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Original Article

Shrinkage of head and neck cancer specimens after formalin fixation

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Abstract

Background: We conducted this study to investigate whether formalin fixation is associated with the shrinkage of head and neck cancer specimens.

Methods: Patients scheduled to undergo operation were eligible for enrollment. Fresh specimens were measured immediately in the operating room, and the measurements were repeated after formalin fixation.

Results: A total of 100 specimens were collected. Nearly half of them were obtained from the oral cavity ($n = 49$), and a large majority were squamous cell carcinoma ($n = 69$). The average decreases in length, width, and depth after formalin fixation were 1.50 mm (4.40%), 1.52 mm (6.18%), and 0.67 mm (4.10%), respectively. There was no significant difference in the shrinkage percentage associated with gender, age, tumor site, tumor size, or histology.

Conclusion: We found that head and neck cancer specimens shrink after formalin fixation. Therefore, we recommend that the specimen be measured immediately in order to avoid the underestimation of tumor size.

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Keywords: formalin; head and neck cancer; shrinkage; tumor staging

1. Introduction

Carcinomas of the head and neck, including cancers arising from the oral cavity, oropharynx, hypopharynx and larynx, represent the sixth most common type of cancers worldwide.¹ Tumor size is an important prognostic factor in head and neck cancer. According to the American Joint Committee on Cancer (AJCC) staging system, the pathological classification of a carcinoma is determined by evidence acquired before treatment, then supplemented and modified by additional evidence acquired during surgery, particularly specimen examination by a pathologist. The pathological T category is

derived from the actual measurement of the unfixed tumor in the resected specimen because up to 30% shrinkage of soft tissue may occur in a resected specimen after formalin fixation.²

Formalin fixation may cause tumor shrinkage and subsequently lead to the underestimation of tumor staging. Siu et al found that esophageal tumors shrink 10% after formalin fixation. Furthermore, the overall shrinkage of the whole esophageal specimen after fixation was 50%.³ A study addressing the effect of tissue fixation and processing on breast cancer size pointed out that there was no difference in measured size between fresh and fixed specimens in most cases (96%).⁴ Jonmarker et al, in their study about prostatectomy specimens, found the average linear shrinkage after formalin fixation was 4.5%.⁵ In non-small cell lung cancer, Hsu et al found that formalin fixation might cause tumor shrinkage and migration from T2 to T1.⁶

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In head and neck cancer studies on margin discrepancy after formalin fixation, the results show that the tumor margin shrank from 47.3% to 22.7%.^{7–9} However, few studies have focused on the extent of tumor shrinkage after formalin fixation in head and neck cancer specimens. Therefore, the aim of this study was to investigate whether formalin fixation affects the size of head and neck cancer specimens.

2. Methods

The study protocol was approved by the institutional review board of Taichung Veterans General Hospital. All patients scheduled to undergo surgical excision due to head and neck cancer were eligible for this study. Those who switched to nonsurgical modalities, had previously undergone chemotherapy or radiotherapy, had inadequate chart records, or were reluctant to participate in this study were excluded. Patients with metastatic lymphoid tissues were also excluded from the current study. All patients were informed about the purpose of the study, and their consent was obtained before enrollment. Basic demographic data, including age, gender, tumor locations, tumor staging, and histological features, were obtained. Clinical treatments were carried out on all patients according to the consensus guidelines of the head and neck cancer team of our hospital. All surgical procedures were performed by a single head and neck surgeon (SA Liu).

All patients underwent surgical resection under general anesthesia. The fresh specimens were measured immediately after resection in the operating room. All measurements were performed by a single observer, a senior otolaryngology resident. The tumor dimensions were measured using a slide caliper (Fig. 1). Precise measurements were achieved by intensive training on the quantification technique in advance. Ten square toy blocks were randomly given to the observer with an interval of 2 days to 2 weeks between sessions. An intraclass correlation above 0.96 was reached before the initiation of study. The maximal diameter of the tumor was

recorded as the “length,” the longest diameter perpendicular to the “length” was recorded as the “width,” and the “depth” represented the longest diameter perpendicular to the plane composite of the “length” and “width.” The locations where the “length,” “width,” and “depth” were measured were marked using silk stitches, then the specimens were placed in 10% neutral buffered formalin. After formalin fixation for 24–48 hours, the measurements were repeated by the same observer using the same slide caliper and the same methods.

We used descriptive statistics in the general data presentation. Comparisons of the dimensions between the fresh and formalin-fixed specimens were analyzed using paired *t* test. Differences in shrinkage between groups were examined using the independent *t* test. Differences in the dimensions before and after formalin fixation were further compared according to various factors (e.g., age, gender, tumor site, etc). All statistics were calculated using SPSS for Windows, version 10.1 (SPSS, Inc., Chicago, IL, USA). A *p* value < 0.05 was regarded as statistically significant.

3. Results

From January 2009 to October 2010, 107 patients with head and neck cancer were scheduled to undergo surgical intervention. Three patients chose nonsurgical treatment, two patients had previously undergone chemotherapy or radiotherapy, and two patients were reluctant to join the study before surgery. In total, 100 specimens were obtained from the remaining 100 patients for measurement and analysis. Among these 100 participants, male accounted for 80% (*n* = 80) of the patients and the average patient age was 51 years old (range: 23–71 years). Nearly half of the primary tumor sites were in the oral cavity (*n* = 49, 49%) followed by the thyroid (*n* = 16, 16%) and oropharynx (*n* = 11, 11%). Most of them were identified as squamous cell carcinoma (*n* = 69, 69%), while the others included mucoepidermoid carcinoma, adenoid cystic carcinoma, papillary carcinoma, follicular carcinoma, and other forms of cancer. Other primary tumor sites included the hypopharynx, larynx, parapharyngeal space, and the submandibular and parotid regions.

After formalin fixation, the average tumor length decreased from 34.06 mm to 32.56 mm, whereas the average width and depth of tumor were reduced from 24.60 mm to 23.08 mm and 16.36 mm to 15.69 mm, respectively. The differences in the dimensions between the fresh and formalin-fixed specimens were statistically significant (all *p* < 0.001). The average shrinkage percentages of the length, width, and depth were 4.40%, 6.18%, and 4.10%, respectively. After dividing participants according to median age, the differences were still statistically significant. However, after dividing participants according to gender, the differences were only statistically significant along the width. Detailed data are presented in Table 1.

We further divided all participants into subgroups according to gender, age, tumor site, pathological features, and tumor size. The differences between or among subgroups in terms of length, width, and depth of each specimen before and after

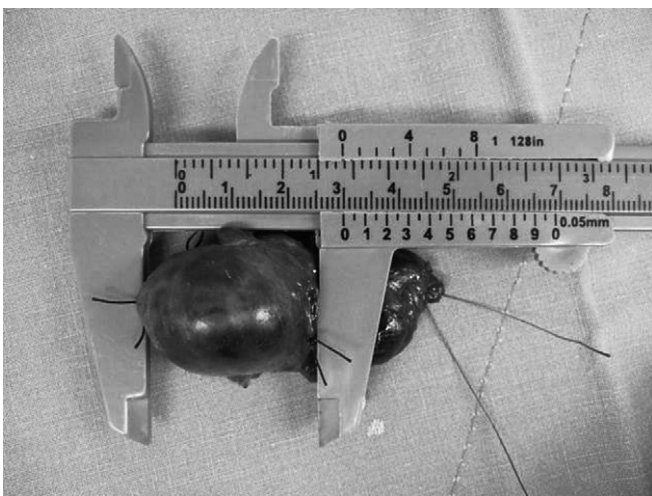


Fig. 1. The dimensions of the tumors were measured using a slide caliper. The measured points were marked using silk sutures.

Table 1
Changes in the dimensions of specimens after formalin fixation.

	Fresh specimen	After formalin fixation	Difference (%)	95% Confidence Interval		P
				Lower limit (%)	Upper limit (%)	
Length (mm)	34.06 ± 1.52	32.56 ± 1.47	1.50 (4.40)	1.02 (2.99)	1.98 (5.81)	
<=52 y	37.10 ± 2.31	35.45 ± 2.27	1.65 (4.45)	0.93 (2.51)	2.38 (6.41)	< 0.001
> 52 y	31.14 ± 1.93	29.78 ± 1.81	1.35 (4.34)	0.69 (2.22)	2.01 (6.45)	< 0.001
Male	33.35 ± 1.70	31.81 ± 1.66	1.54 (4.62)	1.04 (3.12)	2.04 (6.12)	< 0.001
Female	36.90 ± 3.43	35.55 ± 3.11	1.35 (3.66)	-0.09 (0.24)	2.79 (7.56)	0.065
Width (mm)	24.60 ± 1.17	23.08 ± 1.10	1.52 (6.18)	1.02 (4.15)	2.02 (8.21)	
<=52 y	26.80 ± 1.81	25.22 ± 1.69	1.57 (5.86)	0.94 (3.51)	2.21 (8.25)	< 0.001
> 52 y	22.49 ± 1.43	21.02 ± 1.36	1.47 (6.54)	0.68 (3.02)	2.26 (10.04)	0.001
Male	24.19 ± 1.34	22.60 ± 1.27	1.59 (6.57)	1.01 (4.18)	2.17 (8.97)	<0.001
Female	26.25 ± 2.30	25.00 ± 2.09	1.25 (4.76)	0.22 (0.84)	2.28 (8.69)	0.020
Depth (mm)	16.36 ± 1.02	15.69 ± 0.99	0.67 (4.10)	0.33 (2.02)	1.01 (6.17)	
<=52 y	18.89 ± 1.48	18.17 ± 1.48	0.72 (3.81)	0.17 (0.90)	1.27 (6.72)	< 0.001
> 52 y	13.94 ± 1.33	13.31 ± 1.24	0.63 (4.52)	0.21 (1.51)	1.04 (7.46)	0.004
Male	15.66 ± 1.13	14.82 ± 1.08	0.84 (5.36)	0.46 (2.94)	1.21 (7.73)	< 0.001
Female	18.95 ± 2.28	18.90 ± 2.25	0.01 (0.05)	-0.72 (3.80)	0.82 (4.33)	0.893

formalin fixation were compared. We found that there was no significant statistical difference between the male and female participants in terms of length, width, or depth before and after formalin fixation (Table 2). There were also no significant statistical differences among age groups (Table 3), primary sites (Table 4), or pathological features (Table 5) in terms of the dimensional changes before and after formalin fixation. When we divided all of the participants according to median tumor size (32.5 mm) into two groups, there were significant differences between the two groups in terms of length and width. However, when we converted the absolute changes in the dimensions into percentage changes, the difference turned out to be insignificant (Table 6).

4. Discussion

Tumor size is an important factor for determining the correct tumor staging. Tumor staging not only influences the decision-making process in patient treatment plans, but also provides information about the prognosis of head and neck cancer patients. At present, there is no universal, standardized method for tumor size measurement. It is well known that tissues will shrink after formalin fixation. However, different reports have different findings. In 1983, Boonstra et al found that cervical tissue shrank up to 15% after formalin fixation and processing.¹⁰ Esophageal tumors demonstrated 10% shrinkage after formalin fixation in another study.³ It was also reported that prostate specimens decreased by 4.5% in diameter and 13% in volume after formalin fixation.⁵ Two studies

on breast cancer specimens presented different results. Yeap et al reported 4.5% shrinkage in breast cancer diameter after histological fixation.¹¹ In contrast, another study reported no significant change between fresh and fixed states in 96% of breast cancer specimens.⁴ Regarding the variation in shrinkage reported in the abovementioned studies, it is possible that the constitution and type of tissue (e.g., amount of muscle or fat tissues) may influence the overall degree of shrinkage. Therefore, it is reasonable that studies addressing changes in dimensions should be organ-specific and account for tissue composition. To the best of our knowledge, the current study is the first study to investigate the shrinkage of head and neck cancer specimens. We found that the maximal diameter of the head and neck cancer specimens shrank 1.5 mm (4.40%) after formalin fixation. If we used the dimensions of the fixed specimens as measurements of tumor size, underestimation of T staging was found in eight patients, according to AJCC guidelines (5 patients from T3 to T2 and 3 patients from T2 to T1). Similar results was reported by Hsu et al in their study on the effects of formalin fixation on lung cancer tumors (a tumor shift from stage Ib to stage Ia was reported in 9.97% of patients).⁶

Although a variety of proprietary fixatives were developed for surgical specimen preparation, formalin fixation provides the highest histomorphological quality for tissue staining and determining a pathological diagnosis.¹² Neutral-buffered formalin (10%), which contains 4% formaldehyde, is the most common fixative used in the laboratory. The effects of formalin on surgical specimens can be divided into two

Table 2
Changes in the dimensions of specimens after formalin fixation in patients divided into subgroups according to gender.

Difference	Male (n = 80)	Female (n = 20)	P
Length (mm)	1.54 ± 0.25	1.35 ± 0.69	0.759
Width (mm)	1.59 ± 0.29	1.25 ± 0.49	0.596
Depth (mm)	0.84 ± 0.19	0.05 ± 0.37	0.057

Table 3
Changes in the dimensions of specimens after formalin fixation in patients divided into subgroups according to age.

Difference	<= 52 y (n = 49)	> 52 y (n = 51)	P
Length (mm)	1.65 ± 0.36	1.35 ± 0.33	0.539
Width (mm)	1.57 ± 0.32	1.47 ± 0.40	0.843
Depth (mm)	0.72 ± 0.27	0.63 ± 0.21	0.787

Table 4

Changes in the dimensions of specimens after formalin fixation in patients divided into subgroups according to primary tumor site.

Difference	Oral cavity (<i>n</i> = 49)	Others (<i>n</i> = 51)	<i>P</i>
Length (mm)	1.22 ± 0.30	1.76 ± 0.38	0.265
Width (mm)	1.41 ± 0.32	1.63 ± 0.40	0.667
Depth (mm)	0.57 ± 0.22	0.76 ± 0.26	0.575

phases. During early phase, formalin penetrates the tissue by diffusion and accumulates to a concentration sufficient for the next phase to begin.⁶ In the second phase, the molecular changes include the formation of cross-links between proteins, or between proteins and nucleic acids, that involve hydroxy-methylene bridges. Another proposed mechanism is the formation of coordinate bonds with calcium ions. The cross-links and coordinate bonds may alter the three-dimensional structure of the proteins.¹³ This reaction is slow, taking 24–48 hours to complete. A short fixation time leads to incomplete processing, so the duration of fixation can affect the degree of tissue shrinkage.^{5,6}

In our study, there was no significant difference in the tumor-shrinkage rate between subgroups in terms of gender, age, and tumor site. Gender has not been reported as an association with shrinkage after formalin fixation.¹⁴ However, Kerns et al reported that patient age is a significant factor associated with shrinkage in cutaneous specimens. They also found there was 5% greater shrinkage in trunk excisions than head and neck excisions.¹⁴ This disparity might be due to the diverse pathological features in the different studies. Most of our patients had squamous cell carcinoma, whereas patients in the aforementioned study had cutaneous carcinoma. Another possible explanation is that the cutaneous specimens shrank in length and width immediately after excision because of intrinsic tissue contractility, not formalin fixation.^{14,15} In contrast, we didn't measure *in vivo* tumor size and the shrinkage in our study was mostly due to formalin fixation.

Pritt et al, in their study on breast cancer, found no significant difference in shrinkage in terms of various histological features.⁴ Conversely, Hsu et al, in their study on non-small cell lung cancer, pointed out that different histological characteristics were distributed unevenly among subgroups.⁶ In our study, histology was not associated with tumor shrinkage. The reason might be that a large portion of our specimens were squamous cell carcinoma (*n* = 69, 69%). In addition, distinct histological features and dissimilar composition in various organs might cause a different shrinkage rate after formalin fixation. Interestingly, we found that larger

Table 5

Changes in the dimensions of specimens after formalin fixation in patients divided into subgroups according to pathological features.

Difference	SCC (<i>n</i> = 69)	Others (<i>n</i> = 31)	<i>P</i>
Length (mm)	1.65 ± 0.28	1.16 ± 0.47	0.352
Width (mm)	1.61 ± 0.30	1.32 ± 0.46	0.603
Depth (mm)	0.80 ± 0.19	0.40 ± 0.34	0.277

SCC: squamous cell carcinoma.

Table 6

Changes in the dimensions of specimens after formalin fixation in patients divided into subgroups according to tumor size.

Difference	≤ 32.5 mm (<i>n</i> = 50)	> 32.5 mm (<i>n</i> = 50)	<i>P</i>
Length (mm)	0.72 ± 0.25	2.28 ± 0.39	0.001
Width (mm)	0.56 ± 0.19	2.48 ± 0.43	< 0.001
Depth (mm)	0.34 ± 0.19	0.96 ± 0.26	0.062
Length (%)	3.09 ± 1.06	5.13 ± 0.85	0.137
Width (%)	3.73 ± 1.32	7.52 ± 9.99	0.052
Depth (%)	1.87 ± 1.38	4.37 ± 9.25	0.191

specimens tended to shrink more in terms of their absolute dimensions after formalin fixation. When the change in the absolute dimensions was converted into relative percentage, the difference turned out to be insignificant. It is easy to understand that when the shrinkage rate is fixed, larger specimens will shrink more in terms of absolute dimensions.

Most studies on formalin fixation of head and neck cancer evaluated the margin of shrinkage. In an animal study, Johnson et al found that the mean shrinkage rates of the lingual surface, deep tongue, and labiobuccal mucosal margins were 30.7%, 34.5%, and 47.3%, respectively.⁷ Another study on oral cancer showed the mean shrinkage rates of the tongue and buccal mucosa margins were 23.5% and 21.2%, respectively, whereas the mean shrinkage rates of the margins in T1/T2 and T3/T4 tumors were 25.6% and 9.2%, respectively.⁸ Cheng et al, in their study on margin discrepancy in oral squamous cell carcinoma, found that the mean discrepancy rates in buccal mucosa, mandibular alveolar ridge, and retromolar trigone margins were 71.90%, 53.33%, and 42.14%, respectively. The mean discrepancy rates in T1/T2 and T3/T4 tumors were 51.48% and 75%, respectively.⁹ None of abovementioned studies included data on tumor shrinkage. However, because we didn't collect the data on margins, no comparisons could be made. Why there was such a huge difference between mucosal margin shrinkage and main tumor shrinkage might be explained by the different constituents of the mucosal margin are mainly composed of loose connective tissues.

According to the latest version of the *AJCC Cancer Staging Manual*, the pathological classification of a carcinoma is determined by the evidence acquired before treatment, then supplemented and modified by additional evidence acquired during and from surgery, particularly pathological examinations of resected tissues.² However, the pathologists do not routinely measure fresh specimens in the operating theater in Taiwan. In contrast, pathologists always measure specimens that have undergone overnight formalin fixation. Not all medical facilities have their pathologists handle the fresh specimens immediately after tumor resection in the operating room.⁶ Therefore, the surgeons should measure the fresh specimens in order to prevent inaccurate determination of the tumor stage.

The strength of this study is its status as a prospective case series, instead of a retrospective design at a single institute (tertiary referral center) with the proper statistical work-up. However, there are several limitations to the present study. First, the small sample size could reduce the broader

applicability of the results. Secondly, our study included several primary tumor sites and various histological features of the head and neck tumors, which may have different characteristics. Finally, the fixation time ranged from 24–48 hours, and this variation may have affected the accuracy of this study. Further studies that take the fixation duration into account may be needed in order to clarify the relationship between shrinkage and formalin fixation.

In conclusion, we found that head and neck cancers specimens shrank significantly after formalin fixation. The average shrinkage of the maximal diameter of head and neck cancer specimens was 4.40% in our current study. Therefore, we recommend that the tumor size be measured immediately after resection in the operation room in order to avoid the understaging of head and neck cancers.

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