Short Communications

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Optimizing Ideal Thickness of Sections for Epidermis in Intra-Operative Frozen Sections

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Abstract

Aim and objectives: To estimate and evaluate the optimal thickness of tissue section for skin and dermal appendages intra-operatively and to compare the morphology and architecture of the epidermis and its appendages at 7 and 10 microns. **Methodology:** After obtaining clearance from the institutional human ethical committee, 101 skin margins were prospectively analyzed using a two-step embedding technique. After multiple trials and errors, 7- and 10-micron thicknesses were selected for the present study. Artefacts, staining characteristics, cellular morphology, cellular outline, and nuclear outline were assessed and scored as unacceptable or acceptable. The data were entered in a Microsoft Excel spreadsheet and analyzed using SPSS software. **Results:** There was a statistically significant difference between the sections obtained at 7 microns and 10 microns (p-value: <0.001), the latter were better in all the parameters analyzed. However, no difference was noted in the characteristics of the dermal appendages (p-value: >0.05). **Conclusion:** While mucosal margins can be obtained at the usual 5-7 microns, the same thickness is not optimal for skin margins intra-operatively. Frozen sections for the skin margins may be set at 10 microns, to save time, minimize artefacts, and for better readability.

Keywords: Frozen- intraoperative- thickness- skin

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Introduction

Skin biopsies/margins, received in frozen room, are generally performed to assess the sufficiency of the tissue margins (primarily for basal cell carcinoma or squamous cell carcinoma), for removal of the entire lesion and confirmation of the clinical diagnosis, and margin evaluation of other skin neoplasms or assessment of direct skin involvement in primary malignancies of other anatomical sites such as oral squamous cell carcinoma (OSCC) [1]. OSCC is a multifactorial and the most common neoplasm of the head and neck region with grave outcomes despite advancements in therapeutics [2-4]. There are numerous clinicopathological factors that dictate the prognosis in OSCC such as neural or vascular invasion, depth of invasion, tumor-infiltrating lymphocytes, tumor budding, tumor stroma ratio, extra-nodal extension, clinical staging, and, involvement of the adjacent structures like bone, skin, masticatory spaces or base of the skull [4-6]. According to the current staging system for OSCC, the involvement of facial skin upgrades the tumor to c/pT4a [7].

As aforementioned, the involvement of skin and resected margins are crucial predictive and prognostic factors for any malignant lesion. However, from the surgical pathologist's side, the attainability of sections with adequate readability in minimal time dictates the surgical procedure, involving the requirement of revised margins, which in particular is crucial for aesthetic areas in maxillofacial malignancies. According to the literature, a 5–10-micron thickness is usually applied depending on the type of tissue examined. In our surgical oral oncopathology unit, 5-7 microns section thickness is used for mucosal margins. However, the same thickness appears unsatisfactory for the epidermis. The aim of the study is to intra-operatively estimate and evaluate the optimal thickness of tissue section for skin and dermal appendages, to compare the morphology and architecture of the epidermis and its appendages at 7 and 10 microns.

Materials and Methods

A prospective observational study was designed and conducted in the Department of Oral Pathology & Microbiology in association with the Department of Oral Oncology at a private tertiary health care institution of Chennai, after obtaining approval from the institutional human ethical committee board (IHEC/SDC/OPATH-2204/23/273). The present study included 101 skin margins obtained from patients with histologically confirmed oral squamous cell carcinoma with clinical T4a (involvement of the facial skin) to evaluate the optimal

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thickness of tissue section for skin and dermal appendages and to compare the morphology and architecture of epidermis and its appendages at 7 and 10 microns. After multiple trials and errors, cut-off values of 7 and 10 microns were selected, where below 7 there were gross artifactual defects which included curling/chattering of tissues yielding sections beyond readability. Sections more than 10 μ, were thick. Sections at 8 and 9 microns, were sub-optimal to analyze microscopically.

A double-step procedure for embedding of tissues was followed for all the sections as previously described [8]. Briefly, chucks were prepared in advance prior to receiving the tissue by applying a very thin layer of optimal cutting temperature media (OCT media) over the chucks and allowed to freeze inside the cryostat at -16°C to -18°C. The received specimen was then examined thoroughly and appropriate mucosal and skin margins were taken. The following steps included the placement of tissues over the prepared chucks with a thin layer of OCT media, the application of another layer of OCT media (enough to coat the tissue entirely), and placement on the freezing stand with heat extractor in place. After the tissues were set, two sections were cut for all the skin margins, at 7 microns thickness, and at 10 microns thickness, to evaluate two hundred and two sections in total. The respective sections were then carefully retrieved on clean marked glass slides followed by routine fast H&E staining for frozen sections. The sections were mounted in DPX media. The sections were divided into two groups: Group I- sections at 7 microns and Group II- 10 microns. All 202 sections were evaluated by two pathologists (DP and RPK) for five parameters which included, evaluation of artefacts (curl, chatters, detachment of epidermis from dermis), staining characteristics, cellular morphology, cellular outline, and nuclear outline. Epidermis, sweat glands, and sebaceous glands were individually examined for all parameters. The sections were finally semi-qualitatively categorized into unacceptable (score 0), and acceptable (score 1). Agreement between the two observers was observed and analyzed using Kappa statistics. The values of 0–0.20, 0.21–0.39, 0.40–0.59, 0.60–0.79 0.80–0.90, and above 0.90 were considered as none, minimal, weak, moderate, strong, and almost perfect levels of agreement respectively [9]. All the collected data were entered in Microsoft Excel Spreadsheet (2021), a database was generated using SPSS statistics software version 26 (IBM Corp., Armonk, NY, USA) and analysis was done using Chi-square test. A p-value below or equal to 0.05 was considered statistically significant.

Results

We evaluated 101 skin tissue margin sections (202 in total) to obtain an optimal thickness of tissue section for skin and dermal appendages and compared the morphology and architecture of the epidermis and its appendages at 7 and 10 microns. Two hundred and two sections were studied individually by two observers and a Kappa value of 0.835 was estimated, suggesting a strong inter-observer agreement.

Epidermis

For evaluation of epidermis, two sections were taken at 7 and 10 microns. In Group I (7 μ), 85.15% of sections were unacceptable for readability and interpretation. The major flaws that led to difficulty in interpretation were chatter and curling of the overlying skin (Figure 1a-b). In contrast, 95.05% of sections in the second group (cut at 10 microns) were acceptable with only 4.95% of unacceptable sections (Table 1). There was a significant difference between the sections cut at these two thicknesses (p-value: <0.001).

Dermal appendages

Pilosebaceous units including hair follicles with sebaceous glands and sweat glands were examined. For hair follicles with sebaceous glands, overall, most sections were acceptable for histopathological examination (191/202). Group I demonstrated 93.1% (94/101) acceptable sections and only 6.9% (7/101) sections were not acceptable histologically (Figure 1b-c). Similarly, 96.1% of sections were acceptable for readability and interpretation in the second group. No statistically significant difference was noted between the study groups (p-value: 0.269) (Table 1).

For the evaluation of the sweat glands at the skin margins, group I showed 95 acceptable sections and only 6 unacceptable sections. Group II demonstrated 94 acceptable sections and 7 unacceptable sections (Figure 1e-f). No statistically significant difference was seen in the morphology of sweat glands at the skin margins (p-value: >0.500) (Table 1).

Discussion

Surgical pathology is an indispensable part of onco-surgeries which involves the examination of tissues from the patients during surgery for diagnosis of ambiguous cases and the development of treatment strategies for such cases and intra-operative assessment of the surgical margins. The cryostats were introduced in around 1960s; however, the modern frozen technique which is practiced globally was developed in 1905 by Dr Louis B Wilson [10]. Ideally, surgical pathology commences with gross visual examination of the obtained tissue with the naked eye followed by cryotomy and subsequent microscopic examination of the processed tissue. Thus, the pathologist is in a position to inform the operating surgeon about the nature of the lesion, and the necessity of further sampling or investigation, if any. Three T's form the basis of surgical pathology namely, adequate Tissue sampling, simple Technique and minimal possible Time [8].

Previous studies have assessed sensitivity and specificity of over 80% using intra-operative frozen sections [10, 11]. In a single institutional study, Adhikari P et al, reported an accuracy of 97% for frozen sections, similar results were demonstrated by Layfield EM et al. [11- 12], Nayanar SK et al. in another study, from a south Indian cancer centre, reported more true negative cases as compared to true positives [10]. As aforementioned, evaluation of skin margins is mandatory in T4a cases of oral squamous cell carcinoma or primary melanocytic

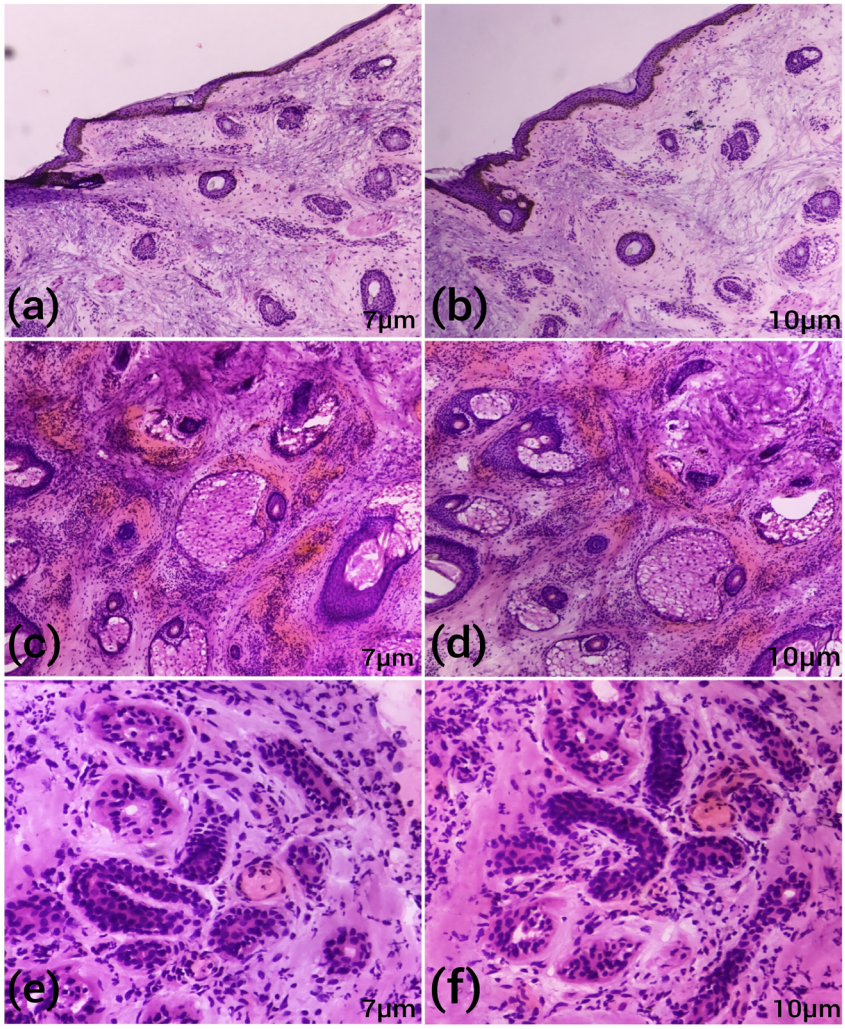

Figure 1. Photomicrographs of H&E Stained Intra-Operative Frozen Section of Skin and Dermal Appendages (a, c, e at 7 Micron Thickness and b, d, f at 10 Microns). a and b- epidermis (40X), c and d-hair-follicles and sebaceous glands (100X), e and f- sweat glands (400X)

or nonmelanocytic malignancies of skin. In any case, a faster procedure with minimal turnaround time (intra-operatively) and minimal artefacts are essential for the success of any oncosurgery.

We hereby used a double-step embedding technique for intra-operative evaluation of skin evaluation margins for the estimation of an optimal thickness. At 7 microns and 10 microns thickness, there was no statistically significant difference in cellular/nuclear morphology and outline of dermal appendages. At many times, tangentially cut hair follicles may be misdiagnosed as basal cell carcinomas (BCC) of the skin [1]. Peri-tumoral clefting, high mitotic index, and apoptotic cells in the histological sections favor a diagnosis of BCC. Additionally, unlike the tumor islands of BCC, a normal hair follicle is surrounded by a fibrous sheath, and histochemical stains may prove fruitful. These structures were adequately demonstrated even at 10 microns. The major difficulty lies in obtaining an optimal section of the epidermis without any artefacts for faster intra-operative diagnosis. Despite being a routine procedure in all onco-centers globally, there is no much literature available on the optimisation of section thickness of skin. We found that the best sections were obtained at 10 microns, with a statistically significant difference

Table 1. Comparative Evaluation of Epidermis, Pilosebaceous Units and Sweat Glands at 7 Microns and 10 Micron Thickness, Intraoperatively; # not significant, *statistically significant

Tissue analysed	Groups	Unacceptable	Acceptable	P value
Epidermis	Group I (7 micron)	86 (85.15%)	15 (14.85%)	0.000*
	Group II (10 micron)	5 (4.95%)	96 (95.05%)	
Pilosebaceous units	Group I (7 micron)	7 (6.9%)	94 (93.1%)	0.269#
	Group II (10 micron)	4 (3.9%)	97 (96.1%)	
Sweat glands	Group I (7 micron)	6 (5.9%)	95 (94.1%)	0.500#
	Group II (10 micron)	7 (6.9%)	94 (93.1%)	

between the study groups. Thus, we believe 10 microns may be considered as optimal sections for skin and its appendages during frozen section procedures; 6-7 microns are reserved for mucosal margins.

Intraoperative assessment of surgical margins is crucial. While negative/clear margins positively improve the survival of the patients, positive margins appear to be the biggest nightmares for the operating surgeons, and pathologists and dismally affect prognosis. We hereby, in our prospective study, demonstrated that 10 microns is the optimized thickness for intra-operative skin sections, unlike the lesser thickness for mucosal margins.

Author Contribution Statement

Kavya Dharmaraj: Design, data acquisition and interpretation, Contributed to conception, initial draft, literature search and critically revised the manuscript; Deepak Pandiar: Conception & design, data acquisition and interpretation, image retrieval and analysis, performed all statistical analyses, literature search and drafted the manuscript; Reshma Poothakulath Krishnan: Contributed to conception, image retrieval and analysis, and critically revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

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Conflict of interest

The authors have no conflict of interest to declare

Ethical Clearance

Ethical approval was granted by Saveetha Dental College-Institutional Human Ethical Committee (SDC-IHEC) with approval number: IHEC/SDC/OPATH-2204/23/273.

Availability of data (if apply to your research)

Available on request.

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