CORRESPONDENCE

Orientation precedes interpretation: comparison of different tissue handling techniques to attain well-oriented small-intestinal endoscopic biopsy

Recent deployment of minimally invasive procedures often yield smaller biopsies, demanding more meticulous tissue handling and proper orientation, ie, obtaining histological section perpendicular to the mucosal surface. But most often the biopsied tissue tends to curl up when suspended in tissue fixative, or undergo tangential or horizontal sectioning resulting in poor orientation.

A well-oriented histological section is the prerequisite for precise assessment of gastrointestinal disorders like degree of villous atrophy, depth of invasion of a tumour, proper delineation of the site of origin of growth and hence further guiding the proper management. Literature review highlights many methods already worked on for orientation of duodenal,[1 2] cervical,[3] urinary bladder[4] and conjunctival biopsies.[5] These methods include use of filter paper, vegetable matrix including cucumber paper, Gelfoam, direct mount on paper before fixation. We hypothesised that the application of albumin and introduction of lens paper could improve the orientation of the tissue, as compared with use of Whatman filter paper. Hence, an attempt was made to compare percentage oriented length of the biopsy when processed in lens paper versus Whatman filter paper, with and without application of albumin. To the best of our knowledge, we could not find any literature as per this protocol. In addition, the tissue fragmentation and loss during handling and processing was also evaluated.

We have analysed consecutive 150 small-intestinal biopsies from patients suspected of non-neoplastic disorders irrespective of age and sex of the individual over duration of 6 months. With 90% CIs, sample size of 75 was taken in each of the four arms under study (figure 1). For the first 75 biopsies received, the tissue in each container was randomly divided almost equally into two groups A and B, and for next 75 cases similarly into C and D. Processing was done as explained in figure 1. To improve the precision, magnifying hand lens (×10) was used to put the tissue pieces with mucosal side up and cut surface down towards the paper. All the sections were examined for:

1. Linear/ curvilinear total length of the biopsy (figure 2).
2. Length of the well oriented biopsy.
3. Fragmentation or loss of the biopsy.

Cases where number of tissue fragments were less than two, were excluded from the study. A 'well-oriented' biopsy was considered as having three consecutive crypts to villous units in perpendicular orientation. All the measurements have been done using the NIS Element software (V.4.3). The formula applied to calculate the percentage length of well-oriented tissue is as follows:

Percentage length well oriented (%) = (Total length of well-oriented fragments/ Total length of all the fragments) * 100

Statistical Analysis was done by IBM SPSS Statistics V.20, and $p<0.05$ was considered significant. Written informed consent from the institutional ethics committee was obtained for conducting this study.

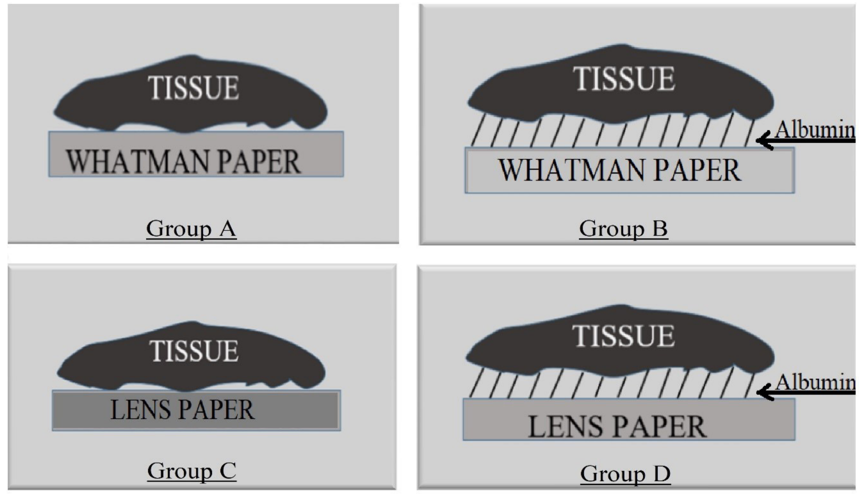

Figure 1 Processing protocols for each of the four groups (A–D); (A) biopsy fragments are placed directly on Whatman paper with a drop of eosin; (B) albumin is applied on Whatman paper, and then the biopsy fragments are placed onto it with application of gentle pressure; (C) biopsy fragments are placed directly on lens paper with a drop of eosin; (D) albumin is applied on the lens paper, and then the biopsy fragments are placed onto it with application of gentle pressure.

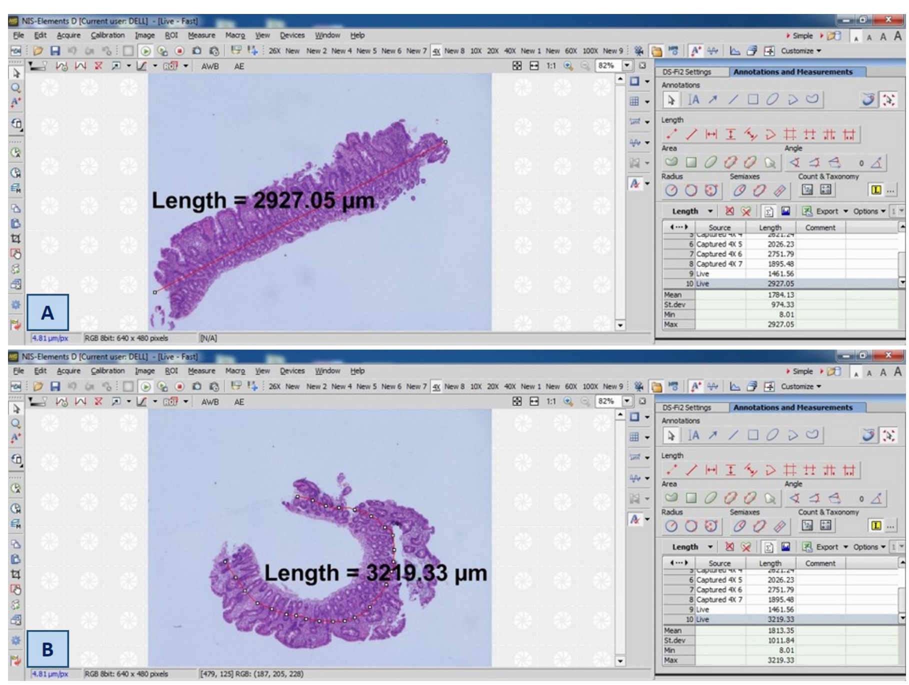

Figure 2 The linear and curvilinear length of the biopsy is measured with the measuring axis kept perpendicular to the villi (NIS Element software V.4.3).

Table 1 Comparison of percentage oriented length of the biopsies and their significance across different groups

	Group A	Group B	Group C	Group D
No of biopsies (total 300)	75	75	75	75
Mean rank (Kruskal-Wallis H test) *	**105.47**	**156.20**	**164.76**	**175.57**
Median percentage orientation (%)	39.72	65.28	72.41	76.90
Cases undergoing fragmentation	14	12	15	12
Percentage fragmentation (%)	18.66	16	20	16

Comparison groups (Dunn post hoc)	Test statistic	P value	Difference in mean rank
A vs B	−50.727	**0.002**	**Significant**
A vs C	−59.287	**<0.001**	**Significant**
A vs D	−70.093	**<0.001**	**Significant**
B vs C	−8.560	1.000	Not significant
B vs D	−19.367	0.997	Not significant
C vs D	−10.807	1.000	Not significant

Parameters used in analysis and significant differences are highlighted in bold
*Highly significant difference between the groups (p<0.001, X^2 29.569, df=3).

The median percentage orientation and mean ranks for each of the four groups have been depicted in table 1. Group A was having the lowest median percentage orientation (figure 3, box plot) and mean rank as compared with other groups, whereas group D was having the highest values for these parameters. Kruskal-Wallis H test for multiple independent samples revealed highly significant difference between the groups ($p<0.001$, X^2 29.569, df=3). Multiple pairwise comparison between any two groups using Dunn post hoc method yielded significant difference between Group A and rest of the other groups (table 1). The comparison among groups B, C and D did not show any significant difference.

These results imply that the null hypothesis of no differences between the mean ranks was rejected, that is, there is significant difference in the percentage oriented length of the biopsy when it is processed using lens paper and albumin as compared with Whatman filter paper. The application of albumin in Whatman paper showed significant difference as compared with Whatman paper alone. The lens paper however did not yield any significant difference with the application of albumin. The number of biopsied tissue pieces in preprocessing and post-processing phase showed fragmentation in range of 16%–20%, without any loss of tissue (table 1).

Meticulous tissue handing during processing and embedding to avoid fragmentation and achieve desirable orientation has been highlighted in the literature.[1] For obvious reasons, larger the size of the tissue, better is the histological section obtained. Ladas *et al*[6] used stereo microscope for orienting the tissue and reported higher percentages (70%) of specimen fulfilling criteria for optimal histological section. Cotruta *et al*[7] oriented the biopsy by direct placement from the biopsy forceps on the nitrocellulose filters and proved its superiority to the freely floating biopsy specimen. Nada *et al*[2] used vegetable matrix for mounting the tissue and proved that it provides better result as compared with the filter-paper mounting. Ravelli and Villanacci[1] suggested cellulose acetate filter for positioning of duodenal biopsies from the biopsy forceps and post fixation embedding the biopsy-filter assembly after 90º rotation.

This study is one of its kind, recommending the use of commonly available laboratory material like lens paper and albumin during processing of free floating endoscopic biopsies, for achieving desirable orientation, satisfactory for interpretation. Although lens paper alone can also serve the purpose to some extent, albumin has to be applied when Whatman filter paper is used to get nearly similar well-oriented biopsies.

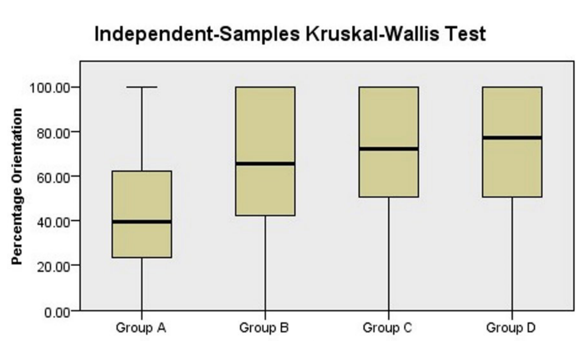

Figure 3 Box plot showing the distribution of percentage orientation between the four groups.

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Contributors NG involved in laboratory work-up, measured various parameters of biopsies and wrote the manuscript. KM codesigned the experiments, analysed the data, corrected and approved the final version of the manuscript. PS had the concept of the work, codesigned the experiments, corrected and approved the final version of the manuscript.

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