RESEARCH ARTICLE

Editorial Process: Submission:02/02/2017 Acceptance:12/03/2017

Evaluation of Volume of Nasopharyngeal Cancers by the Cavalieri Principle

Arzu Tatar[1], Hilal Kiziltunc Ozmen[2]*, Ozgur Yoruk[1]

Abstract

Prediction of tumor volume using the Cavalieri method may be helpful in management of therapy and evaluation of treatment results. The aim of this study was to adapt the Cavalieri stereological method to magnetic resonance imaging for determining volume of nasopharyngeal cancers and assess changes after treatment using the Cavalieri method. Serial MRI images in the sagittal plane were obtained from a total of 33 patients with nasopharyngeal carcinomas (11 with stage T2, 11 with stage T3, and 11 with stage T4 lesions). The images were analyzed retrospectively before and two months following the cessation of radiochemotherapy for comparison. Average tumor volumes before and after treatment in patients with stage T2 were 21. 5±10.5 cm3 and 2.82±3.43 cm^3, respectively (p=0.000). The respective figures were 35.1±19.0 cm3 and 6.27±7.82 cm^3 (p=0.000) for stage T3 cases, and 62.8±27.8 cm3 and 11. 6±11.9 cm3 (p=0.000) for stage T4. Post-treatment tumor volumes were statistically reduced when compared to pre-treatment volumes in all stages.

Keywords: radiochemotherapy- MRI- stereology- nasopharynx- cavalieri method

Asian Pac J Cancer Prev, **19 (9)**, 2403-2407

Introduction

Nasopharyngeal carcinoma (NPC) is the malign epithelial tumor of nasopharynx. Its incidence varies according to geographical and racial characteristics and it is the most common tumor of the head and neck region in South Asia. Due to the surgically inaccessible locations and high radiosensitivity of NPCs, radiotherapy is recommended as the first option in their treatment. In patients with early stage NPC, the prognosis is relatively good with radical radiotherapy, whereas it is relatively poor in moderate and advanced staged patients. Therefore, in patients having moderate and advanced staged tumors, comprehensive strategies, generally consisting of chemotherapy and radiotherapy have been used in order to build up the curative effect (Langendijk et al., 2004; Palazzi et al., 2009).

As in other head and neck tumors, the most important prognostic factor is the stage of tumor in NPC. Until today, various staging systems have been developed for NPC and the most commonly used system among these has been the International Union against Cancer/American Joint Committee on Cancer (AJCC) Tumor, Node, and Metastasis (TNM) Staging System. However, in various studies, T (tumor) system was shown to have limitation in predicting the prognosis of patients with NPC (Palazzi et al., 2009; Tham et al., 2009). In recently conducted studies, primary tumor volume has been shown to be a very important indicator in predicting the prognosis of NPC and has been determined to be better than T classification for evaluating treatment outcomes and the radiosensitivity level of the tumor (Chua et al., 1997; Willner et al., 1999; Chang et al., 2002; Wu et al., 2013). Therefore, knowing the primary tumor volume may help the clinician for planning the treatment and evaluating the treatment outcome. Moreover, in recent years, in order to predict the prognosis more accurately, integration of "tumor volume" into the T staging system has been suggested (Wu et al., 2013; Wu et al., 2014). However, tumor volume has not been integrated to the TNM staging system yet. The primary reason for this may be the difficulty in accurate measurement of the tumor volume in NPC, since NPCs are usually tumors with irregular shape, having aggressive and infiltrative growth pattern. Furthermore, measurement of tumor volume is a time-consuming and labor-intensive procedure. Therefore, in NPC, methods that can determine the tumor volume with high accuracy and that can be performed rapidly and easily are required.

Cavalieri method is a stereological method depending on calculation of 3-dimensional volume by using 2-dimensional images of a geometrical structure (Gundersen et al., 1987; Acer et al., 2008). Numerous studies by applying Cavalieri principles to images obtained from CT, MR or other imaging techniques have been conducted for estimation of volume for many tumors in various anatomical locations (Akgun et al., 2008;

*[1]Department of Otorhinolaryngology, Head and Neck Surgery, Medical Faculty, [2]Department of Radiation Oncology, Ataturk University, Turkey. *For Correspondence:: hkiziltuncozmen@hotmail.com*

Bingol et al., 2016). To our knowledge, no study using Cavalieri method for estimation of the tumor volume in NPC is present.

The aim of this study was to describe and adapt Cavalieri method to magnetic resonance imaging (MRI) for estimation of primary tumor volume in NPC and to evaluate the tumor volume changes measured by the Cavalieri method after treatment.

Materials and Methods

This study was designed retrospectively by the approval of the Ethics Committee of the Ataturk University, Medical School (protocol number: B.30.2.ATA.0.01.00/57). The group of patients was composed of 33 subjects (14 women, 19 men) with NPC who were admitted to the Otorhinolaryngology Clinic in the last 10 years. The mean ages were 43±5 years for women and 49±7 years for men. The patients, who were diagnosed initially as untreated, biopsy-proven nasopharyngeal carcinoma, were included in the study. During evaluation, the patients who were determined to have hepatic, renal, hematological, pulmonary, or any systemic diseases were excluded from the study group.

Treatment plan

All patients were assessed in accordance with the staging criteria determined by American Joint Committee on Cancer (AJCC) in 2002. The T-stage distribution was 11 patients with T2, 11 patients with T3, and 11 patients with T4.

In this study, tumor volume included the gross volume of the primary tumor and the enlarged retropharyngeal lymph nodes. The retropharyngeal lymph nodes and entire nasopharynx were included in the planning target volume and were exposed to a total dose of 70-72 Gy. The other structures such as parapharyngeal spaces, base of skull, and cavernous sinus with potential invasion were irradiated to 50-60 Gy. All patients were treated with three-dimensional conformal techniques throughout the radiotherapy (RT) course. RT was applied 5 days per week during 8 weeks in 1.8 Gy fractions (total 70-72 Gy) by the use of a Multi-collimator linear accelerator (LINAC MLC, Siemens, Erlangen, Germany). Six cures of cisplatin (40 mg/m^2/day) were weekly administrated to the patients with advanced-stage nasopharyngeal cancer (stage IIB-IVB), in addition to radiotherapy. Three weeks after completion of RT, adjuvant chemotherapy (1 to 3 cycles of cisplatin+5-fluorouracil) was optionally administered to the patients with nasopharyngeal cancer.

Determination of nasopharyngeal cancer volumes by stereological method

In this study, we used a combination of Cavalieri estimator and point-counting grid for stereological estimation of the changes in tumor volume before and after radiochemotherapy on MRI of the head and neck. Stereological analyses were performed with a stereology workstation, consisting of a modified light microscope (Leica DM4000 B, Cambridge, UK), a motorized specimen stage for automatic sampling (BioPrecision MAC 5000 controller system; Ludl Electronic Products, Hawthorne, NY), a Charge Coupled Device (CCD) color video camera (Optronics MicroFire, Goleta, CA, USA), and stereology software (Stereo Investigator version 9.0, Microbrightfield, Colchester, Vermont, USA). According to this combination method, the following steps were made:

Step 1. Preparation for MR İmages

In Cavalieri method, firstly the volume of each section is calculated to estimate the regular or irregular organ volume. Then, these data are collected and the total volume is reached. The importance of this method when compared to other methods is that it has a computable mathematical error rate and a mathematical correction (Gundersen et al., 1987; Varoglu et al., 2010). For this purpose, pre and post-treatment sagittal plane MR images of all patients were collected. Consecutive sagittal slices (5-mm thickness) were obtained with a random starting position and the scanning images entirely encompassed whole of the nasopharynx including tumor (gross tumor volume of the primary tumor and enlarged retropharyngeal lymph nodes).

Step 2. Measuring Area and Total Volume on MRI

All MR images were scanned and projected onto a personal computer (PC) screen and then, in each of the serial MR images, the key area (the areas of tumor) was counted via stereology workstation using stereology software.

After determination of the measurement area, point-counting grid, which consists of separated points at equal intervals from each other, was automatically placed with a random angle throughout the delineated area, using stereological software. The density of points within point- counting grid used in our study area was determined by an appropriate coefficient of error (CE). As the next step, all points hitting the area of the tumor were automatically marked using stereological software as in figures 1 and 2; reference volume of structures (tumor) in the MR slices counted by utilizing the following formula (Gundersen et al., 1987), where V is estimated volume, AP is area associated with a point, m is section evaluation interval, t is mean section cut thickness, Pİ is points counted on grid. These applications were performed on other all slices of each group.

Step 3. Precision of the method

The coefficient of error (CE) was used to estimate the precision of total volume measurement. The coefficient of error (CE) for area estimation was the last calculated value. The generally accepted highest limit of CE is 5%. The coefficient of error was calculated by utilizing the following formula (Gundersen HJG et al., 1987), where Nugget is variance due to noise, TotalVar is the total variance. The total variance, nugget, and further details see equations of Gundersen and Jensen (Gundersen HJG et al., 1987).

Statistical analysis

Statistical data were given as number, percentage, mean, and standard deviation. SPSS v.20 was used in the analysis of data. Conformity to the normal distribution was evaluated with Kolmogorov-Smirnov test. T-test was used in dependent sampling in comparison of pre- and post-tumor volumes. Statistical significance level was taken as $p<0.05$.

Results

Average tumor volumes in patients with stage T2 before and after treatment were 21.45±10.50 cm^3 and 2.82±3.43 cm3, respectively ($p=0.000$). These volumes were 35.10±18.97 cm3 and 6.27±7.82 cm^3 ($p=0.000$) for patients with stage T3, respectively, and 62.82±27.79 cm^3 and 11.54±11.94 cm^3 ($p=0.000$) for patients with stage T4, respectively. Post-treatment tumor volumes were reduced significantly, when compared to pre-treatment volumes in all stages (Figure 3).

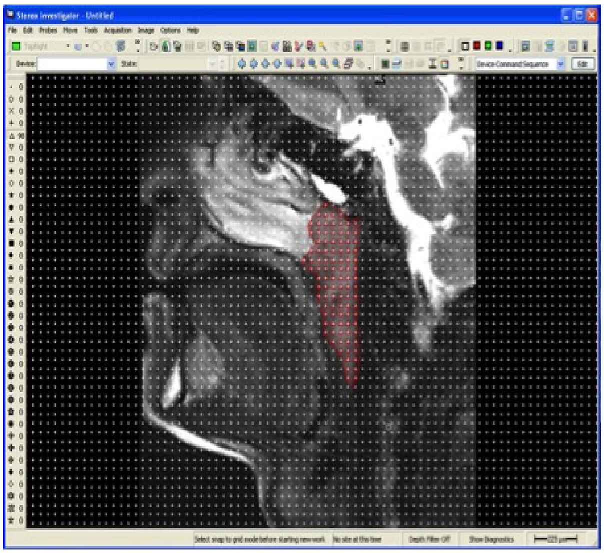

Figure 1. Before Radiochemotherapy, Nasopharyngeal Tumor Area Calculated Using a Point Counting Probe by the Stereo Investigator System

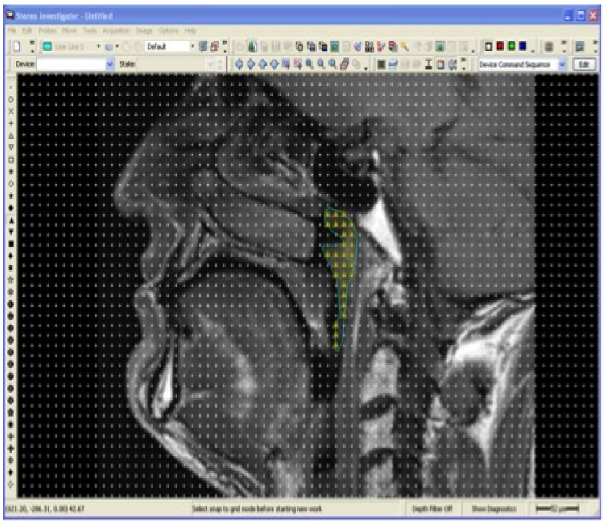

Figure 2. After Radiochemotherapy, Nasopharyngeal Tumor Area Calculated Using a Point Counting Probe by the Stereo Investigator System

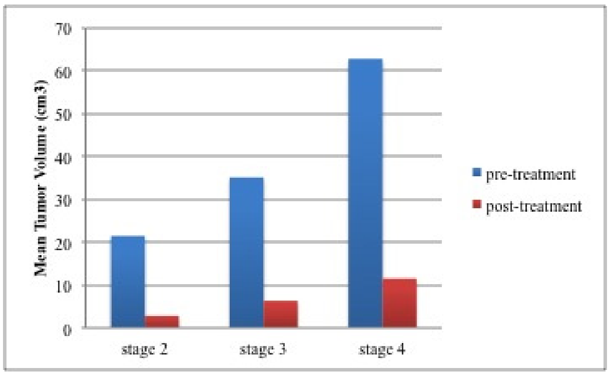

Figure 3. Tumor Mean Volumes± SD of NPC Were Measured by Cavalieri Principle before and after Radiochemotherapy in All Stage Patients

Discussion

Nasopharyngeal cancers are highly radiosensitive tumors, in which radiotherapy is relatedly the fundamental method of treatment. The radiosensitivity level of tumor affects the treatment outcomes; therefore, it is important to identify the radiosensitivity level of the tumor, in order to determine the treatment protocol. As in other cancers, primary tumor volume is considered as a good indicator for determining the radiosensitivity level in NPC (Dubben et al., 1998). In large tumors, the number of clonogenic cells is also increased and the blood supply of the tumor is reduced. Therefore, the number of hypoxic cells increases and eventually necrosis and liquefaction develop at the central region of the tumor. This leads to reduce radiosensitivity of the tumor tissue and poor treatment outcome.

Until today, studies have been conducted in NPC, showing that the primary tumor volume has significant effects on treatment outcome (Chua et al., 1997; Willner et al., 1999; Wu et al., 2013; Wu et al., 2014; Chen et al., 2011). In these studies, it was shown that in cases with small tumor volume, local control could be achieved perfectly by radiotherapy; however, in cases with large tumor volume, local control could be provided with a limited rate by radiation. Tumor volume was shown to be in close relation with the total radiation dose required for enabling local control; in large-volume primary tumors, higher doses of radiation therapy was required when compared to small-volume tumors (Chua et al., 1997; Willner et al., 1999). Moreover, the primary tumor volume has been considered as an independent prognostic indicator for local recurrence and distant metastasis following radiotherapy in patients with NPC and it has been suggested that primary tumor volume can predict the survival rates of NPC patients more accurately, when compared to the AJCC staging system (Wu et al., 2013; Wu et al., 2014; Chen et al., 2011; Guo et al., 2012). Since it is very difficult to achieve the tumor control by only radiotherapy or concurrent chemotherapy in the presence of excessively large tumor, it would be more appropriate to prefer more aggressive therapies, such as adjuvant chemotherapy (Chua et al., 1997; Chang et al., 2002; Wu et al., 2014; Chen et al., 2011).

For cancer patients, accurate prediction of prognosis has vital importance in terms of optimization of treatment strategies. In this regard, by accurate determination of the tumor volume in NPC, the appropriate treatment protocol could be chosen, decision could be made on whether aggressive treatments might be necessary or not; these would contribute to improvement of prognosis of patients. In appropriate cases, needless aggressive treatments could be avoided, enabling reduction of side effects of radiotherapy and chemotherapy.

Although there is strong evidence that the tumor volume is a very significant prognostic indicator for NPCs, probably due to difficulties encountered during accurate measurement of the tumor volume and volume measurements being time-consuming and labor-intensive, it has not been widely used in clinical practice. To date, quantitative techniques, such as geometrical methods, computer-based 3D volumetric reconstructions and stereological approaches, have been described for measuring tumor volume. Among these, geometrical method uses direct geometrical measurements for determining the volumetric features of the radiological images (Stocchetti et al., 2000). This technique assumes that the measured anatomical structure has a known geometrical shape. Since it enables the extensions and the diameter of tumor to be measured roughly, its accuracy is limited. Furthermore, this technique is less applicable in tumors such as NPC having an irregular shape and infiltrative growth pattern. To measure the volume of the nasopharyngeal tumor accurately, the boundaries of the tumor extension in radiological images should be detailed and calculations should be made in the three-dimensional perspective. Computer-based 3D volumetric reconstruction is a technique, which can make the volume estimations more accurately (Albright et al., 1988). However, since this technique requires trained personnel and quite expensive equipment, and is time-consuming, it is inconvenient for clinical and research use.

Another popular technique for determining the tumor volume is Cavalieri method, which is a stereological approach. Stereology is a branch of science that consists of the generation of mathematically unbiased estimates of the geometric properties of three-dimensional characteristics based on data obtained from sampling two-dimensional slices and images of objects (Varoglu et al., 2010; Sahin et al., 2008). The requirements for the application of this method are; slices or images that are obtained to be parallel to each other through the object, these slices or images must be separated by a known distance and samples must be selected in accordance with the systematic-random rule. Until now, the reliability and efficiency of Cavalieri method for estimation of tissue volume has been investigated in numerous studies and its reliability has been proven (Acer et al., 2008; Bingol et al., 2016; Kayipmaz et al., 2011; Eric et al., 2014). Planimetry and point-counting technique are two different methods for volume estimations, using Cavalieri principles (Bingol et al., 2016; Sahin et al., 2008; Odaci et al., 2003). While planimetric technique uses manual tracing for identification of boundaries from the slice images of the related objects, point-counting method uses regular grid of test points. Since planimetric techniques are expensive and time-consuming, they are not suitable to be used in daily routine clinical applications (Varoglu et al., 2010; Odaci et al., 2003). Furthermore, some authors have reported that volume estimations by planimetric technic might end up with over-estimation and various levels of systemic bias (Stocchetti et al., 2000). The unbiased volume estimation of any structure, sliced by using radiological techniques, can be performed rapidly and easily by simple point-counting technique, without necessitating additional equipment or personnel. In addition, the needed time for evaluation of any organ or tissue volume using Cavalieri methods is a few minutes based on tissue size. Therefore, the researchers suggested that the major advantage of the point counting method is the less time consuming (Acer et al., 2008; Sonmez et al., 2010). We also calculated the tumor volumes of NPC within a few minutes in each patient. So, point-counting technique is more appropriate for daily, routine clinical practice. Moreover, point-counting technique offers a more effective and reliable approach, when compared to the planimetric technique (Sahin et al., 2008).

In this study, we applied the Cavalieri method with a simple point-counting approach by making some modification to estimate the changes in tumor volumes (gross volume of the primary tumor and involved retropharyngeal nodes) of the NPC before and after radiochemotherapy in 33 patients. We magnified the MRI images of the patients before measurement. Thus, we tried to obtain results that are more reliable by reducing the potential marginal errors.

As a conclusion, in this study, we presented the application of Cavalieri technique as an easily and quickly applicable, reproducible, low-cost method, with unbiased results for calculating tumor volume in patients with NPC. Prediction of tumor volume by using Cavalieri method may be helpful in management the therapy by facilitating both the prediction of tumor volume and the evaluation of treatment results.

Conflict of Interest

Author A declares that she has no conflict of interest. Author B declares that he has no conflict of interest. Author C declares that she has no conflict of interest. Author D declares that he has no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

Acer N, Sahin B, Usanmaz M, et al (2008). Comparison of point counting and planimetry methods for the assessment of cerebellar volume in human using magnetic resonance imaging: a stereological study. *Surg Radiol Anat*, **30**, 335-9.

Akgun M, Kantarci M, Cayir K, et al (2008). Stereological evaluation of tumor regression rates in lung cancer using CT via the cavalieri method. *Eurasian J Med*, **40**, 109-14.

Albright RE Jr, Fram EK (1988). Microcomputer-based technique for 3-D reconstruction and volume measurement of computer tomographic images. Part 1: phantom studies. *Invest Radiol*, **23**, 881–5.

Bingol F, Yoruk O, Bingol BO, et al (2016). Estimation of the efficacy of chemo-radiotherapy on tumor regression in the patients with laryngeal cancer via computerized tomography using the Cavalieri method. *Acta Otolaryngol*, **136**, 164-7.
Chang CC, Chen MK, Liu MT, et al (2002). The effect of primary tumor volumes in advanced T-staged nasopharyngeal tumors. *Head Neck*, **24**, 940–6.
Chen C, Fei Z, Pan J, et al (2011). Significance of primary tumor volume and Tstage on prognosis in nasopharyngeal carcinoma treated with intensitymodulated radiation therapy. *Jpn J Clin Oncol*, **41**, 537–42.
Chua DT, Sham JS, Kwong DL, et al (1997). Volumetric analysis of tumor extent in nasopharyngeal carcinoma and correlation with treatment outcome. *Int J Radiat Oncol Biol Phys*, **39**, 711–9.
Dubben HH, Thames HD, Beck-Bornholdt HP (1998). Tumor volume: a basic and specific response predictor in radiotherapy. *Radiother Oncol*, **47**, 167–74.
Eric M, Anderla A, Stefanovic D, et al (2014). Breast volume estimation from systematic series of CT scans using the Cavalieri principle and 3D reconstruction. *Int J Surg*, **12**, 912-7.
Gundersen HJG, Jensen EB (1987). The efficiency of systematic sampling in Stereology and its prediction. *J Microsc*, **147**, 229–63.
Guo R, Sun Y, Yu XL, et al (2012). Is primary tumor volume still a prognostic factor in intensity modulated radiation therapy for nasopharyngeal carcinoma?. *Radiother Oncol*, **104**, 294–9.
Kayipmaz S, Sezgin OS, Saricaoglu ST, et al (2011). The estimation of the volume of sheep mandibular defects using cone-beam computed tomography images and a stereological method. *Dentomaxillofac Radiol*, **40**, 165-9.
Langendijk JA, Leemans CR, Buter J, et al (2004). The additional value of chemotherapy to radiotherapy in locally advanced nasopharyngeal carcinoma: a meta-analysis of the published literature. *J Clin Oncol*, **22**, 4604–12.
Odaci E, Sahin B, Sonmez OF, et al (2003). Rapid estimation of the vertebral body volume: a combination of the Cavalieri principle and computed tomography images. *Eur J Radiol*, **48**, 316–26.
Palazzi M, Orlandi E, Bossi P, et al (2009). Further improvement in outcomes of nasopharyngeal carcinoma with optimized radiotherapy and induction plus concomitant chemotherapy: an update of the Milan experience. *Int J Radiat Oncol Biol Phys*, **74**, 774–80.
Sahin B, Mazonakis M, Akan H, et al (2008). Dependence of computed tomography volume measurements upon section thickness: an application to human dry skulls. *Clin Anat*, **21**, 479–85.
Sonmez OF, Odaci E, Bas O, et al (2010). A stereological study of MRI and the Cavalieri principle combined for diagnosis and monitoring of brain tumor volume. *J Clin Neurosci*, **17**, 1499-1502.
Stocchetti N, Croci M, Spagnoli D, et al (2000). Mass volume measurement in severe head injury: accuracy and feasibility of two pragmatic methods. *J Neurol Neurosurg Psychiatry*, **68**, 14–7.
Tham IW, Hee SW, Yeo RM, et al (2009). Treatment of nasopharyngeal carcinoma using intensity-modulated radiotherapy-the national cancer centre Singapore experience. *Int J Radiat Oncol Biol Phys*, **75**, 1481–6.
Varoglu AO, Odaci E, Gumus H, et al (2010). Evaluation of patients with multiple sclerosis using a combination of morphometrical features and clinical scores. *J Clin Neurosci*, **7**, 191-5.
Willner J, Baier K, Pfreundner L, et al (1999). Tumor volume and local control in primary radiotherapy of nasopharyngeal carcinoma. *Acta Oncol*, **38**, 1025–30.
Wu Z, Gu MF, Zeng RF, et al (2013). Correlation between nasopharyngeal carcinoma tumor volume and the 2002 international union against cancer tumor classification system. *Radiat Oncol*, **8**, 87.
Wu Z, Su Y, Zeng RF, et al (2014). Prognostic value of tumor volume for patients with nasopharyngeal carcinoma treated with concurrent chemotherapy and intensity-modulated radiotherapy. *J Cancer Res Clin Oncol*, **140**, 69-76.