not allow fluorescence to appear visually on imaging. Seventeen patients had pigmented BCCs, which showed attenuated or absent fluorescence in the center of the lesion. However, the periphery showed good evidence of fluorescence, which was used to demarcate the lateral margin. Statistical analysis of the effect of BCC pigmentation on the FD results was done and showed no significance ($p = 1.0$).

The fluorescence intensity in ulcerated lesions was decreased as well. Fluorescence diagnosis results and their coincidence with the tumor margin was not affected by its histological subtype ($p = 0.358$), or its location ($p = 0.704$).

5.3. The relation between the FD results and the accumulation factor (AF)

The mean value of the AF (as a measure of fluorescence intensity) of BCCs ranged between 0.77 and 5.6 with a mean value of 2.43. The mean value for AF in BCCs with FD results coinciding with the histopathologic margin was 1.9 ± SD 1.4 and the mean value for AF for BCCs with non-coinciding FD results was 2.7 ± 1.4.

5.4. The relation between the AF and the histological subtypes was assessed

The mean value of the AF of nodular BCCs was 2.6. The mean value of superficial BCCs was 3 and the mean value of infiltrative BCCs was 2.4. The mean value of mixed BCCs was 1.5 and that of adenoid was 2.5. The only case of superficial BCC showed the highest value for AF, while the relation between the AF and the different histological subtypes turned out to be statistically insignificant ($p = 0.706$).

5.5. Side effects and drawbacks of the FD procedure

In 14.81% (4/27) of patients a mild transient erythema within the treated areas was observed after the FD procedure. The erythema was induced by mild irritation to the applied ALA–HCl under occlusion, and it resolved after a few days. This mild inflammation did not affect the outcome of Moh's surgery.

6. Discussion

Fluorescence diagnosis FD of basal cell carcinomas BCC has been studied by a number of research groups. Only a few groups have compared fluorescence imaging with histology. Our study showed that the FD tumor margin coinciding with the clinical tumor margin was sensitive in 44.44% of cases. Similarly, Wetzig et al. [14] showed that FD using Dyaderm digital system on BCCs in the H-zone of the face was sensitive in 38.5% of cases. Their study was comparable to our study, with regard to the number of patients, ALA cream application time and the tumor sizes. As they took all their excisions at a fixed 3 mm safety margin outside that suggested by the FD margin, they concluded that FD is of no clinical benefit in defining the lateral BCC margins or detecting subclinical spread [14]. In the current study, the excision safety margin was taken at a 1 mm safety margin outside that suggested by the FD. Each MMS stage was taken at a further 1 mm distance. Thus, it was possible to calculate the necessary safety margin outside that suggested by the FD. In most of the cases (93.3%), in which the margin suggested by the FD was still positive for BCC, it required only one further MMS stage to reach complete histological clearance.

Thus, if the FD tumor margin diameter appeared to be exactly the same size as the clinical tumor diameter, excision at 2 mm safety margin is needed for complete removal. As for BCCs, in which the FD results show that the FD tumor margin diameter is extending beyond the clinical tumor diameter, an excision with a safety margin of 2 mm is needed if the BCC diameter is ≤1 cm; and at least a 3 mm safety margin is necessary in cases of BCCs with a diameter >1 cm. Based on these findings, FD can be used as a guide in determining the safety margin needed for BCCs less than 1 cm in diameter. In larger lesions this time consuming pre-procedural investigative step does not seem to have added value.

In this study it was shown that ulceration or pigmentation of the lesion did not show fluorescence. It has been previously reported that pigments resulted in lower therapeutic effects of PDT in pigmented BCCs [15]. As we used the fluorescence at the periphery of the pigmented BCCs as a guide for excision, statistically the pigmentation of the BCCs showed no effect on the results of the FD efficacy. Thus, although PDT is not recommended for pigmented BCC lesions, FD may still be used to determine the boundary of pigmented BCCs before surgery. Furthermore, this explains why on correlating the area of BCC indicated by the FD image with the clinically diagnosed BCC area a mean FD tumor area smaller than the mean clinically diagnosed tumor area was revealed, and was considered a less sensitive technology [16]. Hence, FD should be evaluated for BCCs at the tumor margins.

Various studies evaluated the fluorescence intensity of BCC using Wood's lamp [17,18]. We consider FD using the digital Dyaderm digital system to be superior to conventional FD using Wood's lamp, as it can be done in an ambient light situation. With the use of Dyaderm digital system the photobleaching of PpIX is minimized, due to the short exposure time to the excitation light. The data analysis is enhanced by numerous analyzing features including optimal contrast enhancement by false color display and elimination of interferences due to the referencing and standardization of fluorescence images. The property of calculating the fluorescence ratio of diseased skin to normal skin presented as the accumulation factor (AF) is present only in the digitalized FD system. The AF ratio varies from one hyperproliferative condition to another, according to variation in the level of ALA-induced protoporphyrin accumulation. It was found more pronounced in psoriasis (1.77) in comparison to cases of actinic keratosis (1.37) [19], whereas in mycosis fungoides it was found to have a mean of 2.2 [13]. In this study, the mean AF, which is the fluorescence ratio of BCC to normal skin, was 2.4. In another study using ALA as a photosensitizer, the mean ratio was 1.88 [16]. It is to be noted that fluorescence ratios (porphyrin resistance) of BCC to normal skin are 2:1 with the use of ALA, which is different from fluorescence ratio with the use of MAL as a photosensitizer, which is 10:1 [20]. This is related to the enhanced lipophilicity and better penetrative properties of MAL [21]. The mean value of AF of BCCs, in which the FD tumor margin did not coincide with the histologic margin, was higher, although not statistically significant, than the mean value of BCCs, in which the FD margin was coinciding with the histologic margin. This denotes that the AF may be a potential guide to the extent of margin excision beyond the FD tumor margin. Superficial BCC exhibited the highest level of AF, which must be related to its cells being more accessible to the photosensitizer.

Perhaps a better evaluation for this finding would be to measure the vertical thickness of the tumor through vertical sections instead of the transverse sections that we use in MMS.

Our results showed no statistically significant relation between the histological subtype of a BCC and the effectiveness of FD as a guide for the lateral boundary before surgery. Aggressive

histological subtypes of BCCs tend to have a wider subclinical spread. Out of 6 infiltrative BCCs, 5 showed FD results not coinciding with the histologic margin. This was not statistically significant due to small sample size. However, it may be that the increased depth of subclinical spread of infiltrative BCCs does not show as fluorescence at the edges. Also on comparing the AF in the different histological subtypes, there was no statistical significance. Other studies did not compare the histological subtype with the FD efficacy.

7. Conclusion

In conclusion, FD can potentially be useful in guiding the dermatologic surgeon to the adequate excision margin. When the FD tumor diameter is the same size as the clinical tumor diameter; a safety margin of 2 mm can be used. When the FD tumor diameter is larger than the clinical tumor diameter, a safety margin of 2 mm can be used if the tumor diameter is <1 cm and a safety margin of 3 mm can be used if the tumor diameter is >1 cm. In the case of MMS, FD may be useful in limiting the number of stages to one stage, thus significantly decreasing the time consumed to complete the procedure.

Recommendations

FD using Dyaderm digital system may be beneficial in determining the safety margin needed before MMS. However refinement of the technique is needed for BCC larger than 1.5 cm.

Usage of FD before standard surgical excision is recommended to decrease the incidence of incomplete excisions.

Validity of the suggested 2 mm margin for BCCs less than 1 cm diameter as a tissue sparing method needs to be evaluated by a 5-years follow-up study to assess recurrence rates.

Conflict of interest

The DyaDerm system was donated to the Dermatology Department of Cairo University by Biocam GmbH, Regensburg, Germany. The firm had otherwise no relation with the study idea, design, method, study results analysis, manuscript preparation and or publication decision.

Disclosures

None declared.

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