RESEARCH ARTICLE

The shrinkage effect of formalin on renal cell carcinoma: Does it change the stages

Murat Akgul[1], Aysegul Isal Arslan[2], Cenk Yazici[3], Enes Altin[4], Meltem Oznur[5]

Abstract

Objective: To determine the shrinkage effect of formalin on renal cell carcinoma.

Method: The retrospective study was conducted from October to November 2020 at Tekirdağ Namık Kemal University, Turkey, and comprised all radical and partial nephrectomy cases performed by a single surgeon in a single clinic between January 2014 and August 2020. Pre-operative images and post-operative pathology were reviewed by the same clinician. Pre-operative longest tumour diameter of radiological images and pathological specimens measured after formalin fixation shrinkage were compared, and the effect of the difference between the two measurements on the circumference of the tumour was examined. The formalin-related shrinkage rates of renal tumours according to the tumour size and the tumour types were also analysed. Data was analysed using SPSS 20.

Results: Of the 101 cases, 58(57.4%) were of radical and 43(42.6%) of partial nephrectomy. Also, there were 77(76.2%) renal cell carcinoma cases, 22(21.8%) benign renal tumours and 2(1.9%) had other malignant tumours. There were 59(58.4%) males and 42(41.6%) females with an overall mean age of 58.1±12,2 years (range: 30-82 years). The mean radiological size of the renal tumours was 55.3±30.4 mm and it was 52.9±31.6 mm at pathological examination ($p>0.05$).

Conclusion: Formalin fixation of tissues post-surgery caused a difference between the radiological and pathological dimensions. Though the difference was no significant, under-staging due to the shrinkage post-surgery should be considered.

Keywords: Renal tumours, Tissue shrinkage, Formalin, radiological dimension, Pathological dimension.

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Introduction

Renal cell carcinoma (RCC) is one of the deadliest cancers of the genitourinary tract and constitutes 2-3% of all adult malignancies.[1] In 2012, European statistics showed 84.000 new cases of RCC with 34,700 cancer-related death.[2] RCC incidence peaks in the 5th-7th decade with 3-to-2 female predominance.[1,3,4] The Tumour Node Metastasis (TNM) classification is the main staging system for RCC based on tumour size for T1 and T2 stages. According to the classification, tumour ≤4cm is classified as T1a, 4-7 cm as T1b, 7-10 cm as T2a, and >10cm as T2b.[2]

Tumour size is a significant prognostic indicator for RCC. For this reason, clinicians and pathologists must carefully measure the size of the tumour to evaluate their patients precisely. The tumour size used in TNM classification is a pathological definition. Macroscopic and/or microscopic measurement of the renal tumour diameter determines the T1 and T2 stages of RCC. On the other hand, tumour specimens have some processes before definitive pathological evaluation. During these processes, the tumour size may change and this may cause mis-staging of the tumour. The first stage of the pathological process is to embed the specimen to a 10% formaldehyde solution, called formalin, which has tissue-fixation properties. Formalin may cause tissue shrinkage.[5-7] As the tumour size has significant importance for RCC staging, the tissue shrinkage effect of formalin may have an important impact on RCC patients.

The current study was planned to evaluate the shrinkage effect of formalin in renal tumours, to evaluate the shrinkage effect of formalin on benign and malignant renal tumours, to see if tissue shrinkage changes RCC stage, and to identify the shrinkage effect of formalin in RCC subtype pathologies.

Materials and Methods

The retrospective study was conducted from October to November 2020 at Tekirdağ Namık Kemal University, Turkey, and comprised all radical and partial nephrectomy cases performed by a single surgeon in a single clinic between January 2014 and August 2020. Pre-operative images and post-operative pathology were reviewed by the same clinician.

Data related to demographic and clinicopathological properties of patients, including age, gender, histopathological diagnosis and TNM staging, was recorded. As a standard procedure, all patients with the diagnosis of renal mass had a radiological evaluation with

[1,3,4]Department of Urology, Tekirdag Namik Kemal University, Tekirdag, Turkey; [2,5]Department of Pathology, Tekirdag Namik Kemal University, Tekirdag, Turkey.

Correspondence: Murtat Akgul. e-mail: drmuratakgul@gmail.com

contrast-enhanced abdominal computed tomography (CT) or magnetic resonance imaging (MRI), according to their respective creatinine levels. To standardise the study, patients who had only MRI evaluation before the surgery were excluded. The latest CT evaluation of the population was 1 month before the surgery. The largest renal mass diameter was measured at re-format CT images using axial, sagittal and coronal images.

The same procedure was applied on the specimens post-surgery. All specimens were immediately placed into formalin solution without any disruption. As such, it was not possible to cut the specimen to measure the fresh tissue tumour diameter. The specimens were left in formalin solution for 12-24 hours. After fixation, the specimen was cross-sectioned for macroscopic evaluation. The largest diameter of the tumour was selected and measured by a plastic metric ruler at 1mm precision. This measurement was used for staging T1 and T2 tumours. A single pathologist performed all steps of specimen preparation and evaluation.

Data was analysed using SPSS 20. Data in independent groups were analysed for normalcy with Kolmogorov-Smirnov test and was further evaluated with an independent t-test. P<0.05 was considered statistically significant.

Results

Of the 101 cases, 59(58.4%) males and 42(41.6%) females with an overall mean age of 58.1±12.2 years (range: 30-82 years). There were 58(57.4%) cases of radical and 43(42.6%) of partial nephrectomies. Also, there were 77(76.2%) RCC cases, 22(21.8%) benign renal tumours and 2(1.9%) had other malignant tumours. Histopathological diagnosis was clear cell carcinoma 61(60.3%), chromophobe cell carcinoma 6(5.9%), papillary cell carcinoma 8(7.9%), multilocular cystic tumour 2(1.9%), angiomyolipoma 15(14.8%), oncocytoma 7(6.9%), neuroendocrine tumour 1(0.9%) and carcinoid tumour 1(0.9%).

The mean radiological size of renal tumours was 55.3±30.4mm whereas it was 52.9±31.6mm on pathological evaluation, which showed a 4.3% shrinkage rate (p=0.881). The mean radiological size of benign tumours was 64.5±30.9mm and their mean pathological size was 62.4±34.8mm, which showed a shrinkage rate of 3.3% (p=0.765). The mean radiological size of malignant tumours was 54.1±29.7mm and their mean pathological size was 51.7±31.7mm which showed a shrinkage rate of 4.4% (p=0.710). Among the 77(76.2%) RCC cases, 27(35%) radiologically T1b tumours, 7(26%) were staged T1a in pathological evaluation. The mean radiological tumour size of these under-staged tumours was 41.7±1.2mm whereas their mean pathological size was 38.9±2.4mm (p=0.763). Of the 13(17%) radiologically T2a tumours, 4(31%) were reported T1B pathologically. The mean radiological and pathological diameters of these tumours were 71.5±5.3mm and 68.2±4.2mm, respectively (Table 1).

Table-1: Stages of renal cell carcinoma according to radiological and pathological tumour size.

		Pathological Stage (n)				
		T1a	**T1b**	**T2a**	**T2b**	**Total**
Radiological Stage (n)	**T1a**	35				35
	T1b	7	20			27
	T2a		4	9		13
	T2b				2	2
	Total	42	24	9	2	77

Table-2: Formalin-related shrinkage rates of renal cell carcinoma according to the tumour size.

	n	**Tomography size** (mm)	**Pathology size** (mm)	**Shrinkage rate** (%)	***p*-value**
≤ 20 mm	5	17.8±4.2	15.8±3.6	11.2	0.210
20-30 mm	14	28.5±7.4	26.8±3.3	5.9	0.433
30-40 mm	16	36.4±2.7	35.7±9.1	1.9	0.772
40-50 mm	12	45.5±2.1	40.8±9.4	10.3	0.123
50-60 mm	9	56.6±2.34	53.0±4.30	6.4	0.073
60-70 mm	6	65.6±11.5	65.0±3.4	0.9	0.867
70-80 mm	7	76.0±5.6	74.0±3.16	2.6	0.720
≥ 80 mm	8	96.3±11.7	87.5±20.7	9.1	0.095
Total	77	49.0±23.2	46.1±23.1	5.9	0.157

Table-3: Shrinkage rates of renal cell carcinoma according to tumour subtypes.

	n	**Tomography size** (mm)	**Pathology size** (mm)	**Shrinkage rate** (%)	***p*-value**
Clear cell carcinoma	61	46,7±21,6	44,4±20,6	4,9	0,062
Chromophobe cell carcinoma	6	64,0±25,8	61,5±19,0	3,9	0,589
Papillary cell carcinoma	8	57,1±36,1	52,4±29,3	8,2	0,198
Multilocular cystic renal cell carcinoma	2	38,5±13,4	36,0±7,0	6,5	0,579

Formalin shrinkage rates according to the tumour sizes and RCC subtypes showed that although the pathological sizes of the tumours were smaller than the radiological sizes, the differences were not significant (Table 2). The same relation was also observed in RCC subtypes (Table 3). The highest shrinkage rate was observed for papillary cell carcinoma, but the decrease of tumour size was not significant in terms of RCC subtypes (p>0.05).

Discussion

The staging of a tumour is important for the clinical evaluation of patients. Both post-operative treatment and follow-up schedules may change according to tumour

stage. Pathological, radiological and clinical data is used to ensure the proper staging of a tumour. RCC is one of the tumour types in which tumour size has significant importance for tumour staging. The T1a, T1b, T2a and T2b stages are distinctly separated from each other with cut-off tumour sizes of 4cm, 7cm and 10cm, respectively. The estimated 5-year cancer-specific survival rates of RCC have been reported to be 97% for T1a, 87% for T1b and 71% for T2 tumour stages.[8,9] As tumour size is the main determinant for these stages in TNM classification,[2] its prognostic importance is inevitable.

The tumour size that is used for RCC staging is defined as the tumour's largest diameter at pathological specimen. On the other hand, most of the pathological specimens are embedded in formalin solutions to transfer the specimen to the pathology unit. The specimens have to wait in formalin solution for a while before macroscopic and microscopic pathological evaluation. The fixation time of the specimen may last 12-24 hours, according to the pathologist's preference. Formalin (10% formaldehyde solution) causes tissue dehydration and cellular shrinkage to preserve cellular integrity.[10-12] By this effect, formalin may cause tissue shrinkage and the pathological size of the tumour may change.

Studies in different organs like lung, gastrointestinal system, breast, women genital system, and oral cavity demonstrated tissue shrinkage effect of formalin. The rates of shrinkage in different tissues ranged between 2.7-57%.[13-18] This wide range of tissue shrinkage in different organs and tumours might be related to the histopathological properties of that tissue. Even the intracellular and extracellular fluid and protein composition of tissues might affect the shrinkage rate of formalin. Besides the formalin shrinkage, histopathological processes, including embedding and mounting, contribute to further tumour shrinkage.[10] For this reason, the shrinkage rate of a tumour may vary.

Although several studies have demonstrated the shrinkage effect of formalin in different tissues, there are few studies evaluating this effect on RCC. Tran et al. documented a 12.1% size difference between radiological and post-resection tumour diameters, and reported a mean shrinkage rate of 11.4% in the microscopic evaluation of post-fixation specimens.[10] The current study observed a 4.4% post-fixation shrinkage rate. On the other hand, the mean tumour size of patients in the earlier study was nearly half of the current sample.[10] This might explain the differences. Even small changes in smaller diameters might show higher rates of size change. As the tumour size increased, the shrinkage rate might relatively be lower. The tumour properties might also be responsible for this difference. Larger tumours have a larger zone of tissue necrosis, which might also affect the shrinkage rate of formalin fixation. As the tumour size is the main determinant of renal tumour staging, this subject must further be evaluated. Unlike the other studies, the current study evaluated the effect of tissue shrinkage on RCC staging, and observed that nearly one-fourth of radiological T1b tumours became pathologically T1a after formalin fixation. The radiological tumour sizes of these patients were close to 4cm, which was the cut-off value for T1b tumours. A similar relation was also observed in some T2a tumours. Nearly 30% of radiological T2a patients became T1b in the pathological evaluation and these patients' tumour sizes were also close to 7cm, which was the cut-off value for T2a tumours. The clinicians must be aware of possible formalin shrinkage for RCC that has sizes close to the cut-off values for T1b and T2a tumours. For those patients, it may be useful to stage the tumours using the tumour size in fresh tissue.

The current study further evaluated the shrinkage rates of formalin on benign tumours and compared them with malignant tumours. Although the difference was not significant, the shrinkage rates of benign renal tumours were less than the malignant tumours. This difference might be related to the histological properties of tumours. Malignant tumours had a more vascular structure with neovascularisation, whereas neovascularisation is less prominent for benign tumours. Even this property of tissues might be related to tissue shrinkage rate differences between malignant and benign tumours.

Another aim of the current study was to evaluate the formalin shrinkage effect on RCC subtypes. Each subtype of RCC originates from a different part of the renal unit and has different histopathological properties. For this reason, shrinkage effect of formalin may change in these subtypes. Similar to literature, most of the patients in the current study had clear cell carcinoma, followed by papillary, chromophobe and multilocular cystic subtypes.[2,4,9] Shrinkage was observed on all RCC subtypes, but the shrinkage rates were not significant. The most prominent shrinkage was observed in papillary RCC. This might be related to the tumour structure as papillary RCC is a tumour with a high rate of necrosis and haemorrhage.

Fixation is a vital part of the pathological analysis and cannot be oversimplified. In addition to the shrinkage effect, formalin may affect the morphology of the tissue, ribonucleic acid / deoxyribonucleic acid (RNA/DNA) extraction ability, protein evaluation, or immunohistochemical (IHC) staining of the tissue. For this reason, fresh-frozen material could be analysed as the basis of molecular studies, especially for the extraction of

RNA/DNA from the tissue. Several fixative agents have been tried in a pathological area to replace formalin, but none have been found to be good enough. Formalin does not change the real grading system and the main pathological diagnosis of the RCC tissue if applied up to 24 hours. Pathological fixation is applied in a similar procedure with formalin for RCC in regular pathology clinics. Nowadays, formalin remains the favourable fixative agent in majority of pathological analyses.[19,20]

As shown in other studies, it was a significant finding in the current study that formalin fixation during pathological evaluation caused tissue shrinkage for renal tumours.[10,21,22] It might be a question of debate if a formulation can be used to estimate the corrected size for renal tumours using pathological specimen diameter. Tran et al. reported a formulation to estimate the corrected size using both microscopic and post-fixation tumour sizes.[10] On the other hand, the formulations might change as the tumour sizes increase. For this reason, we believe that both Tran et al.'s study and the current study might guide the clinicians to estimate the real tumour size and perform more appropriate staging for renal tumours. According to the current findings, the addition of 5% to the post-fixation tumour diameter might estimate the corrected tumour size. This is an interesting subject that must be further investigated by prospective studies.

The current study has some limitations, the retrospective nature being the main one. The measurements of tumour sizes were performed by the same pathologist to decrease inter-observer bias. The study did not measure the fresh tissue size of the tumours before formalin owing to the policy of the institutional pathology department. All pathological specimens were cut by the pathologist after formalin fixation. The third limitation was related to the small number of benign tumours in the study population. It was not possible to make a significant statistical analysis with the limited number of patients with benign tumours.

Conclusion

Formalin caused shrinkage in both benign and malignant renal tumours. Although the shrinkage rate was not significant between radiological and pathological evaluations, it might lead to under-staging for some RCC patients. The clinicians must be aware of this shrinkage for possible under-staging. The shrinkage effect should be considered when interpreting the real tumour stage of RCC in pathological reports. In terms of RCC subtypes, the most prominent shrinkage rate was observed in papillary cell carcinoma.

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