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Research Article

DETERMINATION OF FORMALIN FIXED TISSUE DIMINUTION AFTER PROCESSING AND ITS SIGNIFICANCE IN SURGICAL MARGINS AND TNM STAGING: A PROSPECTIVE REVIEW OF 42 CASES IN A TERTIARY HEALTH CENTER

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A B S T R A C T

Introduction: Tissues shrink after fixation and post processing before final microscopic assessment by the pathologist. Many studies indicating shrinkage of tissue post-fixation with possible corrective factors have been highlighted in many studies, however, it is imperative to determine shrinkage during tissue processing which forms a critical position in determining surgical margins and in tumour staging using TNM classification, it is the paucity of information regarding that prompted the need for this study.

Methodology: Forty-two tissue biopsies were randomly selected prospectively during surgical cut off and specific block is selected and their volumes were measured using meter rule post-fixation, after automated tissue processing. The tagged block diameter (volume) was then retaken, the percentage shrinkage was then determined. Correlation of shrinkage with age, sex, site of the biopsy and diagnosis was analyzed using SPSS version 16 and presented in simple tables and bar charts.

Results: Forty-two cases were selected during grossing. There were 10 malignant neoplasms, five cases were benign neoplasm, eight were inflammatory lesions and 19 cases are nonneoplastic lesions. The age ranges were between 2 to 85 years with mean age of 38.5. There were 25 females and 17 males. The peak % shrinkage was observed in malignant neoplasm and inflammatory lesions with mean % shrinkage of 43.7 and 61.3 respectively. Overall least shrinkage was seen in non-neoplastic lesions. The benign neoplastic lesions show fairly uniform shrinkage ranging between 46 to 64%. Tissue shrinkage also depends on nature of specimens, site of biopsy, the age of the patient and type of lesion.

Conclusion: Shrinkage of tissue post processing is very significant finding that can give rise to false surgical margins and TNM stage with the resultant impact on prognostication of tumour. There is a need to develop shrinkage corrective factors for each type of malignant neoplasm and that has to be center based depending on the method of tissue processing and processing machine in order to resolve surgical margin incongruities and staging.

INTRODUCTION

Tumour staging and prognostication using the WHO tumour size, node, and metastasis (TNM) has formed a pivotal position over the years in the grading malignant neoplasm.[1, 2] The TNM classification method has evolved from non-specific small and large tumour size classes to a specific cut offs tumour size that is translated into various stages and that may affect prognosis and treatment of patients with cancer, however, there are many modifications that were made on tumour size from 2002 to 2010, where some tumours with pT1 group are subdivided into pT1a and pT1b, similarly tumours in group pT2 were refined to pT2a and pT2b[3,4] and these modifications were done based on the tumour size. The definition on how to obtain the tumour size as fresh tissue, post-fixation or post staining is not clear for many tumour[3, 4]. It is optimistic that tissues reduce in size after formalin fixation and after processing, and the original dimension taken by the surgeon cannot be the same after fixation in 10% buffered formalin, and also tissue processing which involves dehydration, clearing, and impregnation further shrinkage the tissue. Shrinkage of tissue *in vivo* and *in vitro* also depends on the age of the patients, sex, and site of tissue biopsy; although there are many disagreements on different studies of shrinkage and stereology of excised tissue before and after fixation and

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processing of both visceral and cutaneous specimens, [5-11] however, most of the studies have not emphasized the importance of tissue diminution for specific malignant neoplasm as a factor in quality of tissue processing and resolving surgical margins discrepancies in the medical records and in TNM staging. [12-16] There is a paucity of information on shrinkage of tissues and correlation to histological diagnosis along with patient demography, sites and nature of biopsies despite its importance[1] and that prompted the need for this study.

METHODOLOGY

This study was designed to prospectively determine tissue shrinkage after automatic processing (dehydration, clearing, and impregnation). Forty-five tissues were randomly selected during surgical cut up, the dimension (volume) of each tissue block was determined after 24 to 48 hours of formalin fixation using meter rule. The tissue blocks were then submitted for processing and after it has been processed the dimension (volume) was re-taken. The percentages of shrinkage were then calculated by dividing the deficit by the original volume and multiply by 100%. The gender, age, site of biopsy, nature of specimens and diagnosis were recorded from the request cards and analyzed and correlated with shrinkage using SPSS version 16. The result obtained was presented in tables and bar charts.

RESULTS

Forty-five cases were randomly selected during surgical cut off; 42 tissue blocks were used and 3 were discarded (failed selection criteria). There are 10 malignant neoplasm 3 females and 7 males (table 1).

80% shrinkage each as shown in figure 3,5 and 6. Renal cell carcinoma has the least shrinkage (16.7%) among all malignant tumours as shown in figure 3.

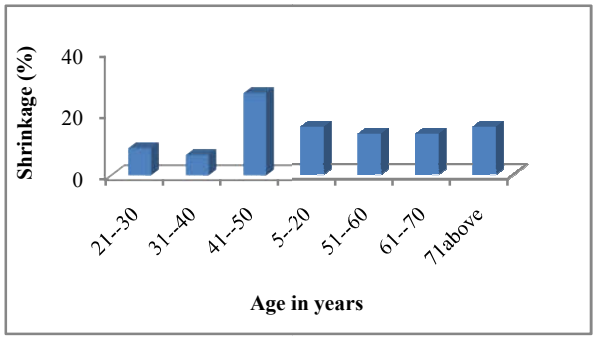

Figure 1 showing percentage of shrinkage and age in years

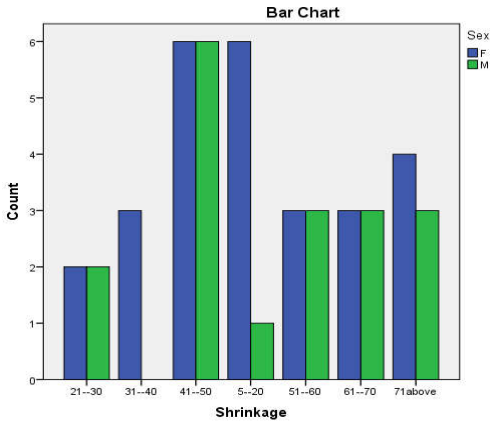

Figure 2 Showing percentage of shrinkage, age, and sex

Table 1 Malignant tumours

S/N	SITE OF BIOPSY	AGE (years)	SEX	SHRINKAGE (%)	DIAGNOSIS
1	Breast	65	F	17	Invasive ductal carcinoma
2	Leg ulcer	45	M	46	Squamous cell carcinoma
3	Thigh mass	35	F	47.8	Dermatofibrosarcoma
4	Lumbar mass	14	M	46	Rhabdomyosarcoma protuberans
5	Nephrectomy	85	F	16.7	Renal cell carcinoma
6	Omental cake	14	M	72	Metastatic sarcoma
7	Prostate trucut	65	M	25	Adenocarcinoma
8	Rectal mass	16	M	63	Mucinous carcinoma
9	Rectal mass	65	M	80	Adenocarcinoma
10	Stomal tissue	32	M	23.5	Squamous cell carcinoma

Table 2 Benign tumours

S/N	SITE OF BIOPSY	AGE (Years)	SEX	SHRINGAKE (%)	DIAGNOSIS
1	Breast lump	30	F	46	Sclerosing adenosis
2	Endometrium	46	F	60	Leiomyoma
3	Axillary mass	47	F	60	Lipoma
4	Ovarian mass	32	F	64	Serous cystadenoma
5	Scalp mass	32	M	50.8	Dermal duct tumour

Their ages range from 14 to 85 years (table 1 and figure 1). Five cases are diagnosed as benign neoplasm; 4 females and one male with ages between 30 to 47 years (table 2). Eight inflammatory lesions have been recorded; 5 females and 3 males with an age range from 14 to 60 years (table 3). There are 19 non- neoplastic lesions with ages between 2 to 65 years (table 4). The age groups 41-50 years show the highest degree of tissue shrinkage (figure. 1) and tissue shrinkage versus sex and age are equal for all ages, except for age group 31-40 and 5-20 which shows variation, (Fig. 2). The tissues with the highest degree of shrinkage includes thyroid tissue (83%) followed by rectal adenocarcinoma and typhoid ileitis having neoplastic tumours which include ovarian tissue that was diagnosed as corpora albicantia shrank by 8.8%, as shown in figure 6. The benign neoplastic lesions generally show fairly equal shrinkage ranging from 46 to 64% (table 2, figure 4). All cases of malignant neoplasm and inflammatory lesion show significant shrinkage as shown in table 1, 3 and figure 3,5). Sites of tissue biopsies from malignant lesions show omental and rectal tissues 72% and 80% shrinkage and least shrinkage are seen in breast, kidney and stomal tissues with 17%, 16.7% and 23.5% shrinkage respectively (figure 7). Sites of biopsies for benign neoplasm and inflammatory lesions show fairly equal shrinkage

ranging from 46%-64% and 42-80% respectively as shown in figure 8 and 9.

Sites of biopsies for non-neoplastic lesions, scrotal tissue (80% shrinkage) and thyroid tissue (83.1% shrinkage) have the highest shrinkage while ovarian tissue

Table 3 Inflammatory Lesions

S/N	SITE OF BIOPSY	AGE (YEARS)	SEX	SHRINKAGE (%)	DIAGNOSIS
1	Appendix	27	F	60	Acute appendicitis
2	Axillary mass	22	F	67	Hidradenitis Suppurativa
3	Bone tissue	36	M	75	Osteomyelitis
4	Caecal mass	51	F	51	Acute inflammation
5	Edge of bowel	14	F	80	Typhoid ileitis
6	Knee mass	18	M	42.4	Mycetoma
7	Ileo-caecal mass	51	F	73	Acute suppurative inflammation
8	Leg ulcer	60	M	42	Inflamed granulation tissue

Table 4 Non-neoplastic lesions

S/N	SITE OF BIOPSY	AGE (YEARS)	SEX	SHRINKAGE (%)	DIAGNOSIS
1	Abdominal mass	2	F	21	Rupture choledochal cyst
2	Adnexal cyst	40	F	15	Non-neoplastic cyst
3	Bilateral tubal ligation	37	F	47	BTL confirmed
4	Cervical tissue	46	F	44.5	Nabothian cyst
5	Skin growth	25	M	54	Epidermal inclusion cyst
6	Fallopian tube	31	F	40	Rupture ectopic tubal gestation
7	Endometrial Curettage	46	F	60	Lower grade endometrial hyperplasia
8	Fibroid tissue	46	F	39.5	Nabothian cyst
9	Ganglion sac	25	M	42.4	Ganglion cyst
10	Ovarian tissue	46	F	8.8	Corpora albicantia
11	Uterine content	22	F	66.2	Products of conception
12	Prostate	65	M	44.8	Nodular hyperplasia
13	Scrotal mass	20	M	80	Hydrocele
14	Skin cyst	22	F	48	Abscess
15	Spermatic cord	20	M	63.7	Normal
16	Testicular tissue	61	M	54.7	Long standing hydrocele
17	Thyroid tissue	40	F	83.1	Diffuse toxic goiter
18	Tonsillar tissue	13	F	50	Lymphoid hyperplasia
19	Trephine biopsy	35	F	20	Normocellular marrow

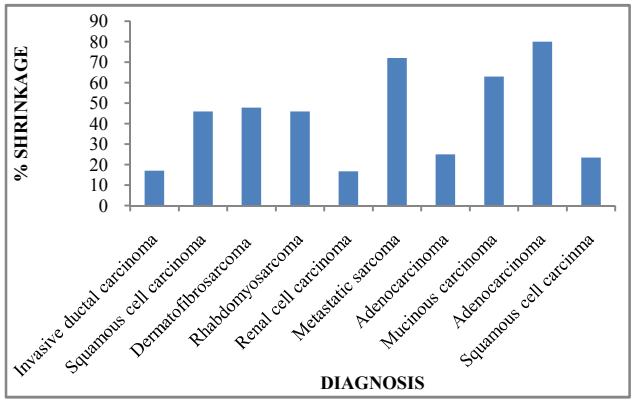

Figure 3 Malignant Neoplasm with percentage shrinkage

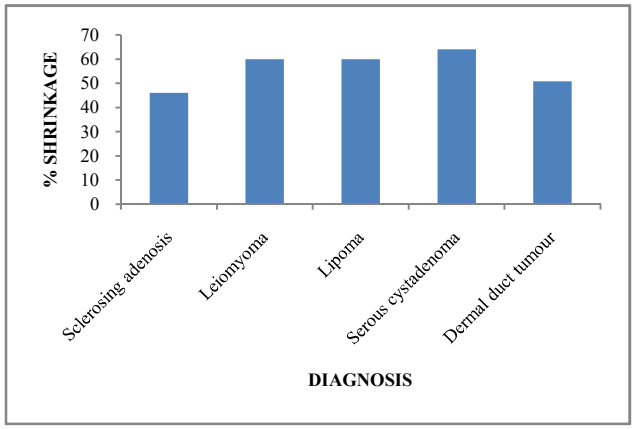

Figure 4 Benign neoplasm and percentage shrinkage

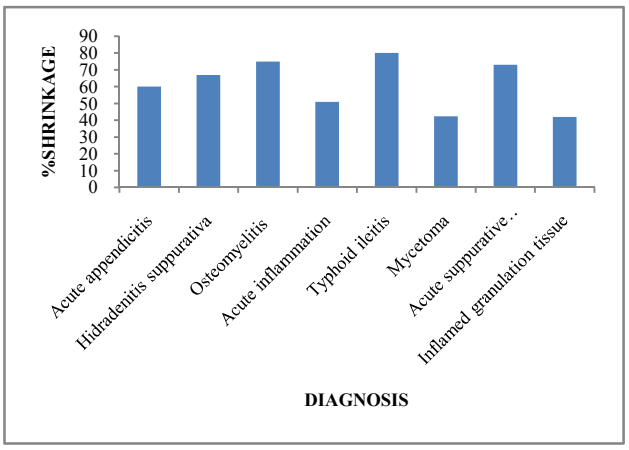

Figure 5 Inflammatory lesion Diagnosis with percentage shrinkage

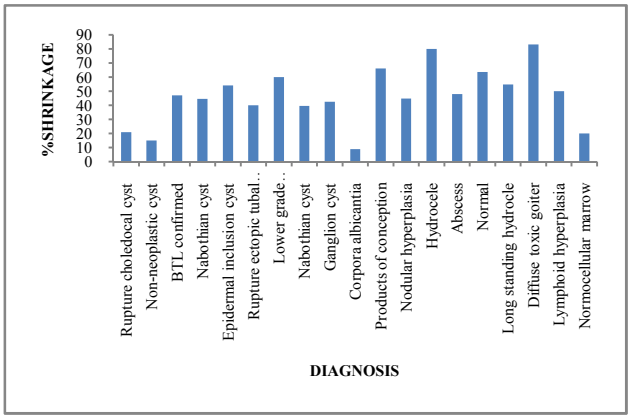

Figure 6 Shrinkage of tissue and diagnosis of non-neoplastic lesion

(8.8% shrinkage), adnexal cyst (15% shrinkage) and trephine biopsy (20% shrinkage) as shown in figure 10.

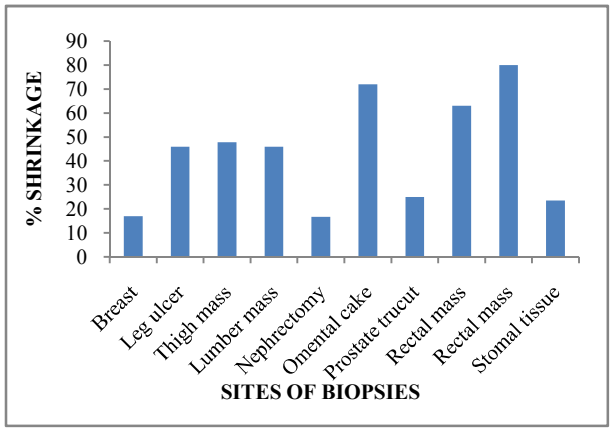

Figure 7 Sites of biopsies and percentage shrinkage (malignant neoplasm)

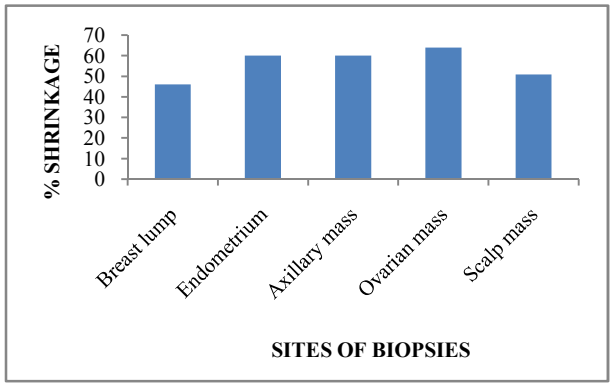

Figure 8 Sites of biopsies and percentage shrinkage (benign neoplasm)

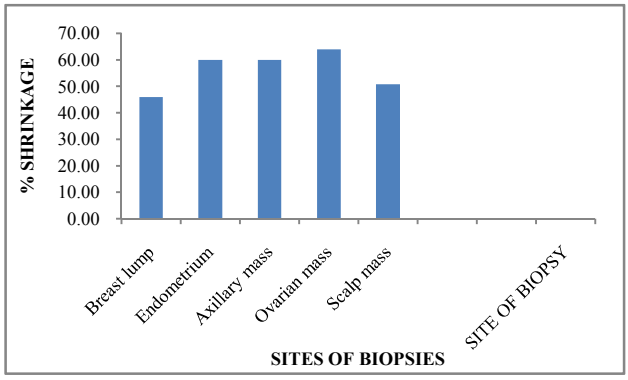

Figure 9 Sites of biopsies and percentage shrinkage (inflammatory lesions)

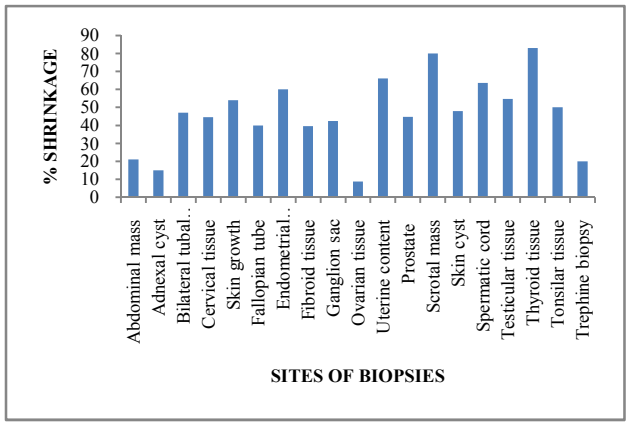

Figure 10 Sites of biopsies with percentage shrinkage (non-neoplastic lesions)

DISCUSSION

There are many studies indicating tissue shrinkage after formalin fixation and following processing and among the factors that are responsible for the shrinkage during tissue processing include alcohol dehydration, clearing, and impregnation in paraffin wax. Embedding in paraffin wax which may further compress tissue if the paraffin is too warm has also been described. [17] Similarly, studies have indicated that the average tumour diameter from fixation to microscopic measurements shrink by 7.1% and the overall percentage of shrinkage from fresh biopsy to microscopic diameter ranges from 4.5% to 41%. [17] In our study, we found marked average shrinkage in malignant neoplasm and inflammatory lesions (figures 3 and 5). Rectal mucinous carcinoma and rectal adenocarcinoma show a high rate of shrinkage of 63% and 80% respectively. Renal cell carcinoma and invasive ductal carcinoma (NOS) show the least shrinkage of 17% each among all the malignant neoplasm study, shrinkage of 2.14 mm for invasive ductal carcinoma, NST following fixation has been reported.[18] Looking at recurrent rates of 16% for a patient with breast cancer with positive margins as against 4% for those with free margins, as indicated in some studies, the 17% shrinkage by malignant breast tumours found in our work can be very significant to give rise to suboptimal tumour staging. It is important to note that malignant tumours usually shrink more than the surrounding normal tissue that can interfere with tumour free margins. Many studies [9,13] have shown that there is no correlation between shrinkage in breast cancer tissue and the use of formalin with graded pH and duration of fixation. In our work, we have found mucinous and adenocarcinoma of rectal tissue with the highest shrinkage rate of 80 and 68% respectively, this may be due to mucin production by the tumour cells and associated host cellular response. [6,19]

Intermediate shrinkage were seen in squamous cell carcinoma, adenocarcinoma of the prostate and rhabdomyosarcoma with 46%, 46% and 25%, respectively (table 1, figures 3,7). The shrinkage noted in those three cases is adequate to alter surgical margins during microscopic interpretation. These findings were in agreement with many written reports in the literature.[15,17,20] Studies correlating shrinkage factors ranging from 1.22 millimeter to 1.5 mm in prostatectomy specimens have been observed based on the findings that linear shrinkage of prostatic tissue after formalin fixation was found to be 4.1%, but that increased to 14.5% following processing.[8] Correction factor of shrinkage of 1.12% to 1.13% for brain tissue post processing was suggested by some authors[7] even though the slightest difference of shrinkage of different parts of the brain parenchyma exists.

Morphometric study[11] of cervical tissue, particularly in the determination of crypt involvement by cervical intra epithelial lesion (CIN), is very crucial in the patient line of management; shrinkage of 2.7% and 12.6% of the original volume in cervical cancer has been described.[11] In our study cervical tissue that was categorized under non- neoplastic lesion shrank by 44.5% of its original post-fixation volume (table 3, figure 3). Skin tissue diagnosed as squamous cell carcinoma show shrinkage of 46% (figures 3,7), this is in agreement with other studies [12] even though some have considered post-fixation rather than post processing. Shrinkage of esophageal cancer was found to be remarkably high (83%) post-fixation.[5] It is important to mention that most of the work on tissue shrinkage

have not solved the matters of shrinkage correction factors that are needed for specific cancers, which can assist in determining correct surgical margins and tumor size. Our observation in the non-neoplastic lesion is that tissue with abundant loose connective tissue and a cystic lesion shrink remarkably high as exhibited by scrotal and thyroid tissue biopsies (table 4, figure 10) with 80% and 83% post processing shrinkage respectively. Tissue with compact stroma like ovarian biopsy show 8.8% shrinkage post processing (figure 10).

Inflammatory lesion shows remarkably high shrinkage ranging between 42 to 80% (table 3, figures 5, 9). This post-processing shrinkage finding is not out of place because it is obvious that inflammation is associated with oedema. That can also explain marked shrinkage of a malignant tumour because of accompanying host immune response associated with oedema. In the few cases of benign neoplasm study (table 2, figures 4,8), the percentage shrinkage appears to be fairly uniform ranging between 46% to 64%. That also can be supported based on the biological behavior of a benign tumour. Site of biopsy is very relevant to shrinkage of neoplastic lesions; reports indicating tissue specimens obtained from trunk or extremities show greater shrinkage (16%) compared to specimens from head and neck region (10.2%).[13] We have found similar findings from our study; where stomal tissue show 23.5% shrinkage compared to thigh mass which shrank by 47.8% as shown in Table 1 and figures 7, 8, 9, 10.

Studies indicating age variations in tissue shrinkage have shown 25% shrinkage for a patient less than 50 years and 20% of patient 50 and 51 years. Fifteen percent shrinkage was observed in patient ≥ 60 years with neoplasm.[15] The surgical margins predicted by formula for shrinkage were within ± 3.5 mm of the actual surgical margins. In our survey, we found 28% shrinkage for the patient between the age of 41-50, and those above 70 years was 15% (table 1 and figure 2). This is in accordance with some study [15] even though their shrinkage was measured after tissue fixation not post processing as in our case.

There was no marked difference found between sex for age and shrinkage as shown in figure 2, however, patients between the age of 2-20 years have shown a female preponderance that can be explained on the ground that all patients < 20years in the survey are female with the exclusion of single male aged 14 years diagnosed as rhabdomyosarcoma (table 1).

A study indicating no changes in tissue size when graded formalin concentration, differing pH, whole mount versus quadrant sections, tissue thickness and different water bath temperature were used has been reported.[8] However, there are inter-laboratory variability due to different methods and automated tissue processing machine in use has been noted.[8] In our study, we used *Shaldom Dublex* automated tissue processor.

CONCLUSION

Tissue specimens show considerable shrinkage after processing[21,22] and the shrinkage after tissue processing from our findings depends on the types of the neoplasm, sites of biopsy, nature of specimens and the age of the patients. Thus, determination of post tissue processing correction factors for every malignant neoplasm is necessary for accurate staging using the TNM staging system, and to our knowledge, that component (shrinkage correction factors) is lacking for many specific malignant tumours. Again, we have observed that development of correction factors should be regional based because of different method and tools used in tissue processing that will serve as an effective means for correct staging/grading that can further assist in appropriate prognostication of cancer, and resolve surgical margin discrepancies, especially in micrographic surgery.

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