Quantifying Tissue Shrinkage Throughout Histological Processing for Cutaneous, Parenchymal and Luminal Tissues

Mina Lee[1], Murray Burns[1] and Michael K. Ghebremariam[2]

[1] School of Biodiversity, One Health & Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow

[2] Division of Pathology, Public Health & Disease Investigation, School of Biodiversity, One Health & Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow

Introduction

Shrinkage of tissues following excision and histological processing is a well-known phenomenon. However, in veterinary medicine it is poorly quantified, particularly for internal organs.

In many circumstances, surgery is the primary treatment modality for neoplasia in canine and feline patients (Balsa and Culp, 2019). The success of surgical oncology is often based on the presence of complete or incomplete margins assessed on histopathology (Liptak, 2020). However, if tissue shrinkage is significant during histological processing, then the clear margins seen by the pathologist will be less than the true tumour-free margins. In addition, studies based on retrospective histopathological findings may recommend surgical margins that are too conservative. For example, one study concluded, based on histopathological analysis of small intestinal carcinomas in dogs, that a 3cm margin from the palpable tumour could be defined as complete (Morrice et al., 2019). However, without quantifying shrinkage occurring during histological processing, it is hard to correlate this research with surgical recommendations. The aim of the present study was to bridge this knowledge gap by quantifying tissue shrinkage during histological processing in cutaneous, parenchymal and luminal tissues.

Methodology

Four clinically normal ewe cadavers were extensively sampled at post-mortem examination. All samples were collected within 6 hours of euthanasia, which is within the time frame of similar studies (Reagan et al., 2016). Samples were obtained from nine cutaneous bilateral locations to assess the effect of sample location on shrinkage. Half of the cutaneous samples were left freely floating in formalin, while the other half were adhered to cardboard during fixation. Five parenchymal organs (the lungs, heart, liver, spleen, and kidneys) and ten luminal organs (entire gastrointestinal tract, uterus, and bladder) were also sampled. When appropriate, a random number generator was used to assign a specific location for tissue excision. One individual was responsible for excising tissue samples from the cadaver while another individual was responsible for measuring sample length.

All samples were fixed in formalin, dehydrated, embedded into paraffin wax, and then mounted onto slides and stained with Haematoxylin and Eosin. Measurements were taken at five different time points: in situ (T0), post excision (T1), after 24-48 hours fixation (T2), post paraffin wax embedding (T3), and on slide, post H&E staining (T4). Shrinkage for cutaneous samples was calculated from T0-T4, and T1-T4 for parenchymal and luminal organs.

Tissue shrinkage was calculated using the formula:

$$Percentage\ Shrinkage = -\left(\frac{Length\ at\ Current\ Time\ Point}{Length\ at\ Previous\ Time\ Point} \times 100 - 100\right)$$

Data processing was carried out using 'RStudio' (R Core Team 2023) and statistical significance was set at $p < 0.05$. Shrinkage is displayed with positive percentage values and increases in length are displayed with negative percentage values.

Discussion

Shrinkage from T0 to T4 was shown to be significant at every cutaneous location, parenchymal and luminal organ group. Mean shrinkage for cutaneous sample groups from T0-T4, including both cardboard and freely floating samples, ranged from 12.57% (thorax) to 19.97% (cranial abdomen). The mean shrinkage from T0-T4 of freely floating cutaneous samples was 20.75%. This was lower than the reported values of 24.0-37.7% in canine cadavers (Reagan et al., 2016), and 35.8% in feline cadavers (Jeyakumar et al., 2015).

From T0-T4, samples from the cranial and caudal abdomen displayed significantly greater shrinkage than the head and thorax. Cutaneous samples fixed on cardboard displayed significantly less shrinkage than freely floating samples from T0-T4 (11.32% and 20.75% respectively). Based on this finding, cardboard should be considered for use during formalin fixation to decrease length shrinkage artefacts.

Parenchymal organs displayed mean overall shrinkage (T1-T4) values between 15.24% (kidneys) and 20.14% (lungs). The difference between organs was not statistically significant. For tumours of the liver, lung, kidney, and spleen, either entire organ or lobe removal is often possible (Balsa and Culp, 2019, Gibson et al., 2022, Wendelburg et al., 2015) unlike tumours of the gastrointestinal tract and bladder. Therefore, decisions around tumour resection margins are inevitable for luminal organs. The results of the present study found mean total shrinkage (T1-T4) in luminal organ samples to be between 8.43% (rumen) and 18.13% (oesophagus). Only the oesophagus and rumen were statistically significantly different from each other. However, this should be interpreted with caution as the rumen was also the only organ that had to be pinned to cardboard, while the others adhered on their own. Unfortunately, post-excisional length shrinkage measurements could not be accurately achieved for parenchymal and luminal samples and therefore shrinkage is only calculated T1-T4. Post-excisional length shrinkage of canine jejunal samples was found to be 28.3% in another study (Clarke et al., 2014). In the present study, jejunal sample shrinkage from T1-T4 was 16.94%. If histological shrinkage is similar between canine and ovine

samples, then combining these values would result in total shrinkage from in-situ to on-slide measurements of 40.5% in dogs.

To better correlate histological analysis to surgical guidelines, it is vital to fully quantify the shrinkage of various tissues throughout histological processing. It is the authors' belief that more research should be carried out in organ shrinkage of various species of animals to allow for more evidence based surgical margin decisions to be made.

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