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Step Section Analysis in Routine Dermatopathology practice- A study.

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Abstract: Background: *Nonspecific and overlapping microscopic features often pose diagnostic challenges in routine dermatopathology practice.*
Objectives*: To detect additional findings on step sections of routine skin biopsies and study the significance of the same in improving the diagnostic accuracy. To formulate possible prerequisites to order step sections and study the economical aspects and turn around time alterations.*
Settings*:Tertiary Hospital based Dermatopathology practice.*
Materials and Methods*:Routine skin biopsies received in department of pathology, Dr B.R.Ambedkar Medical College/Hospital(BRAMCH) were processed. Initial sections were interpreted and step sections were ordered in lesions which showed nonspecific features, clinically non correlating importantly those with clinical suspicion of malignancy. Step sections obtained at different levels at 50 µ intervals were reinterpreted and compared with the initial sections (retrospective step sections).*
Results*: Of the 70 cases studied additional diagnostic findings were present in 23 cases (38.3%) including 2 cases of Squamous cell carcinoma and1 case of Basal cell carcinoma. Diagnosis of 18(30%) cases were revised following step sections analysis including 2 cases of Squamous cell carcinoma and 1 case of dcis with invasion. The possible prerequisites for ordering step sections included nonspecific histopathological features and clinical non correlation. Retrospective step sections promise to be economy friendly and more effective. No significant turn around time changes were observed with respect to dermatopathology practice.*
Conclusions*: This study highlights the essential utility of retrospective step sections to improve diagnostic accuracy and to discover hidden malignancies in routine dermatopathology practice without compromising on economy and turnaround time.*
Key words*: step sections, skin biopsies,diagnostic accuracy,economy,turnaround time.*

I. Introduction

Initial sections from skin biopsy specimens often exhibit nonspecific and overlapping microscopic features and pose diagnostic challenges in routine dermatopathology practice. Incomplete sectioning through, a tissue block creates sampling error resulting in false negative diagnosis. In dermatopathology ,studies suggest that deeper levels provide more accurate diagnosis in one third of skin biopsies[1].The pathologist may require sections at different levels to be examined for final diagnosis and such situations necessitates the need for deeper sectioning[2].Despite the clear importance of diagnosing invasive or in situ skin cancer,few objective data exist to guide the dermatopathologist in deciding whether to examine additional step sections[3].In small biopsy specimens such as needle core and endoscopic biopsies deeper sectioning is routinely used in many laboratories to enhance sensitivity and diagnostic accuracy[4-7]. However, there are not many studies done and currently no standard methods are available to direct the performance of step sections . Standards for handling small skin biopsies have not been universally followed and scope of potential variability exists.

The aim of this study is to analyse the utility of retrospective step sections in detecting additional findings and improving the diagnostic accuracy in routine dermatopathology practice, to formulate possible prerequisites to order step sections, to study the economical aspects and turn around time alterations.

II. Materials and Methods:

Routine small skin biopsies received in the department of pathology, Dr.BRAMCH for which step sections were ordered were processed following standard grossing techniques.

Shave biopsy sample <5mm and punch biopsy sample < 3mm were submitted in toto. Shave biopsy sample> 5mm and punch biopsy sample measuring 3-5mm in diameter were bisected. Punch biopsy Sample measuring 6mm or more in diameter were transsected at 2mm intervals. Elliptical specimens <6mm long were longitudinally bisected & ellipses measuring 6mm or more were serially sectioned transversely at 2mm intervals. Tissues bits were formalin fixed and paraffin-embedded. 5µm thick tissue sections were cut and stained with hematoxylin and eosin(H and E). Initial sections were interpreted by reporting pathologists (R.S.S* and A.N.H**). Step sections were ordered for those lesions which showed non-specific & overlapping

histopathological features on initial sections, clinical non-correlation also including those with clinical suspicion of malignancy but initial slides were non diagnostic and to detect additional findings even though diagnosis was possible on the initial slides. Step sections were prepared as per department Standard Operating Procedure. 3 Step sections at different levels at 50µ intervals were prepared with, each ribbon containing 4-6 sections. Sections were stained with Haematoxylin and Eosin. More deeper step sections were ordered for those lesions with clinical suspicion of malignancy but initial slides did not show definitive evidence of the same. Initial slide was labelled as 1 and first step slide was labelled as 2, the next step slide was labelled as 3 and so forth and subjected for reinterpretation by the reporting pathologists(R.S.S* and A.N.H**). Step sections were analysed and findings were compared with initial sections. Additional findings, detection of hidden malignancies, economical aspects and turn around time alterations were studied.

III. Results

70 cases for which retrospective step sections were ordered out of 300 routine skin biopsies were included in our study (table-1).

Table1: Cases included in the study.

Lichen planus	20 cases
Psoriasis	12 cases
Non specific features	9 cases
Pityriasis rosea	6 cases
Morphea/scleroderma	5 cases
Squamous cell carcinomas	4 cases
Polymorphous light eruption	4 cases
Nipple biopsies	2 cases
Verruca vulgaris	2 cases
Basal cell carcinoma	2 cases
Gluteal papilloma	1 case
Folliculitis decalvans	1 case
Conjunctival nevus	1 case
Actinic keratosis	1 case

Additional diagnostic findings were present in 27/70(38.5%) cases (table-2) ,including 2 cases of squamous cell carcinoma and 1 case of basal cell carcinoma "fig 2 and fig 4".

Detection of additional findings, though diagnosis was possible on the initial slides improved the diagnostic sensitivity and helped to confirm the initial diagnosis. Diagnosis of Lichen planus was possible on initial slide but additional diagnostic findings like civattee bodies and Max joseph's space were revealed on deeper step sections "fig 5".

Table-2: Cases in which additional findings were discovered.

Lichen planus	9/20cases
psoriasis	5/12cases
Pityriasis rosea	3/6 cases
Squamous cell carcinoma	2/4 cases
Basal cell carcinoma	1/2 cases
Nipple biopsies	2/2 cases
Conjunctival nevus	1/1 case
Folliculitis decalvans	1/1 case
Polymorphous light eruption	2/4 cases
Actinic Keratosis	1/1 case

Diagnosis of 18/70 cases (25.71%) were revised (table-3) following step section analysis including detection of three hidden malignancies. 2 cases of squamous cells carcinoma and 1 case of ductal carcinoma in situ(dcis) with foci of invasion were diagnosed on deeper step sections "fig1" and "fig3".

Table-3: Cases in which diagnosis were revised.

Initial Diagnosis	Revised Diagnosis
Non specific findings Prurigo simplex Psoriasis Discoid lupus erythematosus	Lichen planus-3 cases Prurigo nodularis-2 cases
Nonspecific findings Pitiriasis rosea	Psoriasis -4 cases
Non healing ulcers Nipple biopsy	Squamous cell carcinoma-2 cases Dcis with foci of invasion-1 case
Psoriasis P rubra pilaris Conjunctival biopsy with initial non specific finding	Pitiriasis rosea-2 cases Conjunctival nevus-1 case.
Actinic prurigo	Polymorphous light eruption-1 case
Xeroderma pigmentosum	Actinic keratosis-1 case
Gluteal papilloma	Verrucous hemangioma-1 case

The possible prerequisites derived from this study for ordering step sections (retrospective) were as follows:

a) Clinical non-correlation i.e; when histopathological findings on initial sections are not correlating with the dermatological diagnosis.
b) Initial slides show nonspecific features with which certain diagnoses cannot be opined.
c) Clinical suspicion of malignancy is offered but initial slides do not show confirmative evidence.
d) To confirm, clarify and rule out a diagnosis formed by initial slide interpretation.

Retrospective step sections analysis proved to be economically more friendly as they were ordered only when required following interpretation of initial slides unlike with that of prospective step sections.

Turn around time was not significantly altered and showed to be minimally prolonged by4- 6 hours in our study.

IV. Discussion

Step sections are routinely ordered in dermatopathology practice. Literature reveals approximately 30% of small skin biopsies require deeper levels for maximum diagnostic accuracy [1]. In our study 23.3% of skin biopsies required step sections for final diagnosis.

Different types of sections can be obtained from the tissue block based on the requirements.Serial sectioning is defined as obtaining a continuous ribbon of sections from a paraffin block & placing all the sections on multiple slides[7] .Step sections are a form of sampling in which sections are collected at specified depths in the block. Step sections are preferred to serial sections as the intervening unstained sections are available for special stains if needed [11].

Traditionally, deeper levels are obtained, at the request of the pathologist, after the original slides have been reviewed-retrospective step sections[2]. In some laboratories step sections are prepared prior to receipt of the slides by the histopathologist-prospective step sections[1].

Retrospective step section analysis was performed in our study. Additional findings were observed in 27/70 cases (38.3%). 3 cases of hidden malignancies were detected including 2 cases of squamous cell carcinomas and 1 case of dcis with invasive foci from a nipple biopsy. This highlights the importance of step sections in dermatopathology.

Initial sections from biopsies with history of non healing ulcers and with clinical suspicion of malignancy revealed only ulcer with granulation tissue and showed no definitive evidence of malignancy.

On subsequent step section analysis epidermal dysplasia with atypical mitoses, and abundant keratinisation were observed. Well formed Keratin Pearls with concentric laminated whorls of keratinized squames was apparent on 5^{th} step section in one case and 7^{th} step section in the other. Additional findings like clear cell changes and acantholytic foci in squamous cell carcinoma were identified on deeper sections.Clear cell change may appear as a foci in an otherwise typical squamous tumors,but ocassionally they occupy the bulk of the lesion[14,15].

Figure 1: Biopsy from a 45 years male with a non healing ulcer on the lateral aspect of the foot with clinical suspicion of malignancy.

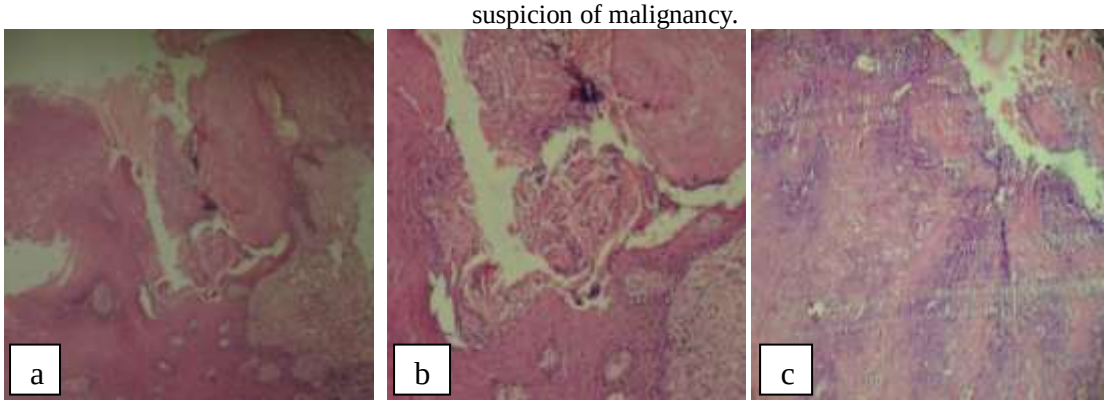


Figure1:(a);Initial section:note an ulcer with granulation tissue, (b);3rd step section;note atypical keratinocytes with attempted keratin pearl formation(H and E,x100),(c);7th step section ;note features of fully evolved squamous cell cracinoma (H and E, x400)

Figure2: Additional findings on deeper step sections in squamous cell carcinoma

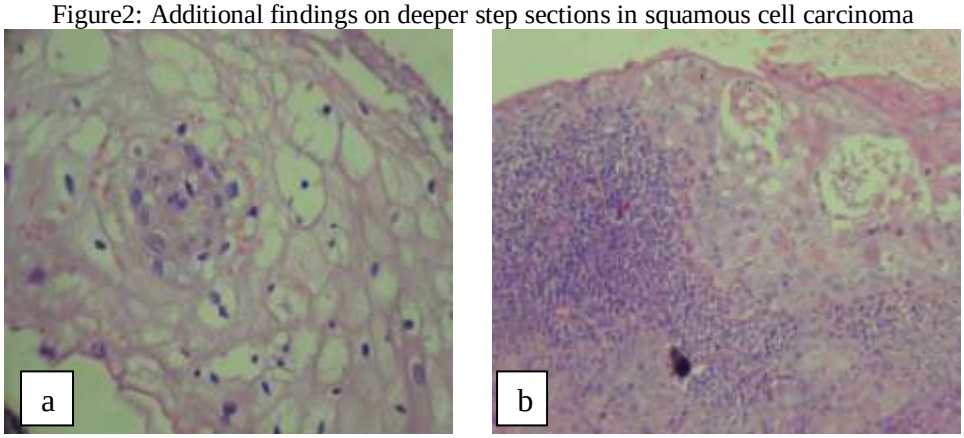


Figure2:(a) note clear cell change and (b)note an acatholytic foci(H and E,x400)

Initial sections of a nipple biopsy with a history of bleeding per nipple and clinical supsicion of malignancy showed only ulceration and crowded glands and failed to reveal malignant features on initial sections.Features of dcis exhibiting micropapillary,cribriform and solid variants were clearly apparent on 3rd step slide. 7th step slide revealed invasive foci[16].

Figure3: Nipple biopsy from a 50 yr female with bleeding per nipple with clinical suspicion of malignancy.

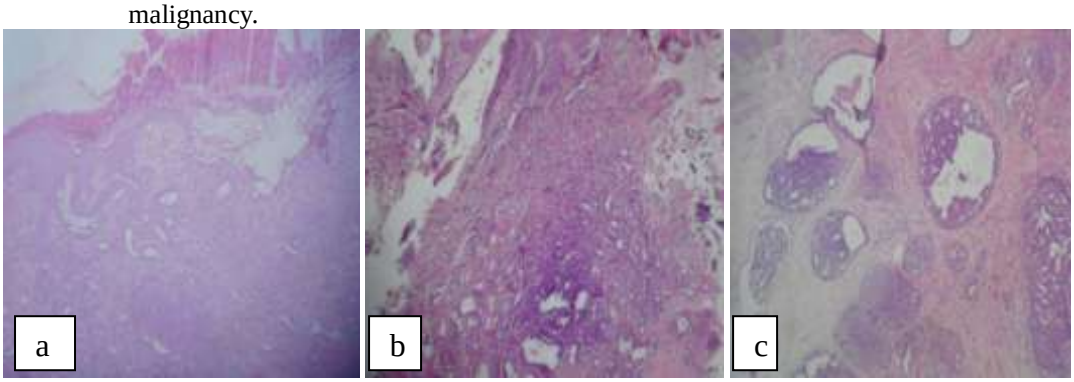
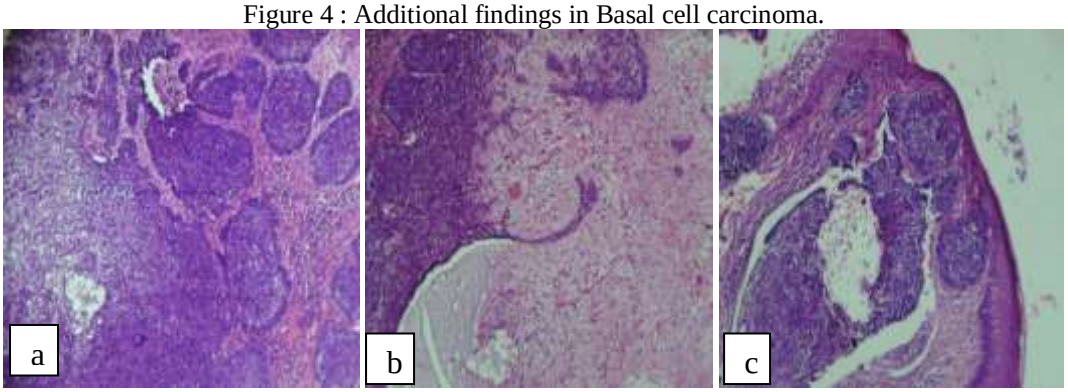


Figure 3:(a)initial section: ulcer with crowded glands (b)and (c)deeper sections: micropapillary, cribriform and solid patterns of dcis with invasion (H and E,x 100x)

Additional findings discovered in basal cell carcinoma on step sectioning included clear cell changes , identification of nodulocystic foci and areas of myxoid changes. Clear cell change may be due to accumulation of lysosomes rather than glycogen and represents a degenerative phenomenon[17].Solid Basal cell carcinoma(BCC) containing large aggregates of tumor cells occasionally show disintegration of their cells in the

centre of tumour masses resulting in cyst formation[18].Serial transverse cross sections(bread loafing) at 4 mm intervals of elliptical excision specimens from facial BCC is sensitive in detecting residual tumor. Complete histologic margin control using en face tissue orientation (Mohs technique) is recommended to identify residual tumor and reduce the risk of tumor recurrenceafter elliptical excision of facial BCC[21]

Figure 4 : Additional findings in Basal cell carcinoma.



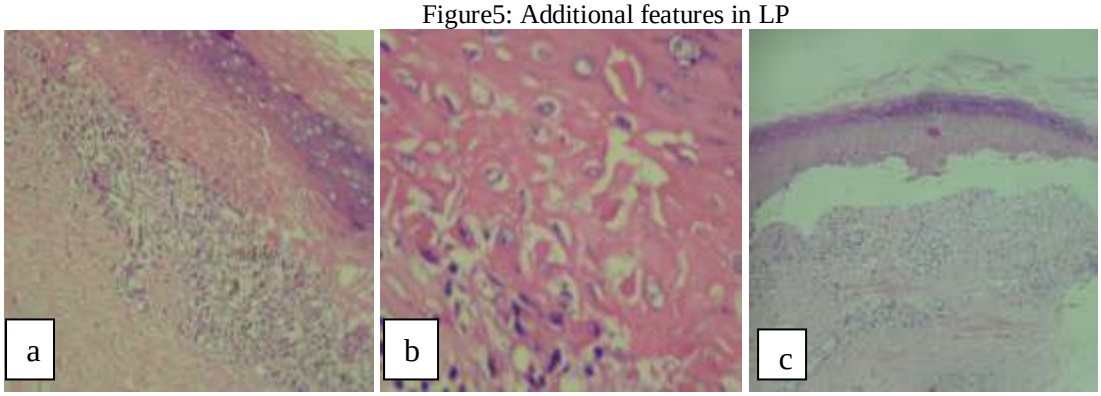
(a);initial sections showing features of conventional Basal cell carcinoma.(b);deeper sections; note myxoid areas(c);deeper sections ;note foci of nodulocystic areas (H and E,x400).

Examination of multiple levels has been cited as means of avoiding diagnostic error and subsequent malpractice claims[20]. The optimal number of deeper sections to be ordered for maximal diagnostic accuracy have not been standardized. Data from this study suggests that at least three step sections might be necessary for diagnosing benign lesions and upto five or more step sections for detecting hidden malignancies.Literature suggests that atleast 5deeper sections might be necessary to detect significant number of hidden malignant lesions[3]. However there is currently no generally accepted standard method to direct the performance of step sections[10] .

Recent retrospective studies have demonstrated the utility of step sections for detecting cutaneous malignant tumors[8] and for identifying stromal invasion in cases originally diagnosed as melanoma in situ[9].

In cases of lichen planus though diagnosis was possible on initial slides, diagnostic findings like Civatte bodies and max joseph's space were detected on 3rd step slide. On Step sectioning areas of wedge shaped hypergranulosis are found to be contiguous to intraepidermal adnexal structures,namely acrosyringia and acrotrichia[19].

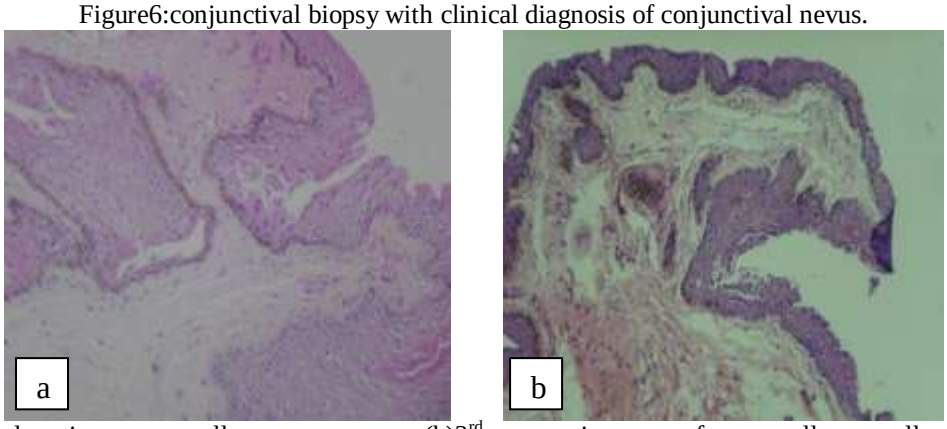
Figure5: Additional features in LP



(a):initial sections;band like inflammatory infilterate and melanin incontinence is seen (b)deeper sections;civattee bodies are well seen.(H and E,x400x)(c);deeper sections:note Max joseph's space (H and E,x100x).

Initial sections from a cojunctival biopsy with clinical diagnosis of cojunctival nevus did not reveal nevus cells. 4thstep slide revealed nests of nevus cells "fig-6".

Figure6:conjunctival biopsy with clinical diagnosis of conjunctival nevus.



(a);initial sections;nevus cells are not apparent (b)3rd step section;nests of nevus cells are well apparent. (H and E,x 100x).

In cases of proriasis, early findings like capillary dilation, edema in papillary dermis with a lymphocytic infiltrate surrounding the capillaries, focal spongiosis were obviously evident on the 2nd slide. Micro munroe abscess in well developed lesions which were not seen in the initial sections were occasionally observed on the 3rd step slide.

There is a great deal of variability in how dermatopathology laboratories deal with the problem the problem of nondiagnostic initial slides[10]. Diagnostic labs must balance the utility of deeper levels with additional time required and expenditure incurred and the anticipated impact on patient care.

Economical aspects of retrospective step section analysis depends on the technical work load ie; retrieval of paraffin block, cutting sections, staining and labelling of sections.

Prospective step sections obviously do not require any of these additional procedures however, there are increased supply costs for prospective sections (staining materials, glass slides, etc) and the costs for an increased storage volume must be considered[1] .

Proper supervision and training of the laboratory technician in terms of orientation of the tissues will minimize the chances of deeper sections[2] and hence reduce economic burden on the patients.

It was observed from our study that retrospective step sections are more economical when they were ordered with a valid prerequisite. The most important prerequisite was clinical suspicion of malignancy but initial slide was nondiagnostic. This observation correlated with that of Henry R carag et al; which states that the most significant factor correlated with the discovery of malignant lesion on deeper sections was a clinical diagnosis of malignancy, also significant were a history of skin cancer and presence of ulceration on the initial slide[3].

In our institutional based dermatopathology practice the TAT is 48-60 hrs normally. Retrospective step section preparation and analysis required an extra 4- 6 hrs. Hence no significant TAT changes were noted. In laboratories with rapid TAT and deeper levels available with in hours, the impact of prospective step sections is less significant[1].

V. Conclusion

This study highlights the essential utility of retrospective step sections in improving diagnostic accuracy in routine dermatopathology practice without compromising the economy and turnaround time. Discovery of hidden malignancies affirms the potential benefits of step section analysis. Retrospective step sections promise to be more cost effective when ordered with a valid prerequisite.

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