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Original Article

Utility of prospective step sections in diagnostic skin histopathology for small biopsies

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Keywords:

Prospective step sections;
Skin biopsy;
Turnaround time.

ABSTRACT

Background: Small skin biopsies have a cosmetic advantage and prospective step sections could potentially improve turnaround time without compromising diagnostic information.

The study aimed to examine the use of prospective step sections of small skin biopsies and its effect on histopathological diagnosis and turnaround time.

Materials and Methods: This was a hospital based cross-sectional study at Department of Pathology and Department of Dermatology, BPKIHS from June 2011- June 2012.

Diagnoses /comment on three levels of prospectively taken 3mm biopsies were compared with those of 5mm biopsies taken from the same/similar lesion from 100 patients. Additional sections from 5mm biopsies were taken retrospectively. Percentage, proportion, mean, standard deviation, diagnostic sensitivity, kappa statistics and paired sample t- test were used as statistical tools.

Results: Of 100 cases, 80 were diagnosed using 5mm biopsies while 73 were diagnosed using 3mm biopsies. On additional step sections of 3mm biopsies, 3 more cases were diagnosed. When compared with 5mm biopsies, the sensitivity of the 3mm biopsy rose from 90% to 93.8% after additional sections while the measure of agreement rose from 0.751 to 0.826.Mean turnaround time for prospectively sectioned 3mm biopsies was 2.56 days and that of retrospectively sectioned 5 mm biopsies was 4.64 days with their difference being statistically significant.

Conclusion: A statistically significant decrease in turnaround time and an increase in sensitivity and agreement after step sections elucidates the utility of prospective step sectioned 3 mm biopsies in diagnostic skin histopathology.

INTRODUCTION

Small skin biopsies have an advantage over larger ones in terms of its cosmetic value. However, its utility is compromised by the apparently lesser amount of information gathered in comparison to larger skin biopsies.[1] The inability to bisect these smaller specimens creates problems regarding tissue orientation during histopathological examination and may affect diagnostic accuracy.[1] Step sectioning methods are used in laboratories for increasing the retrieval of diagnostic information in biopsies including skin biopsies with studies suggesting that deeper sections

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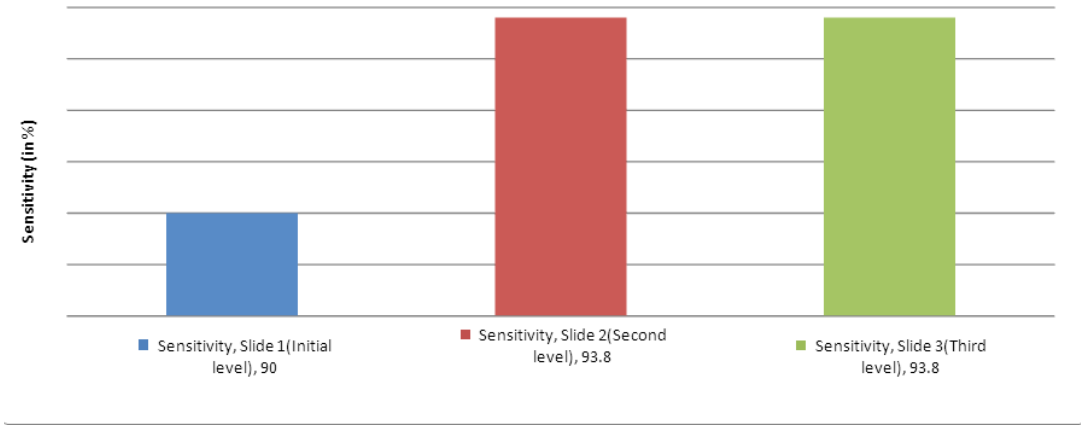

Figure 1: *Change of sensitivity with additional sections.*

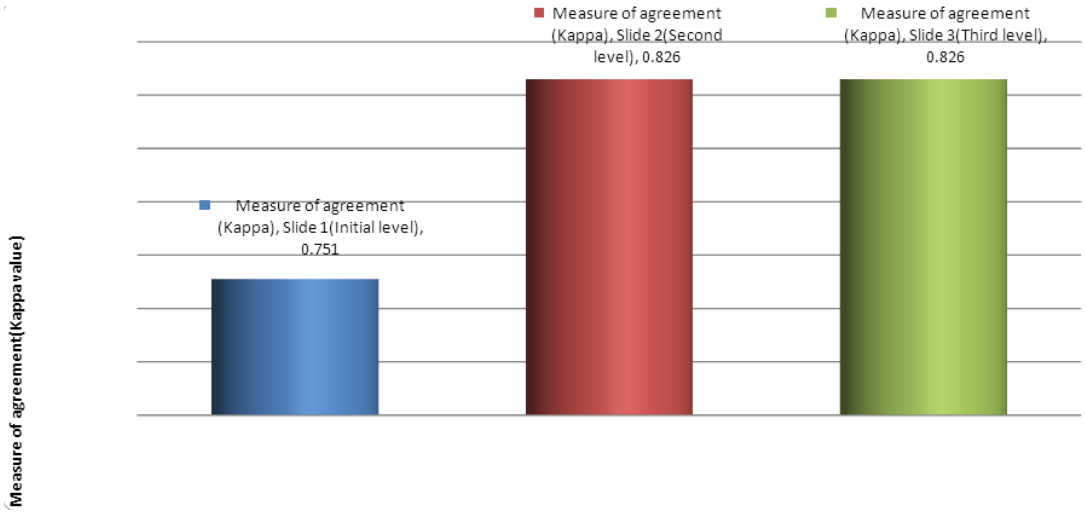

Figure 2: *Increase in agreement (strength of association) of small specimens (3 mm) after additional sections with that of large specimen (5mm) showing rise from 0.751(good strength of association) to 0.826(very good strength of association).*

increase diagnostic accuracy in about one third of skin biopsies.[2,3] Similar increases have also been noted on small skin biopsy specimens.[2,4] However, some of these studies used a large number of step sections and focussed on only particular disease entities.[2]

Although studies suggest an intensive sectioning protocol, they have also been criticized for recommending a tendency to request limitless deeper sections in pursuit of a diagnosis.[5,6] However, most laboratories use 3 levels of step sections for these small specimens.[2] Usually, these are done retrospectively (i.e. on pathologist's request after examining the first slide available for reporting). Some studies show discrepancy in diagnosis on further step sectioning in biopsies and the limited diagnostic information provided by the first of the three levels of step sections for some biopsies have also been highlighted by some.[2,7] Thus, the possibility of inaccurate or incomplete diagnostic information is likely in the absence of step sections especially for smaller specimens. Yet not all of these studies incorporated small skin biopsies. So, a study confined to small skin biopsies to examine such possibilities is one of the overriding rationales of this study. At the same time, the study would examine a wide range of disease entities encountered in dermatopathology practice instead of focussing on a single entity. In addition, it seems logical to assume that prospective step sections (sections taken prior to pathologist's request) reduce the turnaround time.[4]

The aim of the study was to examine the use of prospective step sections of small skin biopsies and its effect on histopathological diagnosis and turnaround time.

MATERIALS AND METHODS

This was a cross-sectional study conducted at the Department of Pathology and Department of Dermatology at BP Koirala Institute of Health Sciences, Dharan, Nepal over a period

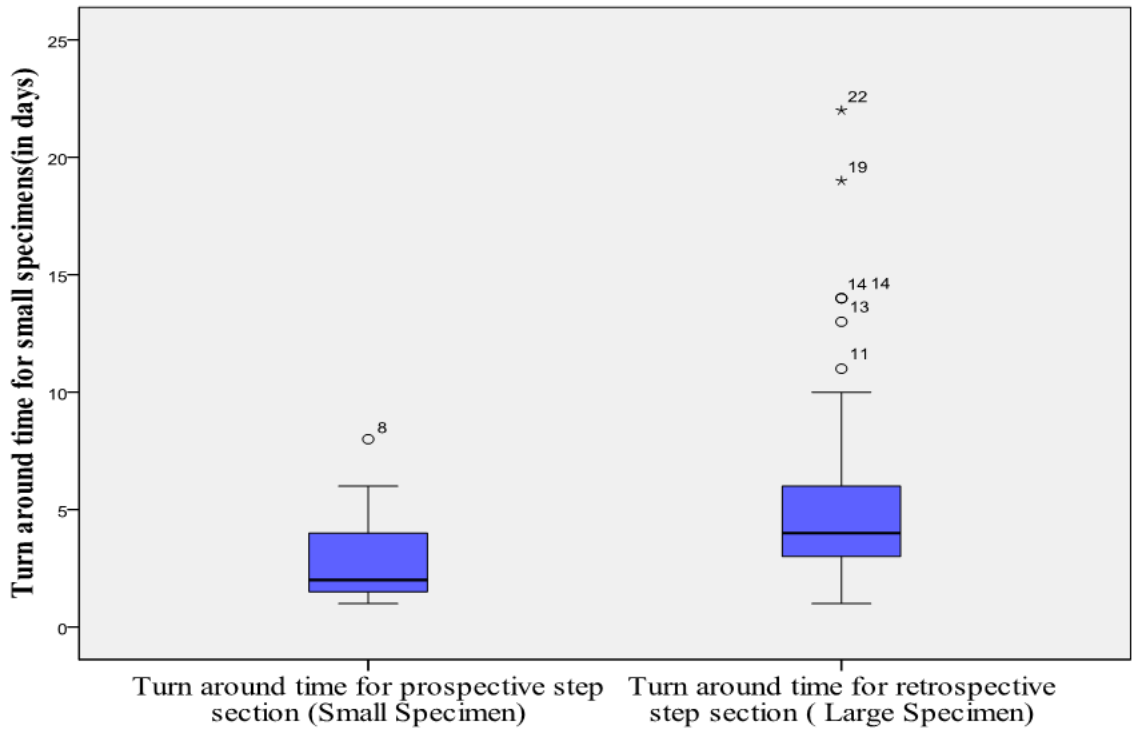

Figure 3: *Box-plot chart comparing the turnaround times between small specimen: 3 mm (prospective step sections) and of large specimen: 5 mm (retrospective step sections).*

of one year (June 2011 to June 2012).The study included patients from whom two punch biopsies / shave specimens (5≥ mm and ≤ 3 mm in maximum diameter) of the same / similar skin lesion were available.

Larger punch biopsy/shave specimen (i. e. ≥5 mm) was subjected to routine histopathological examination. Any additional section requested by the pathologist was taken and the turnaround time and final diagnosis noted. The turnaround time was defined as the time elapsed from grossing of the specimen to availability of all slides ready for reporting.

The smaller of the punch biopsy/shave specimen (i.e. ≤ 3 mm) was subjected to prospective step sectioning (sections taken prior to pathologist's request). For each small specimen (i.e. ≤ 3 mm), 3 slides each containing 1 ribbon of tissue containing 4 to 6 sections was obtained at 50µm intervals from the paraffin block.

In order to avoid bias, the sections from the smaller specimen were reviewed at a later date than that for the larger specimen. In addition, the pathologist was blinded from the final diagnosis given for the larger specimen. In order to avoid inter-observer variability, sections from both the larger and smaller specimens were reported by the same pathologist.

For the small skin biopsy, a diagnosis was made using slide 1 (the initial section of the prospective step sections) only. Then slides 2 and 3 (the remaining sections of the prospective step sections) were reviewed for any additional information and classified as providing no additional information, more accurate diagnosis, exclusion of malignant neoplasm, benign neoplasm or inflammatory condition diagnosed or malignant neoplasm diagnosed. Any change of diagnosis based on information on slides 2 or 3 was noted. The turnaround time for the prospective sections was noted as well.

For all specimens, clinic notes, operative reports, and gross specimen descriptions were reviewed for clinical diagnosis and size and location of lesion. History of skin cancer or immunosuppression, size of the specimen, and presence of ulceration on the initial level were noted as well.[2]

For calculating the sample size, the sensitivity of histopathological examination of large specimen(≤ 5 mm) was considered as 90% (referent) and the sensitivity of histopathological examination of smaller specimen (without additional sections) as 65%.[2-4] The assumption of sensitivity for small biopsies with three sections was considered as 10 % difference from the referent(large specimen).

With alpha error of 5% and power of 90%, for comparison

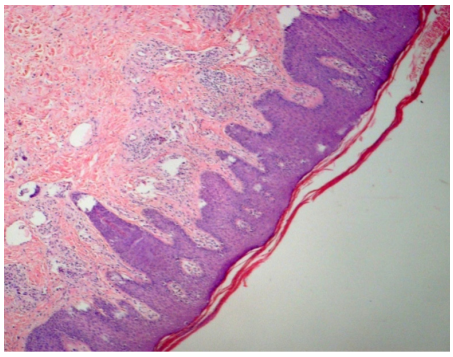

Figure 4: *Pityriasis rubra pilaris, initial level (HE stain, X400).*

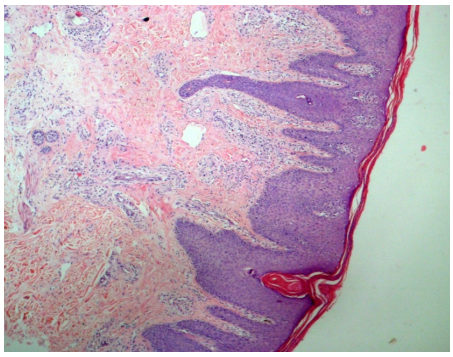

Figure 5: *Pityriasis rubra pilaris with follicular plugging, second level (HE stain, X400).*

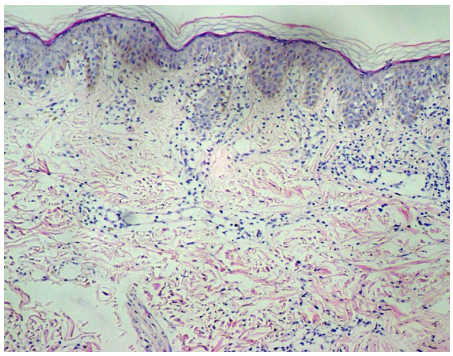

Figure 6: *descriptive, initial level (HE stain, X400).*

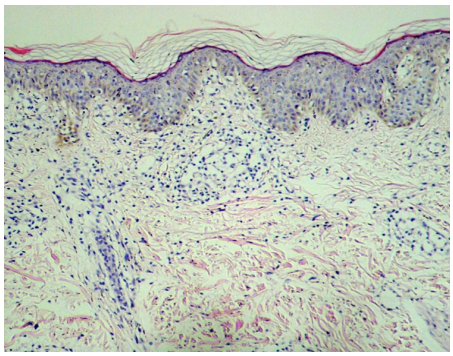

Figure 7: *Borderline Tuberculous Hansen's disease, second level (HE stain, X400).*

of these sensitivities and then using the following formula for sample size calculation, the sample size calculated for the study was 100.

$n= [(Z\alpha +Z\beta)2 \times p \times q \times 2]/d^2$ where,

$Z\ \alpha$ = Z value for α level (i.e. p=0.05 where CI= 95 %).

$Z\beta$ = Z value for β level (i.e. p= 0.10 where power= 90%

p= average percentage between the two groups

q= 100-p

d= clinically meaningful difference between two groups.

Master chart was prepared in MS excel 2007 and converted into SPSS (Statistical package for Social Sciences) version 17.0 for statistical analysis after validity test of data. For descriptive statistics, percentage, proportion, mean, standard deviation and various diagrammatic presentations were done. For inferential statistics, the diagnostic sensitivity was assessed for interpreting the difference among these methods. Inter-sample variability of the information in between the large sample histological examination and different levels of small sample was assessed using Kappa statistics. For comparison between categorical variables, $\chi 2$ tests were used. Change in turnaround time between the groups was assessed by the comparison between the two means.

RESULTS

Out of 100 cases 53 were female and 47 were male. Gender as a variable (for being diagnostic) was not statistically significant. All small specimens received were punch biopsies of 3mm. All large specimens received were punch biopsies of 5mm. Age ranged from 8 months to 79 years. Mean age was 36.19 years (standard deviation: 17.98). Age as a variable (for being diagnostic) was not statistically significant.

Comparison of first level (slide 1) of small punch biopsy specimen (3 mm) with that of large punch biopsy specimen (5 mm:referent specimen) showed sensitivity of 90 % [95% Confidence interval 80.7%-95.3%], specificity of 95% [95% Confidence interval 73.1%-99.7%], positive predictive value as 98.6% [95% Confidence interval 91.6%- 99.9%], negative predictive value as 70.4% [95% Confidence interval 49.7%- 85.5%] and measure of agreement (Kappa) of 0.751(good strength of association).

Comparison of second level (slide 2) of small punch biopsy specimen (3 mm) with that of large punch biopsy specimen

(5 mm: referent specimen) showed sensitivity of 93.8% [95% Confidence interval 85.4%-97.7%], specificity of 95% [95% Confidence interval 73.1%-99.7%], positive predictive value as 98.7% [95% Confidence interval 91.9%- 99.9%], negative predictive value as 79.2% [95% Confidence interval 57.3%- 93.1%] and measure of agreement (Kappa) of 0.826(very good strength of association). The third level (slide 3) showed no change of diagnosis. Hence the sensitivity, specificity, positive predictive value, negative predictive value and measure of agreement remained the same as that of Slide 2 (second level) (fig. 1 and fig. 2).

The frequencies of lesion at various sites were 53 for extremities (not palms/soles) and 34, 6, 5 and 2 for trunk, head and neck (not scalp), scalp and sole respectively. Location as a variable (for being diagnostic) was not statistically significant.

The turnaround time (for small 3mm specimen) ranged from 1 day to a maximum of 8 days. Mean turnaround time was 2.56 days (Standard deviation: 1.373). The turnaround time (for large 5mm specimen) ranged from 1 day to a maximum of 22 days. Mean turnaround time was 4.64 days (Standard deviation: 3.492) (fig.3). Among the large 5mm specimens, additional sections were ordered after reviewing the available slide in 59 cases. As the values of turnaround time were in normal distribution, paired sample t-test was used. The difference between the turnaround times for large specimen (5mm) and small specimen (3mm) was statistically significant (p value <0.001) .

Presence of ulceration occurred in 3 cases. In 2 cases, a diagnosis could still be given. However, in 1 case, only a descriptive diagnosis could be given. Size of the lesion varied from 1 cm to 7.5 cm (mean: 2.4 cm and standard deviation: 1.46628).Size of lesion as a variable (for being diagnostic) was not statistically significant in the initial level. It remained not statistically significant in additional sections.

Out of 100 cases, 27 were descriptive with 8 Vasculitides, 8 Spongiotic dermatitides, 13 infectious conditions, 16 lichenoid dermatoses, 3 sclerosing dermatitides, 2 vesiculobullous conditions, 11 psoriasiform dermatoses, 4 neoplastic entities and 8 miscellaneous conditions. Second level of the small specimen (3mm) revealed no additional findings in 91% whereas 6% showed more accurate diagnoses and for 3% an inflammatory condition was diagnosed (fig.4 and fig.5). Third level of the small specimen (3mm) revealed no additional findings in 97% whereas 3% showed more accurate diagnoses.

DISCUSSION

Among the cases evaluated, results revealed that small punch biopsy (3 mm) specimens in the initial level (first level) showed good sensitivity, specificity, positive predictive value and negative predictive value. Besides, measurement of agreement between large punch biopsy specimens (5 mm) and small punch biopsy specimens using kappa statistics showed good strength of association.

Thus, even in the absence of additional step sections, this study indicates that a small punch biopsy is able to retrieve adequate diagnostic information. This is in agreement with studies done by Todd P et al that showed that specimens as small as 2 mm punch biopsies were adequate for diagnostic purposes in a wide range of dermatological conditions and were not statistically different when compared to clinical pathology results obtained by elliptical biopsy.[8-10] However, some consider a 2 mm punch biopsy too small to represent all compartments, and often insufficient to demonstrate a recognisable pattern.[11-14] In addition, small biopsies have problems with orientation of specimen while processing the slides by the histotechnologist and have frequent problems related to crushing artefacts.[15,16]

Three mm is the smallest size likely to give sufficient tissue for consistently accurate histological diagnosis and less likely to cause significant scarring.[14] However in relation to scarring, good depth of punch biopsy is as important for absence of scarring as is smaller size of punch biopsy. Biopsies reaching upto the subcutaneous fat are preferable diagnostically and also heal faster and with a less prominent scar.[17] The subcutaneous fat has a rich network of fine capillaries that helps in the formation of granulation tissue and the healing process. Moreover, when biopsies reach up to fat, due to normal elasticity of the dermis, surrounding skin tends to slide over the fat leaving a defect that is oval and smaller than the punch size. On the contrary, biopsies that have the relatively avascular reticular dermis in their base tend to form slough in their base with higher chance of secondary infection and consequent bad scar.[8,13]

With additional sections, sensitivity, negative predictive value and positive predictive value improved from slide 1 (initial level) to slide 2 (second level). The measure of agreement improved from good agreement to very good agreement. Besides, 6 cases showed more diagnostic features.

The third level (slide 3) showed no change of diagnosis. Hence the sensitivity, specificity, positive predictive value, negative predictive value and measure of agreement remained the same as that of Slide 2 (second level) but 3 cases showed more diagnostic features.

These findings illuminate the positive diagnostic role of additional step sections in skin histopathology especially in small biopsies. All small specimens received in this study were punch biopsies of 3 mm. All large sample specimens were punch biopsies of 5 mm. Hence, this study could

specifically compare 3 mm punch biopsies (after step sections) with 5 mm punch biopsies.

In a similar study on prospective step sections on small skin biopsies by Bruecks et al , 88% of the cases were diagnostic using initial level only.[4] In this present study, 73% were diagnostic using initial level (80% had been diagnostic using 5 mm specimen) increasing to 76% on subsequent step section. These results need to be interpreted with the underlying knowledge of several factors that affect the presence or absence of diagnostic features in a punch biopsy specimen.

Choice of lesion, choice of site for biopsy and proper technique of a punch biopsy has a profound effect on the diagnostic information that can be retrieved from punch biopsies.[8] In general, a fully evolved untreated lesion is taken. However, if blisters are present, the smallest of vesicles is chosen and the roof is kept intact. An exception to this rule is that when dermatitis herpetiformis is suspected, biopsy should be taken from a non-excoriated papule rather than a vesicle or a bulla.[8,18] For annular lesions such as granuloma annulare, porokeratosis or dermatophytosis, specimen from the advancing edge is most likely to give diagnostic information. For most other conditions, focus of maximal induration or elevation will usually give the best result.[8] Normal skin is not included as it may be inadvertently sectioned by the technician especially in small biopsies where the lesion is not appreciated grossly. In such instances, it may lead to a report of normal skin or "non-specific dermatitis".[8]

Likewise, biopsy from legs is usually avoided as stasis change can complicate its interpretation. Biopsies from the leg or foot show thickening of the small venules and capillaries in the superficial and deep plexuses with an apparent increase in their number. Besides, a sparse inflammatory infiltrate of lymphocytes and histiocytes (containing hemosiderin) may add to the pre-existing inflammation due to the primary condition being biopsied. In other instances, non-visualization of the deep dermal plexus and subcutaneous fat in biopsies may miss panniculitis and use of forceps, especially in small specimens, cause compression artefacts making cellular identification impossible.[8]

Visualisation of diagnostic histological features is also dependent on the stage of the disease. Hence the overall diagnostic sensitivity needs a clinico-pathological correlation and is largely subjective.[19]

Studies on deeper sections on three levels of histological sections by Luo YV et al (although done on cervical biopsies) had concluded that the first of 3 levels (initial level) contributed little to reaching a diagnosis but the control of inter-observer variation(subjectivity) seemed to be superior to preparation of additional levels as a strategy for reducing diagnostic error.[7] A study by Hill CB et al (2005) on different levels of biopsies reiterated similar emphasis on the profound effect of subjectivity on diagnostic sensitivity in biopsies.[15] However, the initial level in the present study we were able to recognise 73% of cases out of the 76% finally recognised on additional sections. Studies by Carag HR et al[2] and Maingi CP et al[3] where deeper sections revealed a diagnosis in 37.3 % of the cases, had concluded that step sections were particularly helpful when skin cancers were suspected and that their use was paramount in assessing the presence or absence of cutaneous malignancy rather than in diagnosing inflammatory skin processes. The majority of the cases in the present study were non-neoplastic (1 intradermal nevus and 1 superficial spreading melanoma were identified). Besides, suspicion of a neoplastic skin lesion is usually accompanied by an excisional skin biopsy rather than a punch biopsy. Hence the exclusion of such excisional boipsies where additional sections would be more likely to reveal a diagnosis may have resulted in a fewer number of diagnostic cases with step sections.

There have been several studies to determine the optimum number of levels or additional step sections for small biopsies. Most of these studies have tended to focus on core biopsies, particularly of breast and prostate gland with recommendations ranging from 2 to 5 additional levels.[20-25] In the present study too, with additional sections, the agreement (strength of association) of small specimens (3 mm) with that of large specimen (5 mm) increased from good strength of association to very good strength of association. Thus, 3 levels of sections are in agreement with the recommendations. However, generalizations are still difficult given the wide range of study conditions and a variety of specimens.[20]

Nischal U et al have recommended 5 mm punch biopsies in granulomatous conditions.[13] In the present study, use of 3mm biopsy missed 3 granulomatous conditions in the initial level that were identified in the second level after step sections (fig.6 & 7). Thus, use of a 3mm biopsy with additional step sections may be considered for granulomatous conditions as an option at sites where 5mm biopsies are not preferred (e.g. facial regions).

Bahram et al have suggested a punch biopsy for diagnosing inflammatory dermatoses and recommend 3 mm to 4 mm specimen adequate.[16] Majority of the cases evaluated in this study were infective/ inflammatory dermatoses as well where a diagnosis was possible using these small specimens. Hence, a similar recommendation can also be agreed upon.

The mean turnaround time for the specimen (time elapsed from grossing of the specimen to availability of all slides ready for reporting) are influenced by factors such as the volume of cases to be processed and the number of histotechnologists working. Retrospective step sections

are influenced by other additional factors such as retrieval of blocks, labelling, cutting, and mounting.[1,4] The mean turnaround time for prospective step sections for small specimen (3 mm) was 2.56 days (standard deviation:1.373). Among the large 5mm specimens, additional sections were ordered after reviewing the available slide in 59 cases. The mean turnaround time for these large specimens (5 mm), whose step sections were retrospective taken, was 4.64 days (standard deviation: 3.492).

These turnaround times were statistically different in the present study. The turnaround times for a few cases deviated widely from the median. These were due to suspension of histological processing by the histotechnologists for a short period of time because of their ongoing strike. Lessened turnaround times for skin biopsy have a positive influence on patient management by reducing delay. Hence, use of such prospective step sections can be beneficial for the patient and satisfying for the medical personnel involved as well.

Overall, a statistically significant decrease in turnaround time and an increase in sensitivity and agreement (with diagnosis obtained from large specimen evaluation) after step sections has helped to elucidate the utility of prospective step sections of small 3 mm specimens in diagnostic skin histopathology.

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