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碩士論文

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Master Thesis

比較使用低分次性強度調控放射治療與傳統治療在末期犬鼻腔腫瘤之效果

Comparison of Hypofractionated Intensity-Modulated Radiation Therapy Versus Traditional Treatment Modalities in Advanced Stage of Canine Nasal Tumors

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誌謝

快樂的時光總是過得特別快，回想過去的總總，還是覺得自己很幸運可以進入小動物腫瘤醫學的領域。大五實習時，憑著一股傻勁，兩輪內科加上兩輪選修幾乎三分之一的大五都沈浸腫瘤的世界裡。然而，等到真正進了研究室、開始看診之後，才發現往往最難的不是腫瘤本身，而是對病患整體的考量和如何與飼主應對進退。

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中文摘要

鼻腔腫瘤在狗是不常發生的惡性腫瘤，局部侵犯性強但在診斷時甚少發生轉移。 使用經驗性口服藥物通常會暫時改善鼻腔腫瘤的臨床症狀，但症狀還是會間歇性的反覆出現。鼻腔腫瘤的治療主要以局部控制為主，未接受治療的中位存活時間約為 3.1 個月。除了一般的規律分次性放射治療，低分次性放射治療療程較不密集，主要為了緩解影響病患生活品質的臨床症狀。

回溯國立台灣大學附設動物醫院於 2011 至 2015 年的病例，共有 35 隻患犬符合經病理學確診或臨床上強烈懷疑鼻腔腫瘤的病患。15 隻患犬接受低分次性強度調控放射治療被歸類為放療組，非放療組有 20 隻患犬，其中 17 隻接受除了放射治療以外的治療方式，3 隻沒有接受任何治療。

本研究大部分患犬臨床分期較末期（82.8%為第三或第四期），先前文獻末期病患比例較低，本研究治療成果與先前病患接受放射治療或化學治療的文獻不相上下。與非放療組相比，放療組患犬有顯著較長的無疾病進展期與存活時間。非放療組 9 隻患犬有接受化學治療，與同組沒接受化學治療的患犬的生存時間有顯著差異。

以多變數分析各因子與疾病進展的關係時，臉部變形 (P=0.012)與是否接受放療 (P=0.014)仍具顯著差異，危害比分別是 3.741、0.347。以多變數分析各因子與生存時間的關係時，只有是否接受放療 (P=0.005)仍具顯著差異，危害比是 0.259。

這是第一篇犬隻鼻腔腫瘤以低分次性強度調控放射治療的研究。即使本研究大部分患犬臨床分期較末期，經過低分次性強度調控放射治療後可以獲得一段可

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觀的無疾病進展期與存活時間。本研究的結果也指出當鼻腔腫瘤的患犬無法接受放射治療時，傳統的化學治療仍然可以提供病患一些幫助。

關鍵字：犬鼻腔腫瘤、強度調控放射治療、低分次性放射治療、末期

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ABSTRACT

Canine nasal tumors is an uncommon malignancy, usually have a locally invasive behavior and rarely metastasis at the time of diagnosis. Clinical signs of nasal neoplasia usually are intermittent and progressive when empirical therapy administered. Treatment options of nasal tumors focus on local control. The MST of untreated canine nasal carcinoma is about 3.1 months. Besides regular fractionated radiation therapy for canine nasal tumors, coarse fractionated radiation therapy is less intensive and mainly focus on relieving clinical signs that affects patient's quality of life.

The medical records of histologically confirmed or clinically highly suspected nasal malignancy from 2011 to 2015 at National Taiwan University Veterinary Hospital were reviewed and 35 of client-owned dogs met the inclusion criteria. 15 patients treated with hypofractionated intensity-modulated radiation therapy (IMRT) were assigned to RT group. Non-RT group consisted of 20 patients either with treatment except RT (n=17) or no treatment (n=3) for further comparison.

From the result of present study where most patients were advanced stage (82.8% are stage III and IV), our treatment outcome was comparable with previous studies of radiation therapy or chemotherapy in canine nasal tumor patients even though lower ratio of late stage patients were presented in most of the studies. Progression free interval and overall survival were significant longer in RT group when compared with non-RT group. In non-RT group, receiving chemotherapy (n=9) offered significant longer overall survival when compared with those patients that only received palliative oral medications.

Facial deformity (P=0.012) and received radiation therapy or not (P=0.014) remained statistically significant in multivariable analysis of progression free interval.

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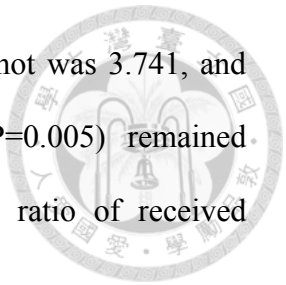

Hazard ratio of facial deformity and received radiation therapy or not was 3.741, and 0.347, respectively. Only received radiation therapy or not (P=0.005) remained statistically in multivariable analysis of overall survival. Hazard ratio of received radiation therapy or not was 0.259.

This is the first IMRT study delivers weekly fractionated radiation therapy in canine nasal tumor patients. From the result of present study where most patients were in late stage, a durable progression free interval and comparable survival was obtained from our radiation treatment procedure. The result from present study also indicate that if radiation therapy is not an amenable option, traditional chemotherapy can still offer some benefit to canine nasal tumor patients.

Key words: Canine nasal tumor; IMRT; Hypofractionated RT; Advanced stage.

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Chapter 1 Literature review

1.1 Canine nasal tumor

Approximately 1% of canine tumors arise from the nasal cavity and paranasal sinuses (MacEwen et al., 1977). Medium-to-large breeds, long-nosed breeds, urban living, and tobacco smoke environment may be the predisposed factors (Bukowski et al., 1998; Patnaik, 1989; Reif et al., 1998; Reif and Cohen, 1971; Stunzi and Hauser, 1976). Near two-thirds of canine nasal tumors are epithelial origin, such as carcinoma, adenocarcinoma, squamous cell carcinoma (SCC), transitional cell carcinoma (TCC), etc (Madewell et al., 1976). Nasal tumors are considered locally invasive and the metastasis is relatively low at the time of diagnosis (Patnaik, 1989).

Clinical signs of nasal neoplasia usually start with unilateral (few bilateral) epistaxis or mucopurulent discharge and are usually intermittent and progressive (MacEwen et al., 1977; Rassnick et al., 2006). These signs may wax and wane while empirical therapy (e.g., antibiotics, steroids or NSAIDs) administered (Rassnick et al., 2006). The severity of disease will affect patients' clinical signs, which include respiratory signs (e.g., dyspnea, open-mouth breathing, sneezing, stertor), and ophthalmic signs (e.g., ocular discharge, exophthalmus) (Madewell et al., 1976; Patnaik, 1989; Rassnick et al., 2006). Facial deformity is a clinical manifestation that often indicates malignancy (Lobetti, 2009; Strasser and Hawkins, 2005). Rarely, the patients only have neurologic deficits (e.g., acute blindness, behavior change, obtundation, seizure, circling and paresis) due to cranial invasion of the tumor.

Tissue biopsy is the gold standard of definitive diagnosis. Diseases except neoplasia include fungal or bacterial infection, idiopathic rhinitis, nasal parasites or foreign body, bleeding disorder, systemic hypertension and trauma may have similar

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clinical signs and must be ruled out (Beck et al., 1999; Burrow, 2004; Saunders et al., 2003). Coagulopathy must be ruled out before performing tissue biopsy. Various techniques can obtain adequate tissue sample. These include nasal flushing, transnostril blind biopsy by bone curette or cup forceps, or transnostril aspiration and core biopsy (Rudd and Richardson, 1985; Withrow, 1982). If patients suffered from the facial deformity or the tumor invaded oral cavity, then directly punch biopsy of the lesion is enough to get diagnostic samples. In order to prevent penetrating the cribriform plate, we should ensure that the transnostril instrument does not penetrate farther than the medial canthus of the eye. In rare case of severe hemorrhage, ipsilateral carotid artery can be permanently ligated (Clendenin and Conrad, 1979). It is widely accepted that nasal biopsies can be easily non-diagnostic in nasal neoplasia due to local inflammation surrounding the tumor (Meler et al., 2008). Inadequate sampling includes small biopsy size and superficial inflammatory cells, and both need further exams when clinical and histologic finding are discoordinated.

Computed tomography (CT) is the imaging modality of choice for staging nasal tumors, as osteolytic lesion of bordering bone of the nasal cavity and mucosal thickening was found on CT images more often than on magnetic resonance images (MRI) (Drees et al., 2009). MRI is more sensitive for identifying intracranial involvement (Moore et al., 1991). Several CT-based staging systems have been proposed for canine nasal tumors (Adams et al., 2009; Adams et al., 1998; Kondo et al., 2008; Theon et al., 1993). The correlation between clinical stage and overall survival is still controversial (Adams et al., 2009; Adams et al., 1998; Kondo et al., 2008; Mason et al., 2013; Buchholz et al., 2009). While cross-sectional imaging like CT and MRI can provide more detail of tumor extent, which is important to assess patient's prognosis, some conventional radiography signs may be sufficient to

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distinguish nasal neoplasia from rhinitis. In one study, radiographic signs with the highest positive predictive value (PPV) for rhinitis were absence of frontal sinus lesions and lucent foci in nasal cavity (PPV of each 82%), and invasion of surrounding bones for neoplasia (PPV 88%) (Russo et al., 2000). The study also indicates that there was moderate agreement between observers about the diagnosis, which means a high accuracy for radiologists examining dogs with nasal diseases (Russo et al., 2000). Although there are few limitations of conventional radiography, the sensitivity is comparable to that of cross-sectional imaging when tumors are in advanced disease. CT has better abilities to assess osteolytic lesion, shorter anesthesia time, lower cost and available in most facilities when comparing with MRI.

Treatment options of nasal tumors focus on local control. The MST of untreated canine nasal carcinoma is 3.1 months, and with the 1- and 2-years survival probability of 12% and 2%, respectively (Rassnick et al., 2006). In the same study, epistaxis is a negative prognostic factor that indicated shorter MST (3 V.s 7.2 months) (Rassnick et al., 2006).

Palliative medical treatments may relieve some clinical signs without prolonging patient's survival (Mason et al., 2013). Immunotherapy and cryosurgery also did not show survival benefit in studies with small case number (MacEwen et al., 1977; Withrow, 1982).

Chemotherapy is not often used in treatment of canine nasal tumors, and will be discussed in the following section of introduction. So far, the treatment of choice for canine nasal tumors is radiation therapy, including regular fractionated and hypofractionated treatment, which will also be discussed in the following section of introduction.

Prognostic factors of canine nasal tumors are still controversial. Negative

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prognostic factors such as age (Fujiwara et al., 2013; LaDue et al., 1999), long nose breed (Fujiwara et al., 2013), duration of clinical signs (Gieger et al., 2008), tumor staging (Adams et al., 2009; Adams et al., 1998; Buchholz et al., 2009; LaDue et al., 1999; Theon et al., 1993), dyspnea before RT (Fujiwara et al., 2013), epistaxis at the time of diagnosis (Rassnick et al., 2006) and histologic diagnosis (Adams et al., 2009; Adams et al., 1987; Theon et al., 1993).

Cross-sectional imaging especially CT scan can assess whether cribriform plate involved or not, which will affect disease-free survival (DFS) and overall survival (OS) (Adams et al., 2009; Adams et al., 1998). Kondo *et. al.,* proposed a CT-based staging system that only stage IV disease (brain involvement with the destruction of the cranium or the cribellum and the tumor mass being invaded into the brain tissue) had significant poor survival (Kondo et al., 2008). Another CT-based staging system proposed by Adams *et. al.,* also indicated that patients with cribriform plate destruction had a shorter survival. Furthermore, the strength of this staging scheme of OS was improved slightly when histologic type was combined with CT-based staging method over CT-based staging method alone (Adams et al., 2009). However, many studies are lack of standard follow up protocol and using clinical signs to assess whether the tumor recur or not. This might lead to inadequate analyzing of tumor recurrence because similar clinical signs can also result from rhinitis secondary to therapy (Thrall and Harvey, 1983). Since the decision of euthanasia varies from owner to owner and additional treatments are not uniformed, comparison of the survival time is difficult.

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1.2 Non-radiation therapy in canine nasal tumors

1.2.1 Surgery

Survival benefit from cytoreductive surgery alone is still in conflict. Surgical procedure alone provided about 4 months survival (Bradley and Harvey, 1973; Cook, 1964; Delmage, 1973; Hoerlein and Evans, 1962; MacEwen et al., 1977; Norris, 1979). Although the median survival time from surgery alone patients cannot be compared directly, it is close to the median survival time of the untreated patients, which is about 3.1 months. No significant prolongation of patients' survival may due to high post-operative complications rate (Henry et al., 1998; Holmberg et al., 1990; Laing and Binnington, 1988; MacEwen et al., 1977).

1.2.2 Palliative medical treatment

Palliative medical treatment (NSAID, steroid, antibiotics, analgesics) can sometimes provide temporally partial relief of clinical discomfort but offered little benefit in survival.

In one study, 38 canine nasal tumor patients either diagnosed confirmed by histopathologic exam or according to their aggressive imaging presentation treated with palliative oral medications (NSAIDs, antibiotics, or additional analgesia) had a MST about 3.5 months, even 86.8% of these patients were stage III and IV (Mason et al., 2013). Another larger multi-institutional retrospective study about palliative medical treatment reported by Rassnick *et. al.,* that included 139 cases achieved median survival time of 95 days (95% CI, 73 to 113 days), and the estimated probabilities of survival 6, 9, 12, and 24 months after diagnosis were 34%, 22%, 12%, and 2%, respectively (Rassnick et al., 2006). In this study, only 48 patients were

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staged through CT image according to Adams system (Adams et al., 1998), and 5 patients (10%) were classified as T3, and 18 (38%) were classified as T4 (Rassnick et al., 2006). The only significant negative prognostic factor was epistaxis ($P<0.001$) but not clinical stage.

1.2.3 Chemotherapy

Chemotherapy may have some benefit in clinical outcome, but rarely used as a single treatment regiment in canine nasal tumor patients. Previous study used chemotherapeutic agents such as cisplatin alone (2-8 treatment cycles) showed a response rate of 27%, tolerable toxicity (transient side effects attribute to cisplatin), and the MST was 5 months (range, 2-32 months) (Hahn et al., 1992). Another study used the combination of chemotherapeutic agents such as doxorubicin and carboplatin (Langova et al., 2004). The adverse effect of this protocol were mild and self-limited, with only 2 out of 8 patients experienced side effects such as neutropenia, diarrhea and vomiting (Langova et al., 2004). Although the response rate was 75% with the MST 210 days, the case number was small (n=8) and lack of control group to prove the benefit from this chemotherapy protocol (Langova et al., 2004). These chemotherapy protocols are well tolerated with favorable clinical outcome. However, due to the small case number and limited follow up modalities, further studies are warranted to confirm these findings.

1.3 Radiation therapy in canine nasal tumors

1.3.1 Combination of radiation therapy and surgery

Studies of combining radiation therapy and surgery in canine nasal patients

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had different conclusions about the effect on survival. When cytoreductive surgery was performed in most patients that were given megavoltage radiation did not improve patients' survival time (50 patients received regular fractionated radiation therapy while 17 patients received hypofractionated radiation therapy) (Adams et al., 1987). In another study, 56 dogs were treated with Monday-Wednesday-Friday, 4 Gy per fraction over 4 weeks radiation therapy alone, and 21 patients had partial tumor resection prior to radiation therapy. Result of the study indicated that cytoreductive surgery before radiation therapy did not affect the clinical outcome (Theon et al., 1993). Result from another study that included 6 patients had surgical treatment prior to Cobalt-60 radiation therapy, when compared with other patients in the same study, surgery or not did not have significant difference in survival (McEntee et al., 1991).

When orthovoltage radiation therapy combined with neoadjuvant (pre-RT) surgical treatment, reported MST was 221 days and 1- and 2-years survival rate was 37% and 17%, respectively. Acute toxicity was moderate to severe for the skin and eyes. 70% of dogs had chronic ocular toxicity requiring medication. Treatment failure may due to differential absorption of orthovoltage radiation by tissues of different density and poor penetration nature of orthovoltage radiation (Northrup et al., 2001).

There were two studies evaluated canine nasal tumor that received radiation therapy followed by surgical procedure. First study delivered radiation by Cobalt-60 in ten 4.2 Gy daily fraction. Post-radiation surgery was performed in 13 patients, while MST was significant longer than RT alone group (MST 1431 V.s 591 days, respectively), patients treated with radiation therapy and surgery were significantly more likely to develop rhinitis (relative risk, 3.17; 95% confidence interval, 1.40 to 7.17) or osteomyelitis-osteonecrosis (relative risk, 5.13; 95% confidence interval, 1.40 to 18.6) (Adams et al., 2005). Another study evaluated 16 patients treated with

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adjuvant (post-RT) dorsal rhinotomy and achieved MST 457 days. Although this study only recruited patients that did not achieve complete remission after radiation therapy, this treatment strategy provide similar survival to previous reports of radiation alone (Bowles et al., 2014).

1.3.2 Regular fractionated radiation therapy

It is difficult to compare with different studies of radiation therapy in canine nasal tumors, due to different tumor types, staging systems, treatment protocols, planning modalities, response assessment criteria and follow-up schedule. Dose of definitive radiation therapy usually ranged from 42Gy to 54Gy, divided into 10 to 18 fractions in 2 to 4 weeks, which MST ranged from 8 to 19.7 months, and the 1- and 2-years survival rates range from 43-68% and 11-44%, respectively (Adams et al., 2005; Adams et al., 2009; Adams et al., 1998; Adams et al., 1987; Bowles et al., 2014; Cancedda et al., 2015; Correa et al., 2003; Hunley et al., 2010; LaDue et al., 1999; Lana et al., 2004; Lawrence et al., 2010; McEntee et al., 1991; Morris et al., 1994; Nadeau et al., 2004; Theon et al., 1993; Yoon et al., 2008). In one retrospective study that evaluated prognostic factors in 130 canine patients that either treated with orthovoltage or Cobalt-60 radiation therapy in regular fractionation, and the median survival of all dogs was 8.9 months (LaDue et al., 1999). In that study, shrink and boost technique had more side effects and did not prolong survival. Patients treated with orthovoltage radiation had better outcome might due to most of the patients had exenteration (LaDue et al., 1999).

In one study with high percentage of late stage patients (87% of modified Adams CT stage III and IV), regardless of patients with advanced stage of tumor, definitive radiation therapy still can provide a comparable survival benefit with MST

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427 days (Mason et al., 2013).

In a study that compared 2 groups of patients received definitive radiation therapy (6 Gy, 5 fractions, twice a week or 3 Gy, 10 daily fractions) that either combined with firocoxib or not (Cancedda et al., 2015). The median progression-free interval was 228 days (range, 73-525) in patients combined RT with firocoxib and 234 days (range, 50-475) in RT alone group. Median overall survival was 335 days (range, 74-620) in patients with concurrent firocoxib and 244 days (range, 85-505) in RT alone group. There was no statistical significant in progression free interval and overall survival between these two groups (Cancedda et al., 2015).

1.3.3 Concurrent chemoradiation therapy (CCRT)

Radiosensitizers are medications given concurrently to improve treatment outcome while combining with radiation therapy. In Veterinary Radiation Oncology, previous studies include giving gemcitabine and slow release cisplatin, either showed significant hematologic toxicity (neutropenia) and acute local tissue complications or similar survival time compared with other RT alone studies (Lana et al., 2004; LeBlanc et al., 2004). Radiosensitizers regimen and schedule need to be optimized for further clinical benefit.

In the study used Gemcitabine as radiosensitizers in 15 canine nasal tumor patients, they proposed a twice weekly 50 mg/m^2 concurrent protocol which induced severe adverse effects including significant hematologic and local tissue toxicity. Thus, in the author's opinion, this treatment combination should be modified in order to reduce severe side effects and gain clinical benefits (LeBlanc et al., 2004).

The use of slow-release cisplatin implant though not available in general practice, two previous studies suggested that it might have clinical benefit in

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combination with radiation therapy (Lana et al., 2004; Lana et al., 1997). First study evaluated 13 patients treated with daily-fractionated radiation therapy with the median dose of 49.5 Gy. The median survival was 580 days and longer than their historic control of 325 days. The study also indicated that only concurrent slow-release cisplatin implant affected survival and P value was 0.023 (Lana et al., 1997). The second study conducted by the same author increased patient number to 51. Even though there were 17 out of 51 patients (33%) that had cribriform involvement, the median survival time 474 days was still comparable to previous studies (Lana et al., 2004). However, there were 2 and 6 patients in each study that had severe tissue toxicity around the implant and need to remove it (Lana et al., 2004; Lana et al., 1997).

Combination of Cobalt-60 radiation therapy and low dose cisplatin in treating canine nasal carcinomas compared with RT alone group had no significant improvement in overall survival (429 V.s 324 days) or tumor control (330 V.s 270 days). Furthermore, 6 out of 18 patients in combination group did not complete the treatment protocol due to azotemia or tumor progression. Although all squamous cell carcinoma patients and 2 out of 3 undifferentiated carcinoma were in combination group, when excluded these patients in survival analysis, there was still no significant difference (Nadeau et al., 2004).

1.3.4 Hypofractionated radiation therapy

Besides curative intent radiation therapy for canine nasal tumors, coarse fractionated radiation therapy is less intensive and mainly focuses on relieving clinical signs that affect patient's quality of life rather than prolonging patient's survival time. Usually the treatment will deliver 6 to 9 Gy weekly or biweekly, total dose of 26-40

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Gy, which offered 66% to 100% of patients a temporarily resolution of clinical signs for about 4 to 10 months, a median survival times of 4.8 to 14.7 months and 1- and 2-years survival rates of 25-58% and 9-15%, respectively (Belshaw et al., 2011; Buchholz et al., 2009; Gieger et al., 2008; Maruo et al., 2011; Mason et al., 2013; Mellanby et al., 2002; Morris et al., 1994; Yoon et al., 2008). Acute toxicity of hypofractionated radiation therapy is mild and self-limited to maintain the quality of life.

In addition to weekly palliative radiation therapy, 4Gy of five consecutive daily-fractionated radiation treatment protocol was applied in some studies to maintain the quality of life while limit the side effects (McDonald et al., 2012; Tan-Coleman et al., 2012). 6 patients were retreated by using the same protocol that offered median response duration of 129.5 days, MST of 309 days and acute effects were mild and while only 1 patient had late effect (leukotrichia and KCS) (Tan-Coleman et al., 2012). Another study used this treatment protocol in 80 canine and feline patients of different tumor types. There were 5 canine and 2 feline nasal tumor patients that achieved MST of 7.3 months. A study reviewed the patients that did not received previous surgical treatment before radiation therapy and there were 5 patients using 4Gy of 5 consecutive daily-fractionated radiation treatment protocol (Gieger et al., 2008). This treatment strategy limited time for tumor cells to repair and repopulation while reducing the chance of late toxicity by lowing dose per fraction when compared with most palliative protocols that use 6 to 9 Gy weekly fractionation.

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Chapter 2 Introduction

Canine nasal tumors are usually locally invasive rather than easily distant metastasis. Therefore, effective local treatment is the goal to prolong patient's survival. Radiation therapy is the treatment of choice that can extend the life expectancy and maintain life quality. Other traditional treatment modalities including palliative oral medication and surgery either cannot provide longer survival or high complication rate. Chemotherapy alone may have some benefit in canine nasal tumor patients and further well-designed study is warranted.

Most of the studies of radiation therapy in canine nasal tumors were focused on regular fractionated treatment protocols. Benefit from combination treatment of chemotherapy or surgery with radiation therapy is still controversial. Hypofractionated radiation therapy usually considered as palliative treatment in advanced stage patients. Lower complication rate, relieving clinical discomfort, and possible prolonging patient's survival are the purposes of hypofractionated radiation therapy. By using advanced technique, more conformal treatment planning can deliver radiation dosage more precisely while sparing normal tissue damage. Intensity-modulated radiation therapy (IMRT) recently utilized in canine nasal tumor patients and provide comparable survival outcome and less radiation induced side effects. However, no study discussed about hypofractionated treatment strategy delivered through IMRT in canine nasal tumors.

The aim of this study is to compare the efficacy of hypofractionated intensity – modulated radiation therapy with our historical control and previous studies in advanced stage canine nasal tumors.

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Chapter 3 Materials and Methods

3.1 Patient selection

The medical records of client-owned dogs with histologically confirmed or clinically highly suspected nasal malignancy from 2011 to 2015 at National Taiwan University Veterinary Hospital were reviewed. The age, sex, neuter status, breed, body weight, histopathological diagnosis, clinical signs before treatment, clinical signs duration before first visit, improvement of clinical signs of all the dogs were obtained from the medical records.

Patients treated with hypofractionated radiation therapy are assigned to RT group. Combination of other treatments (Pre-, post- or concurrent chemotherapy, steroids, NSAIDs, nasal cavity exenteration), dose per fraction and total radiation dose is not limited. Non-RT group allows treatments except radiation therapy.

3.2 Tumor staging

Pretreatment evaluation consisted of full physical examination, complete blood count, serum biochemical profile, coagulation profile, and thoracic radiograph. If swollen lymph nodes noted duration physical examination, then fine needle aspiration will be performed.

Tumor staging computed tomography (CT) studies were performed using a multislice scanner (Activion™ 16, Toshiba, Japan). Post-contrast CT scans were acquired after intravenous administration of 640mg/kg nonionic contrast agent (Iopamiro®, 370 mg/ml, Bracco s.p.a., Milano, Italia) manually, with 1-3 mm of slice thickness. Magnetic resonance imaging (MRI) studies were performed using in house

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Vet MRI (VET-MR, Esaote, 0.2 Tesla field). Based on image findings, tumors were staged according to the modified Adams system (Adams et al., 2009). Table 4.

3.3 Medications

Chemotherapy regiments used in this study including Doxorubicin (Adriblastina Rapid Dissolution, Pfizer, Milano, Italy), Carboplatin (Paraplatin, Bristol Myers Squibb, Latina, Italy), Vincristine (Hospira, Victoria, Australia), and Chlorambucil (Leukeran, Aspen, Feucht, Germany).

Oral medications include prednisolone (Donison, China chemical & pharmaceutical, Hsinchu, Taiwan), piroxicam (Pirocam, Kojar, Taichung, Taiwan), meloxicam (Mobic, Boehringer Ingelheim, Koropi, Greece), firocoxib (Previcox, Merial, Toulouse, France), isotretinoin (Roaccutane, Roche, Eberbach, Germany), and tramadol (Tramtor, Patron, Kaohsiung, Taiwan). Oral medication prescribed depending on the attending clinicians.

Anesthesia medications include propofol (Braun, Melsungen, Germany), fentanyl (UBI pharmaceutical, Hsinchu, Taiwan), midazolam (Dormicum, Roche, Basel, Switzerland), and Zoletil (Virbac, Carros, France).

3.4 RT procedure

Individual thermoplastic immobilization masks with sternal recumbency treatment position were applied to each patient. Planning CT scans were performed using either a dual source scanner (Dual CT 9710, Siemens, 2 slice) or a 16 slice CT scan (Discovery CT 590, GE, 16 slice). Treatment plans were generated individualized by using a computer treatment planning system (Pinnacle 9.0 or 9.8 version, Elekta).

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RT group patients were treated with external beam megavoltage radiation delivered either with a 4 MV linear accelerator (Precise®, 250MU/min, Elekta, Stockholm, Sweden) or a 6 MV linear accelerator (Synergy®, 500MU/min, Elekta, Stockholm, Sweden). A multileaf-collimator (40*2 of 10mm leaves) was used to make the planning more conformal, reduce the side effects of surrounding organs at risk, and optimize the homogeneity of radiation dose.

The gross tumor volume (GTV) usually contained nasal discharge because it was difficult to distinguish tumor from discharge. Clinical target volume (CTV) was defined including GTV and any adjacent tissue that considered at risk for possible extension of microscopic disease. Planning target volume (PTV) included the regions 3–4 mm outside the CTV for possible patient movement and positioning errors. Dose-volume histograms were calculated to ensure coverage of over 90% of the GTV and over 80% of the PTV by the prescribed dose. The surrounding organs at risk (eyes, lens, and brain) were contoured according to the planning CT images.

Neither wedges nor lead blocks were used during the treatment procedure. In order to achieve adequate dose in superficial target tissue, equivalent bolus material was used in the treatment plan when indicated.

For those patients with lymph node metastasis, invaded lymph nodes were irradiated by photons in the separated treatment planning and the dosage was the same with those prescribed to the tumor. Biologically effective dose (BED) was calculated to take into account the different fractionation protocol using the following formula:

$$BED=nd\ [1+d/(\alpha/\beta)]$$

Where n represents the fraction number, d is the dose per fraction, and α/β is the α to β ratio. BED can most conveniently be identified simply by a single subscript meaning the α/β ration of the relevant tissue; thus, an α/β of 3 Gy was used for late

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responding tissue and α/β of 10 Gy was used for early responding tissue (Fowler, 2010).

3.5 Treatment response and side effects

Clinical improvement defined as the relief of clinical signs observed by the owner or clinical practitioner. The response of radiation therapy was defined by the post radiation therapy CT scan. Complete remission (CR) means disappearance of all target lesions. Partial remission (PR) means more than 50% volumetric regression of the tumor. Stable disease (SD) means less than 50% volumetric regression or volumetric progression. Progression disease (PD) means more than 50% volumetric progression.

Owners were asked to update any side effects of chemotherapy or radiation toxicity regularly. Side effects of chemotherapy were retrieved from medical record and graded according to VCOG-CTCAE v1.1 grading system (Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events (VCOG-CTCAE) Following Chemotherapy or Biological Antineoplastic Therapy in Dogs and Cats V1.1., 2011). Table 1. Side effects were classified as activity, appetite, hematology, and gastrointestinal. Patients were assessed during each recheck depending on each attending clinician.

Side effects of radiation therapy were classified as acute toxicity and late toxicity and using a standardized toxicity grading system (LaDue and Klein, 2001). Table 2. and Table 3. According to the medical record, acute toxicity was defined as complications occurred within 4 weeks after last radiation therapy. A clinical evaluation was performed between 2 to 4 weeks after the last radiation therapy to

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assess the remission of clinical signs and acute toxicity. Late toxicity was defined as complications that occurred after at least 6 months of last radiation therapy. Acute radiation toxicity evaluated the organs such as skin (erythema, desquamation, and alopecia), eye (conjunctivitis, scleral injection, KCS, and ulceration), and oral mucus membrane (injection, mucositis, and ulceration). Late radiation toxicity evaluated the organs such as skin/hair, central nervous system, eye (cataracts, KCS, keratitis, and vision loss), and bone (painful palpation, radiographic change, and necrosis).

3.6 Statistic analysis

For all patients, the definition of presenting signs duration in this study is the interval from the time clinical signs first presented to the time when patient first visited NTUVH. Time to progression defined as the day of measurable progression through CT scan, distant metastasis or deteriorated clinical signs such as refractory nasal discharge or epistaxis that was not responsive to empirical treatment. Progression free interval (PFI) defined as the time from either the first treatment day (non-RT group) or the day finished radiation therapy (RT group) to the day of time to progression. Overall survival time (OS) calculated from the day of first visit NTUVH until the day euthanasia or death.

Mann-Whitney U-test was performed in order to compare whether these two study groups have any significant difference in the demographic or tumor features such as age, sex, body weight, histopathological diagnosis, clinical signs before treatment, clinical signs duration before first visit, clinical stage, and chemotherapy or not.

In non-RT group, Pearson chi-square was applied to evaluate correlation

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between each following variables such as clinical stages, duration of presenting signs, histopathology diagnosis, and concurrent treatment with clinical improvement due to lack of follow up CT scan. In RT group, Pearson chi-square was applied to evaluate correlation between each following variables such as clinical stages, duration of presenting signs, histopathology diagnosis, and concurrent treatment with treatment response due to every patient had clinical improvement and cannot analysis.

Univariable analysis (Cox proportional hazards model) was performed to demonstrate the differences of each variable in progression free interval (PFI) and overall survival (OS) of all patients. Variables including age (≤ or >median age), sex, histopathological diagnosis (carcinoma/adenocarcinoma or sarcoma), clinical signs before treatment, clinical stage (stage IV or stage I-III), received RT or not, and received chemotherapy or not were tested for prognostic significance.

Patients of clinical stage I to III were combined together for statistical needs. Histopathological diagnosis was grouped according to their different radiobiological behavior (compared with sarcomas, carcinoma or adenocarcinomas generally have higher α/β ratio). For each variable, the risk (hazard ratio, HR) of tumor progression or tumor related death during the follow-up period was estimated with the corresponding 95% confidence intervals and P values. Further multivariable analysis (Cox proportional hazards model) will be performed in those variables that achieved statistically significant.

For overall survival analysis, the cause of death that related to nasal tumor (either tumor itself or euthanasia due to tumor progression) was recorded as events. Patients that alive at the time of study closure (June, 2016) were censored. If the cause of death was unrelated to tumor progression, the data will be excluded for survival

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analysis.

The Kaplan-Meier method was used to calculate both PFI and OS, and to calculate the one- and two-year OS and PFI rates in both study groups. Furthermore, the Kaplan-Meier method was also used to compare whether clinical stage, histopathology diagnosis (carcinoma/adenocarcinoma or others, squamous cell carcinoma or others), concurrent treatment, and duration of presenting signs will have significant difference in PFI and OS.

All statistical analyses were performed using commercial software (SPSS Statistics v. 21, IBM, Somers, NY). P values < 0.05 were considered significant.

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Chapter 4 Result

4.1 Demographic

35 of client-owned dogs met the inclusion criteria and their medical records were reviewed. The median age at the onset of disease for all dogs is 10 years old (range, 6 to 13 years old) and the median body weight was 14.2 kg (range, 2.36 to 46.5kg).

Sex distribution was 23 males and 12 females, of which 17 males and 6 females were sexually intact. Mongrel breed was the most common breed, which accounted for 7 (20%) patients, and followed by Shiba (n=5), Beagle (n=4), Golden Retriever (n=4), Maltese (n=2), Labrador Retriever (n=2), Shetland Sheepdog (n=2), and Toy Poodle (n=2). Other breeds including one of each following breed: West Highland White Terrier, Scottish Terrier, Schnauzer, Husky, English Bulldog, Dachshund, and Corgi.

According to modified Adams staging system (Adams et al., 2009), 33/35 patients were assessed through CT scan findings. 18/35 (51.4%) are stage IV, 11/35 (31.4%) are stage III, 3/35 (8.6%) are stage II, and 1/35 (2.9%) is stage I.

Medium duration of clinical signs before first visit is 60 days (range, 3-283), and clinical signs include epistaxis (n=25/35, 71.4%), nasal discharge (n=21/35, 60%), sneezing (n=17/35, 48.6%), facial deformity (n=15/35, 42.9%), hard to breath (n=12/35, 34.3%), ocular discharge (n=4/35, 11.4%), seizure (n=2/35, 6%), and ataxia (n=1/35, 3%).

Three patients didn't receive any treatment after diagnosed nasal tumor due to the owner's will. Other 32 patients received treatments except radiation therapy including NSAID (25/32, 78.1%), chemotherapy (15/32, 46.8%), steroid (13/32,

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40.6%), nostril exenteration (2/32, 6.3%), and isotretinoin (2/32, 6.3%).

34/35 patients had histopathologic diagnosis, including 11/35 (31.4%) adenocarcinomas, 8/35 (22.9%) carcinomas, 7/35 (20 %) squamous cell carcinomas, 3/35 (8.6%) rhinitis, 2/35 (5.7%) chondrosarcoma, 1/35 (2.85%) transitional cell carcinoma, 1/35 (2.85%) fibrosarcoma, and 1/35 (2.85%) undifferentiated sarcoma.

4.2 Non-radiation therapy group

20 canine nasal patients did not receive radiation therapy. The median age at the onset of disease for non-RT patients is 10 years old (range, 7 to 13 years old) and the median body weight was 15.5 kg (range, 3.42 to 46.5kg).

Sex distribution was 13 males and 7 females, of which 10 males and 4 females were sexually intact. Mongrel breed was the most common breed, which accounted for 4 (20%) patients, and followed by Beagle (n=3), Golden Retriever (n=3), Labrador Retriever (n=2), Shiba (n=2), and Shetland Sheepdog (n=2). Other breeds including one of each following breed: West Highland White Terrier, Schnauzer, English Bulldog, and Maltese.

Two patients did not performed CT scan due to the owner's will. According to modified Adams staging system (Adams et al., 2009), 18/20 non-RT patients were assessed through CT scan findings. 11/20 (55%) are stage IV, 5/20(25%) are stage III, and 2/20 (10%) are stage II.

Medium duration of clinical signs before first visit is 41.5 days (range, 3-148), and clinical signs include epistaxis (n=14/20, 70%), nasal discharge (n=13/20, 65%), facial deformity (n=11/20, 55%), sneezing (n=9/20, 45%), hard to breath (n=7/20, 35%), ocular discharge (n=4/20, 20%), seizure (n=1/20, 5%), and ataxia (n=1/20, 5%).

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Whether the duration of clinical signs ≤ 34.5 days (median of 18 patients that had clinical staging) or not, it was not correlated with the clinical stage, and the P value was 0.27.

All of non-RT group patients had histopathologic diagnosis, including 7/20 (35%) squamous cell carcinomas, 6/20 (30%) adenocarcinomas, 2/20 (10%) carcinomas, 2/20 (10%) chondrosarcoma, 1/20 (5%) transitional cell carcinoma, 1/20 (5%) fibrosarcoma, and 1/20 (5%) rhinitis.

4.2.1 Treatment and toxicity

Three patients didn't receive any treatment after diagnosed nasal tumor due to the owner's will. Other 17 non-RT patients received treatments including NSAID (n=13), chemotherapy (n=9), steroid (n=7), isotretinoin (n=2), and nostril exenteration (n=1).

Of 9 patients in this group that received chemotherapy, 6/9 received carboplatin 1-2 doses (median, 1 dose) which dosage ranged 200-300 mg/m^2 (median, 300 mg/m^2), 3/9 received doxorubicin 2-3 doses (median, 2 doses) which dosage ranged 20-30 mg/m^2 (median, 30 mg/m^2), 2/9 received chlorambucil metronomic chemotherapy 4 mg/m^2 daily, and 1/9 received one dose of 0.53 mg/m^2 vincristine.

Adverse effects were well tolerated and self-limited. 4/17 (23.5%) Grade I anorexia, 3/17 (17.6%) Grade I lethargy, 1/17 (5.9%) Grade I neutropenia, 1/17 (5.9%) Grade II neutropenia, 2/17 (11.8%) Grade I vomiting. Table 5.

4.2.2 Treatment response

Of 17 patients in this group that received treatment, clinical improvement was

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noted in 5/9 patients that received chemotherapy and 2/8 patients that received palliative oral medications. Mean and median progression free interval is 86.8 and 17 days (range, 0-324 days) and 8 and 0 days (range, 0-52 days), respectively. Mean and median progression free interval of 17 patients in non-RT group that received treatment was 49.7±23.1 (95% CI, 4.4-95) and 0 day (range, 0-324), respectively. Standard deviation and 95% CI of median progression free interval was unable to analysis due to the median was zero.

Mean and median overall survival time of 9 patients that received chemotherapy was 264.1±49.6 (95% CI, 166.9-361.3) and 215±10.4 (95% CI, 194.5-235.5) days, respectively (range, 59-579 days). Mean and median overall survival time of 8 patients that received palliative oral medication was 75.2±22.7 (95% CI, 30.8-119.6) and 38±1.7 (95% CI, 34.8-41.2) days, respectively (range, 7-247 days). Of 3 patients that did not received any treatment, the median overall survival time was 38 days (range, 38-108 days). Mean and median overall survival in non-RT group was 160.2±32.9 (95% CI, 95.8-224.6) and 126±30.2 (95% CI, 66.8-185.2) days, respectively.

The cause of death of 9 patients that received chemotherapy including 6/9 due to tumor progression and 3/9 were euthanized. The cause of death of 8 patients that received palliative oral medications including 5/8 due to tumor progression and 3/8 were euthanized. Of 3 patients that did not received any treatment, the cause of death including 2/3 due to tumor progression and 1/3 were euthanized.

4.2.3 Correlated factors of clinical improvement in non-RT group

When we evaluated the factors that might affect clinical improvement of non-RT group patients, there was no statistically significant compared with clinical

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stage, histopathologic diagnosis of whether carcinoma/adenocarcinoma or not, the duration of clinical signs ≤ 45 days or not, and whether patients received chemotherapy or not. P value was 0.987, 0.323, 0.772, and 0.162, respectively. Although there was only 1 out of 7 nasal SCC patient (14.3%) versus 6 out of 10 non-SCC patients (60%) that had clinical improvement, there was no significant difference and the P value was 0.059. Table 7.

4.2.4 Kaplan-Meier of PFI and OS in non-RT group

We used Kaplan-Meier curve to analyze whether following factors have different progression free interval (PFI) or overall survival (OS), including clinical stage (stage II, III, IV), histopathology diagnosis (carcinoma/adenocarcinoma or others), histopathology diagnosis (squamous cell carcinoma or others), duration of clinical signs ≤ 41.5 days or not, and treatment (chemotherapy, palliative oral medication, or no treatment). None of factors above achieved significant difference in PFI Kaplan-Meier curve analysis. In theses factors, duration of clinical signs ≤ 41.5 days or not was the factor that closest to achieve significant difference, and the P value was 0.059. Table 8., Fig. 1., Fig. 2., Fig. 3., Fig. 4., Fig. 5

There was significant difference of OS Kaplan-Meier curve in non-RT patients that received chemotherapy or not. When versus palliative oral medication, P=0.006 and versus no treatment, P=0.002. Compared palliative oral medication with no treatment, there was no significant difference and P=0.746. Other factors did not achieve significant difference in OS Kaplan-Meier curve analysis and duration of clinical signs ≤ 41.5 days or not was still the factor that closest to achieve significant difference (P=0.065). Table 9., Fig. 6., Fig. 7., Fig. 8., Fig. 9., Fig. 10

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4.3 Hypofractionated radiation therapy group

15 canine nasal patients received hypofractionated radiation therapy. The median age at the onset of disease for RT patients is 10 years old (range, 6 to 13 years old) and the median body weight was 13.1 kg (range, 2.36 to 32.8kg).

Sex distribution was 10 males and 5 females, of which 7 males and 2 females were sexually intact. Mongrel breed and Shiba was the most common breed, and each breed accounted for 3 (20%) patients, and followed by Toy Poodle (n=2). Other breeds including one of each following breed: Golden Retriever, Beagle, Corgi, Dachshund, Husky, Maltese, and Scottish Terrier.

According to modified Adams staging system (Adams et al., 2009), all RT patients were assessed through CT scan findings. 7/15 (46.6%) are stage IV, 6/15(40%) are stage III, and both stage I and stage II had 1/15 (6.7%) patient.

Medium duration of clinical signs before first visit is 95 days (range, 10-283), and clinical signs include epistaxis (n=11/15, 73%), nasal discharge (n=8/15, 53%), sneezing (n=8/15, 53%), hard to breath (n=5/15, 33%), facial deformity (n=4/15, 26.7%), ocular discharge (n=2/15, 13%), seizure (n=1/15, 6.7%), and ataxia (n=0/15, 0%). Whether the duration of clinical signs ≤ 95 days (median of 15 patients that had clinical staging) or not, it was not correlated with the clinical stage, and the P value was 0.658.

14 of 15 RT group patients had histopathologic diagnosis, including 6/15 (40%) carcinomas, 5/15 (33.3%) adenocarcinomas, 2/15 (13.3%) rhinitis, and 1/15 (6.7%) undifferentiated sarcoma.

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4.3.1 Treatment and toxicity

Each RT group patient received weekly radiation therapy of single dose ranged from 7 to 15 Gy (median, 8Gy), with 2 to 5 fractionation (median, 5 fractionation) and the total dose ranged from 30 to 42.5 Gy (median, 35 Gy). All radiation dosage was delivered in 14 to 36 days (median, 28 days).

Adverse effects induced by radiation therapy were mild and manageable. Acute toxicity including 12/15 (80%) Grade I skin toxicity, 1/15 (6.7%) Grade II skin toxicity, 7/15 (46.7%) Grade I oral mucus membrane toxicity, 4/15 (26.7%) Grade II oral mucus membrane toxicity, 9/15 (60%) Grade I ocular toxicity, 1/15 (6.7%) Grade II ocular toxicity, and 1/15 (6.7%) Grade III ocular toxicity. Late toxicity including 14/15 (93.3%) Grade I hair/skin toxicity, 7/15 (46.7%) Grade I ocular toxicity, 1/15 (6.7%) Grade II ocular toxicity, 1/15 (6.7%) Grade III ocular toxicity. None of the patients experienced late toxicity of bone or central nerve system. Table 6.

Six RT patients received pre-, post-, or concurrent chemotherapy. All of RT patients received either NSAID or steroid. One patient received nostril exenteration before radiation therapy.

Of 6 patients in this group that received chemotherapy, all received carboplatin 1-6 doses (median, 2 dose) which dosage ranged 250-300 mg/m^2 (median, 300 mg/m^2). Total 17 doses of carboplatin were given to these 6 patients, 5/17 were prior to radiation therapy, 2/17 were concurrent with radiation therapy, and 10/17 were after radiation therapy. 2/6 received two doses doxorubicin before radiation therapy and the dosage was 1mg/kg and 30 mg/m^2, respectively.

Adverse effects were well tolerated and self-limited. 1/6 (16.7%) Grade II anorexia, 2/6 (33.3%) Grade I anorexia, 1/6 (16.7%) Grade II lethargy, 2/6 (33.3%) Grade I lethargy, 2/6 (33.3%) Grade I neutropenia, 2/6 (33.3%) Grade I vomiting.

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Table 5.

4.3.2 Treatment response

Clinical improvement was noted in all patients that received radiation therapy. Three patients were expired but not because of tumor progression (2 renal failure and 1 bleeding disorder) and excluded for MST calculation. 2 of these 3 patients did not recur and excluded for progression free interval calculation. Mean and median progression free interval in RT group was 370.4±136.2 (95% CI, 103.5-637.3) and 160±33 (95% CI, 95.4-224.6) days, respectively (range, 23-1426 days).

Post radiation therapy CT scan for response assessment was arranged 13-48 days (median, 21 days) after last radiation treatment. 4/15 (26.7%) patients were complete remission, 6/15 (40%) patients were partial remission, and 5/15 (33.3%) patients were stable disease. Mean and median overall survival in RT group that received chemotherapy was 377.8 ±31.5 (95% CI, 316-439.6) and 372±34 (95% CI, 305.4-438.6) days, respectively (range, 296-485). Mean and median overall survival in RT group that did not received chemotherapy was 712.1±235.6 (95% CI, 250.3-1174) and 372±56.3 (95% CI, 261.7-482.3) days, respectively (range, 80-1619). There was no significant difference in overall survival in RT group between patients received chemotherapy or not (P=0.414). Mean and median overall survival in RT group was 572.8±145.9 (95% CI, 286.8-858.9) and 372±26.5 (95% CI, 320.1-423.9) days, respectively.

Besides 3 patients that were expired not related to tumor progression, the cause of death of RT group patients including 3/12 due to tumor progression and 7/12 were euthanized. Two patients were still alive at the time the study closed, and one of them remained complete remission.

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4.3.3 Correlated factors of treatment response in RT group

All patients in RT group had clinical improvement after radiation therapy, and therefore Pearson chi-square method is not amenable to analysis the data. When evaluated the factors that might affect treatment response assessed through post treatment CT scan after radiation therapy, there was no statistically significant compared with clinical stage, histopathologic diagnosis of whether carcinoma/adenocarcinoma or not, the duration of clinical signs ≤ 95 days or not, and whether patients received chemotherapy or not. P value was 0.435, 0.211, 0.87, and 0.87, respectively. Table 10.

4.3.4 Kaplan-Meier of PFI and OS in RT group

We used Kaplan-Meier curve to analyze whether following factors have different progression free interval (PFI) or overall survival (OS), including clinical stage (stage I, II, III, IV), histopathology diagnosis (carcinoma/adenocarcinoma or others), duration of clinical signs ≤ 95 days or not, and received chemotherapy or not. None of factors above achieved significant difference in PFI Kaplan-Meier curve analysis. Nonetheless, when compared clinical stage III with stage IV, it nearly achieved significant difference, and the P value was 0.052. Table 11., Fig. 11., Fig. 12., Fig. 13., Fig. 14.

There was no significant difference of OS Kaplan-Meier curve in RT group patients when analyzing the factors above. Compared clinical stage III with stage IV, P value was 0.102 that was still the factor that closest to achieve significant difference. Table 12., Fig. 15., Fig. 16., Fig. 17., Fig. 18.

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4.4 Compare two study groups

Compared patients' characteristics in RT group and non-RT group, there were no significant difference in age, sex, body weight, histopathologic diagnosis, clinical signs, modified Adams' clinical stage, and received chemotherapy or not.

Duration of clinical signs before first visit in RT group was significant longer than non-RT (95 days V.s 41.5 days, respectively. P=0.012). Table 13.

4.4.1 PFI Kaplan-Meier curve of RT and non-RT group

When compared progression free interval in RT and non-RT group patients through Kaplan-Meier curve, there was a significant difference between them (P=0.002). Mean and median progression free interval in RT group was 370.4 ±136.2 (95% CI, 103.5-637.3) and 160±33 (95% CI, 95.4-224.6) days, respectively. Mean and median progression free interval of 17 patients in non-RT group that received treatment was 49.7±23.1 (95% CI, 4.4-95) and 0 day (range, 0-324), respectively. Standard deviation and 95% CI of median progression free interval was unable to analysis due to the median was zero. Fig. 19.

We then separated non-RT group patients into two subgroups: chemotherapy and palliative oral medication. After further grouping, we compared progression free interval of these two groups with RT group. Mean and median progression free interval in non-RT-chemo group was 86.8±40.2 (95% CI, 7.9-165.6) and 17±25.3 (95% CI, 0-66.7) days, respectively. Patients in non-RT group that did not received chemotherapy but only palliative oral medication (non-RT-POM group) had mean and median progression free interval 8±6.5 (95% CI, 0-20.7) and 0 day (range, 0-52), respectively. Standard deviation and 95% CI of median progression free interval in

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this subgroup was unable to analysis due to the median was zero. The only significant difference obtained between RT group and two subgroups was RT group versus non-RT-POM group (P=0.00001). Other comparisons such as RT group versus non-RT-chemo group and non-RT-chemo group versus non-RT-POM group though were close to but did not reach statistically significant (P=0.06 and 0.073, respectively). Fig. 20.

4.4.2 OS Kaplan-Meier curve of RT and non-RT group

When compared overall survival in RT and non-RT group patients through Kaplan-Meier curve, there was a significant difference between them (P=0.001). Mean and median overall survival in RT group was 572.8 ±145.9 (95% CI, 286.8-858.9) and 372±26.5 (95% CI, 320.1-423.9) days, respectively. Mean and median overall survival in non-RT group was 160.2±32.9 (95% CI, 95.8-224.6) and 126±30.2 (95% CI, 66.8-185.2) days, respectively. Fig. 21.

We then separated non-RT group patients into two subgroups: chemotherapy and palliative oral medication. After further grouping, we compared overall survival of these two subgroups with RT group. Mean and median overall survival in non-RT-chemo group was 264.1±49.6 (95% CI, 166.9-361.3) and 215±10.4 (95% CI, 194.5-235.5) days, respectively. Mean and median overall survival in non-RT-POM group was 75.2±22.7 (95% CI, 30.8-119.6) and 38±1.7 (95% CI, 34.8-41.2) days, respectively. Significant difference obtained between RT group and two subgroups were RT group versus non-RT-POM group (P=0.00002) and non-RT-chemo group versus non-RT-POM group (P=0.002). RT group versus non-RT-chemo group though was close to but did not reach statistically significant (P=0.058). Fig. 22.

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4.4.3 Univariable analysis

Univariable analysis (Cox proportional hazards model) was performed to demonstrate the differences of each variable in progression free interval (PFI) and overall survival (OS) of all patients. Following variables including age (≤ or >median age), sex, histopathological diagnosis (carcinoma/adenocarcinoma or sarcoma), clinical signs before treatment, clinical stage (stage IV or stage I-III), received RT or not, and received chemotherapy or not were tested for prognostic significance.

When evaluated the relationship between these variables and tumor progression, facial deformity (P=0.006), stage (P=0.021), and received radiation therapy or not (P=0.004) achieved statistically significant. Hazard ratio of these three variables was 3.414 (95% CI, 1.431-8.145), 2.620 (95% CI, 1.158-5.927), and 0.309 (95% CI, 0.138-0.689), respectively. Age (≤ 10 years old or >10 years old, P=0.946), sex (P=0.508), histopathological diagnosis (carcinoma/adenocarcinoma or sarcoma, P=0.982) clinical signs (sneezing, P=0.936; nasal discharge, P=0.899; epistaxis, P=0.473), and received chemotherapy or not (P=0.898) were not statistically significant. Table 14.

When evaluated the relationship between these variables and overall survival, facial deformity (P=0.004), stage (P=0.014), and received radiation therapy or not (P=0.002) achieved statistically significant. Hazard ratio of these three variables was 3.239 (95% CI, 1.470-7.140), 2.835 (95% CI, 1.231-6.531), and 0.273 (95% CI, 0.119-0.624), respectively. Age (≤ 10 years old or >10 years old, P=0.524), sex (P=0.556), histopathological diagnosis (carcinoma/adenocarcinoma or sarcoma, P=0.863) clinical signs (sneezing, P=0.937; nasal discharge, P=0.821; epistaxis, P=0.147), and received chemotherapy or not (P=0.474) were not statistically

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significant. Table 15.

4.4.4 Multivariable analysis

Facial deformity, stage, and received radiation therapy or not were three variables that both achieved statistically significant in univariable analysis of PFI and OS. We then further evaluate these variables by multivariable analysis.

Facial deformity (P=0.012) and received radiation therapy or not (P=0.014) remained statistically significant while stage lose significance (P=0.154) when evaluated these three variables by multivariable analysis of progression free interval. Hazard ratio of facial deformity and received radiation therapy or not was 3.741 (95% CI, 1.330-10.524), and 0.347 (95% CI, 0.148-0.810), respectively. Table 16.

Only received radiation therapy or not (P=0.005) remained statistically significant while either facial deformity (P=0.167) or stage (P=0.086) lose significance when evaluated these three variables by multivariable analysis of overall survival. Hazard ratio of received radiation therapy or not was 0.259 (95% CI, 0.102-0.661). Table 17.

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Chapter 5 Discussion

5.1 Efficacy

In this study, we evaluated thirty-5 cases respectively. In order to demonstrate the treatment outcome, patients were grouped according to whether they received radiation therapy or not into RT and non-RT group. In RT group, all patients had clinical improvement after receiving radiation therapy. Response rate assessed by post-radiation therapy CT scan was 66.7%, and the rest of patients remain stable disease. Mean and median progression free interval in RT group was 370.4 ±136.2 (95% CI, 103.5-637.3) and 160±33 (95% CI, 95.4-224.6) days, respectively (range, 23-1426 days). Mean and median overall survival in RT group was 572.8 ±145.9 (95% CI, 286.8-858.9) and 372±26.5 (95% CI, 320.1-423.9) days, respectively (range, 80-1619 days). Whether patients in RT group received chemotherapy or not had no significant difference in PFI (median, 200 days V.s 154.5 days, P=0.324) and OS (median, 372 days V.s 372 days, P=0.414). The one- and two-year survival rate was 58.5% and 25%, respectively. Compared with patients of non-RT group, PFI (median, 160 days V.s 0 days, P=0.002) and OS (median, 372 days V.s 126 days, P=0.001) were significant longer in RT group.

Compared with previous studies of definitive radiation therapy in canine nasal tumor patients, the dose of definitive radiation therapy ranged from 42Gy to 54Gy, divided into 10 to 18 fractions in 2 to 4 weeks. The biologically effective dose (BED) of α/β ratio 10 and 3 was calculated as the formula aforementioned. BED_3 and BED_{10} was ranged from 98 to 135 Gy and 58.8 to 81.9 Gy, respectively (Adams et al., 2005; Adams et al., 2009; Adams et al., 1998; Adams et al., 1987; Bowles et al., 2014; Cancedda et al., 2015; Correa et al., 2003; Hunley et al., 2010; LaDue et al., 1999;

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Lana et al., 2004; Lawrence et al., 2010; McEntee et al., 1991; Morris et al., 1994; Nadeau et al., 2004; Theon et al., 1993; Yoon et al., 2008). Our study administered weekly radiation therapy of single dose ranged from 7 to 15 Gy (median, 8Gy), with 2 to 5 fractionation (median, 5 fractionation) and the total dose ranged from 30 to 42.5 Gy (median, 35 Gy). All radiation dosage was delivered in 14 to 36 days (median, 28 days). The median BED_3 and BED_{10} of our study was 117.3 Gy and 59.5 Gy, respectively. Not only our biologically effective dose, but also treatment outcome including median survival time and the 1- and 2-years survival rates were comparable with these previous studies.

Hypofractionated radiation therapy in canine nasal tumor patients divided total radiation dosage range 16 Gy to 40 Gy into 4 to 5 weekly or biweekly course fractions. This treatment strategy usually focuses on patient's life quality and resolution of clinical signs rather than prolonging patient's survival time (Belshaw et al., 2011; Buchholz et al., 2009; Gieger et al., 2008; Maruo et al., 2011; Mason et al., 2013; Mellanby et al., 2002; Morris et al., 1994; Yoon et al., 2008). BED_3 and BED_{10} was calculated and ranged from 88 to 144 Gy and 43.2 to 68.4 Gy, respectively. While comparing with these studies, our treatment outcome was comparable or better than most of these studies and summarized in Table 19. Although the calculations of biologically effective dose are more accurate in daily dosing treatment protocols and direct comparison with a weekly protocol may be inaccurate, comparing BED of these treatment protocols may still offer some information that hypofractionated radiation therapy usually have similar or lower BED_3 and BED_{10} that accounts for suboptimal tumor control but less incidence and severity of radiation induced toxicities.

Canine nasal tumors locate in complex anatomical region that surrounded by the organs fragile to radiation such as eyes and brain. These organs at risk can be

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spared through more conformal radiation therapy. Intensity-modulated radiation therapy (IMRT) use high speed multileaf collimator in order to complete a more conformal treatment planning and allowed dose escalation while sparing normal tissue damage. So for there were two studies evaluated the efficacy and toxicity of IMRT in treating canine nasal tumor patients (Hunley et al., 2010; Lawrence et al., 2010). Our treatment outcome was no better than these studies and was summarized in Table 18.

The first study mentioned above delivered radiation dose through either helical tomotherapy or IMRT in 31 patients with nasal tumors and compared with 36 2D RT historical control. Definitive treatment plan delivered 42 Gy in 10 daily fractions. Although the MST was not significant different from historical control group (420 V.s 411 days, P=0.71), acute and late side effects were reduced significantly. 61% of dogs in IMRT group did not experience any ocular side effects. Mean dose to eye in IMRT group versus control group was significantly lower (12.5 Gy V.s 33.6 Gy, respectively. P=0.0001). Also, the rate of late ocular effect in IMRT group versus control group was significantly lower (26% V.s 64%, respectively. P=0.0041) (Lawrence et al., 2010).

The second IMRT study mentioned above of 12 canine nasal tumor patients also delivered regular Monday-Wednesday-Friday fractionation for total dose to 54 to 63 Gy. 67% patients had clinical resolution and the MST was 446 days. 1- and 2-years survival rate was 50% and 25%, respectively (Hunley et al., 2010).

When compare with different studies of radiation therapy in canine nasal tumors, considering of different radiation strategies, delivery facilities, patients' condition, concurrent treatments, follow-up schedule and response assessment criteria, the optimal radiation treatment protocol has yet to be determined. Though the biologically effective dose in our study was similar to two IMRT study

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aforementioned, our MST was no longer than previous studies. This may owing to the formula of biologically effective dose applied in our study did not account for treatment time and potential doubling time of tumor. Treatment outcome is also influenced by these 2 factors. If tumors progressed rapidly, it might be less sensitive to radiation because rapid tumor growth will form hypoxic region that allowed continued tumor growth. Hypofractionated radiation therapy may have more benefit in slow-growing tumors that are more responsive because of adequate oxygenation and a lack of tumor cell repopulation between fractions (Gieger et al., 2008).

5.2 Influential and prognostic factors evaluation

Although there was no significant difference when we evaluated the relations between variables and clinical improvement in non-RT group, histopathologic diagnosis of squamous cell carcinoma (n=7) versus other diagnosis (n=10) was closest but no significant difference (P=0.059). Some of the previous literature suggested that when compared with other histopathologic type of nasal tumor, nasal squamous cell carcinoma have a more aggressive clinical behavior and poorer survival.

SCC was clinically aggressive and had tendency to invade surrounding bone tissue in 5 out of 8 cases (Rogers et al., 1995). Survival times of dogs with squamous cell or undifferentiated carcinoma were significantly poor than for dogs with adenocarcinoma or sarcoma (P <0.02 or 0.03) (Adams et al., 1987). Result from another study showed that patients had anaplastic/poorly-differentiated carcinomas or squamous cell carcinomas had a significant shorter disease free survival than the sarcoma group (P=0.011) (Adams et al., 2009). A study evaluated nasal nonkeratinizing SCC treated with Cobalt-60 definitive radiation therapy and the MST

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was 165 days that indicated a poor prognosis of this histopathologic subtype (Correa et al., 2003).

However, the relationship between histopathologic diagnosis and prognosis is still in conflict. In the study that evaluated 139 untreated canine nasal epithelial origin tumor patients, the histologic subtype of carcinoma was not a prognostic factor for survival (Rassnick et al., 2006). In another previous study, they categorized according to patients' histopathologic diagnosis into 3 groups: anaplastic carcinoma, undifferentiated carcinoma, squamous cell carcinoma; carcinoma, adenocarcinoma; sarcomas. There was no statistically significant in survival (P=0.09) (Adams et al., 2009). Another study also did not show survival benefit compared sarcomas with carcinomas (LaDue et al., 1999).

Duration of clinical signs before first visit ≤ 41.5 days or not in non-RT group did not achieve statistical significant in PFI or OS analysis (P=0.059 and 0.065, respectively). Duration of clinical signs before first visit ≤ 95 days or not in RT group did not achieve statistical significant in PFI or OS analysis (P=0.422 and 0.497, respectively). Previous study that administered median dose of 8Gy with total dose 32Gy weekly in 63 canine nasal tumor patients and also found the duration of clinical signs had no significant difference in survival (Maruo et al., 2011). Another study also indicated that either duration of clinical signs ≥ 3 months had no significant difference in survival (P=0.37) (Mellanby et al., 2002). Conversely, a multi-institute study evaluated 48 cases treated weekly median 8 Gy (range, 4-10) with total dose of 24 Gy (range, 16-40). The only factor significantly associated with longer response was the duration of clinical signs for ≥90 days before diagnosis. The median response duration was 80 days in patients <90 days of clinical signs duration versus 150 days in ≥90 days of clinical signs duration (P=0.001) (Gieger et al., 2008).

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Of 9 patients in non-RT group that received chemotherapy, most of them received carboplatin (n=6) and doxorubicin (n=3). Clinical improvement was noted in 5 out of 9 patients and offered median overall survival time 215±10.4 (95% CI, 194.5-235.5) days (range, 59-579 days). The result from our study is comparable to previous study used chemotherapeutic agents such as cisplatin alone (Hahn et al., 1992) or the combination of doxorubicin and carboplatin (Langova et al., 2004) that offered MST about 5 months (range, 2-32 months) and 7 months, respectively. Larger case number and control group are warranted to establish the benefit from chemotherapy.

2 out of 8 patients that received palliative oral medications had clinical improvement and provided median overall survival 38±1.7 (95% CI, 34.8-41.2) days, respectively (range, 7-247 days). Compared the survival of patients received palliative oral medication with no treatment in our study, there was no significant difference and P=0.746. The result from our study is similar to previous studies that MST reported about 3.1 to 3.5 months (Mason et al., 2013; Rassnick et al., 2006). This indicated that palliative medical treatment (NSAID, steroid, antibiotics, analgesics) offered little benefit in survival.

When evaluated the factors that might affect treatment response assessed through post treatment CT scan after radiation therapy, there was no statistically significant. Post radiation therapy CT scan for treatment response assessment was arranged 13-48 days (median, 21 days) after last radiation treatment. 10 out of 15 patient had response (complete and partial remission) to radiation therapy and other patients were stable disease. The time interval between last radiation treatment and follow up CT scan may be too short to assess treatment response since some of the tumor still shrinking at the time CT was performed. Therefore, the response rate of our study may be underestimated.

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There was no significant difference of PFI and OS Kaplan-Meier curve in RT group patients when analyzing the relationship with clinical stage (III V.s IV), and P value was 0.052 and 0.102, respectively. When further group the patients in to stage IV and stage I to III, analyze this variable by using Cox proportional hazards model, and there was significant difference in hazard ratio (HR) of PFI and OS. HR of PFI was 2.620 (95% CI 1.158-5.927), and P value was 0.021. HR of OS was 2.835 (95% CI 1.231-6.531), and P value was 0.014. However, either PFI or OS of clinical stage lose statistically significance in multivariable analysis and P value was 0.154 and 0.086, respectively. Four major staging system of canine nasal tumor were proposed (Adams et al., 2009; Adams et al., 1998; Owen, 1980; Theon et al., 1993) and applied in different studies. In present study, modified Adams staging system (Adams et al., 2009) was applied to stage the patient and 18 out of 35 (51.4%) were classified stage IV as cribriform was involved. 11 out of 35 (31.4%) are stage III, and late stage patients (stage III and IV) accounted for 82.8% of all patients.

Staging system of canine nasal tumor is still in conflict that the value of predicting prognosis is not solid. Some studies suggest staging system is able to offer more information about survival. Previous studies indicated either Adams' stage system is related to survival (P=0.046) and nearly for relapse free interval (P=0.059), or when combine Adams' stage I and II compared with III and IV showed statistically different in progression or relapse free interval and median survival time (Adams et al., 1998; Buchholz et al., 2009). While other studies fail to show significant difference in survival by using stage system proposed by Theon and Adams (Adams et al., 2005; Kubicek et al., 2016; Lana et al., 2004; Maruo et al., 2011; Mellanby et al., 2002).

Facial deformity was the only variable of clinical signs that achieve statistically significant in hazard ratio of PFI (P=0.006) and OS (P=0.004). HR was

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3.414 (95% CI, 1.431-8.145) and 3.239 (95% CI, 1.470-7.140), respectively. Facial deformity remained statistically significant (P=0.012) in multivariable analysis of PFI and HR was 3.741 (95% CI, 1.330-10.524). Facial deformity is a clinical manifestation that often indicates malignancy (Lobetti, 2009; Strasser and Hawkins, 2005). Previous study indicated that facial deformity patients had shorter median survival time and may due to the limitation of orthovoltage radiation that had differential absorption in tissues of different density and poor penetration (Northrup et al., 2001). However, facial deformity failed to predict treatment outcomes in other studies (Buchholz et al., 2009; Maruo et al., 2011).

Although most of our patients in present study were advanced stage (82.8% are stage III and IV), our treatment outcome was comparable with previous studies of radiation therapy or chemotherapy in canine nasal tumor patients even though lower ratio of late stage patients were presented in most of the studies. When analyze either radiation therapy or chemotherapy would affect clinical outcome by using Cox proportional hazards model, only received radiation therapy or not had significant difference in PFI and OS, and P value was 0.004 and 0.002, respectively. Patients receiving radiation therapy had significant lower hazard ratio of 0.309 (95% CI, 0.138-0.689) in PFI and 0.273 (95% CI, 0.119-0.624) in OS. Received radiation therapy or not remained statistically significant in multivariable analysis of PFI (P=0.014) and OS (P=0.005). HR was 0.347 (95% CI, 0.148-0.810) and 0.259 (95% CI, 0.102-0.661), respectively. Patients in non-RT group that received chemotherapy had significant longer OS than those only received palliative oral medication in Kaplan-Meier analysis (P=0.002). These results indicate that either receiving radiation therapy or chemotherapy can improve survival outcome in canine nasal tumor patients.

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5.3 Treatment failure and retreatment

Although most patients of nasal tumor will respond to radiation therapy, which may relieve the clinical signs, most of them still have a poor prognosis. Failure of local control is the main reason that the patients expired or be euthanized. Treatment failure in our study may due to more late-stage patients than in previous studies, fractionation schedule that could favor repopulation of tumors with shorter potential doubling time (potential doubling time about 5 days in human head and neck cancer) (Begg et al., 1992), and positioning error that could reduce tumor control probability.

Major dose-limiting factor is the radiation tolerance of normal tissue that is related to the ability of normal tissue sparing. By the advanced technology, a reasonable approach to improve treatment outcome through a more conformal radiation therapy that allowed dose escalation and sparing normal tissue damage. While advanced technique makes radiation therapy more conformal and precisely, these may cause miss target or normal tissue damage once position error occurred. A study retrospectively used IMRT planning system replanned 10 canine nasal tumor patients that were previously treated by helical tomotherapy. Daily setup shift were recorded by on board image system and applied to IMRT plans. Mean setup error magnitude in any single dimension was at least 2.5mm, and mean composite offset vector was 5.9±3.3mm. A loss of equivalent uniform dose for target volumes of up to 5.6% was noted which corresponded to a potential loss in tumor control probability of 39.5% (Deveau et al., 2010).

In order to improve treatment outcome, boost and shrink technique were utilized in previous studies. However, these techniques not only cause more severe

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acute toxicity, but also have no benefit in prolonging survival. The result from this study indicated that dose escalation of definitive radiation therapy was unable to improve treatment outcome but induced more side effects (LaDue et al., 1999; Thrall et al., 1993).

When failed in local control, reirradiation may be an option to prolong patients' survival. In a recent study that reviewed 9 patients of canine nasal neoplasia received second radiation therapy after the tumor recurred. The median dose delivered in the first and second radiation therapy was 50 Gy (range, 44-55) and 36 Gy (23-44), respectively. Median time to progression after the first and second courses was 513 days (95% CI 234-1180 days) and 282 days (95% CI 130-453 days), respectively. There was no significant difference in median time to progression between the first and second treatment. The median survival time was 927 days. Although acute toxicity caused by the first and second radiation therapy was mild and no significant difference, there was 2 patients experienced severe late toxicity (Bommarito et al., 2010). Furthermore, a case report of a brachycephalic dog that received three courses of radiation therapy in order to manage recurred nasal tumors. Total dose was 117 Gy given to the tumor by using intensity modulated radiation therapy (IMRT). The side effect including self-limited inappetence, anorexia, diarrhea, and clinical signs consistent with esophagitis. Bilateral diminish tear production developed near the end of life. The patient expired due to metastatic disease 694 days after completion of the first radiation therapy course. IMRT appeared to improve the patient's quality of life while spare severe side effects of normal tissue, and also prolong survival (Rancilio et al., 2016). In a retrospective study, median survival time for 8 dogs that received multiple radiation protocols was 654 days and this was significantly longer than the median survival time of 356 days for the 86 dogs that received one protocol of

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radiation therapy with no adjuvant therapy (P = 0.042) (Sones et al., 2012). The result from these studies indicated that reirradiation might be a treatment option of recurred canine nasal tumors.

Besides IMRT, other techniques use “on-board” imaging to correct positioning errors such as image-guide radiation therapy (IGRT) and stereotactic radiation surgery (SRS) are more available in veterinary medicine. Such techniques allow extremely precise dose delivery perhaps benefits in treating well-defined tumor near to complex surrounding organ at risk. 57 canine nasal tumor patients were treated with SRS with the median dose of 30 Gy (range, 18.57-56 Gy). MST of these patients was 8.5 months (Kubicek et al., 2016). Treatment planning did not contour either clinical target volume or planning target volume (possible microscopic extension, patient motion or setup uncertainty) (Kubicek et al., 2016), and may underestimate the extent of disease and lead to geographic misses since most recurrence occurs within the PTV volume (Thrall et al., 1993). More studies about advanced radiation procedure are warranted to elaborate different treatment outcome in canine nasal tumor patients.

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5.4 Limitation of this study

Due to the nature of retrospective study, data was retrieved from our medical records that may lead to several limitations. Duration of clinical signs before first visit obtained either from medical record or referral veterinary that was evaluated in different basis. Underestimating clinical signs based on medical records may also cause bias in data analysis.

Radiation therapy and post-treatment follow up protocol were not unified. Treatment response evaluation CT scan was close to last treatment that may under estimate efficacy of radiation therapy. Evaluation of acute and late radiation side effects by different clinicians and lack of standardized questionnaire to investigate clinical improvement in order to quantified treatment outcome. Rather than cross-sectional imaging such as CT or MRI, evaluation of tumor progression in most of our patients were according to clinical signs or radiograph that that is either not sensitive enough or may cause false positives, such as rhinitis that mimics clinical signs of tumor recurrence.

More advanced stage patients in present study that may different from clinical nature. Epithelial origin tumors were account for about 80% in our study and squamous cell carcinoma patients were all in non-RT group that may have influence in treatment outcome. Small sample size with non-uniformed treatment modalities limited data analysis and significant differences to be demonstrated.

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Chapter 6 Conclusion

This is the first IMRT study delivers weekly fractionated radiation therapy in canine nasal tumor patients. From the result of present study where most patients were in late stage, although local recurrence is still the most common cause of death, a durable progression free interval and comparable survival was obtained from our radiation treatment procedure.

In RT group, all patients had clinical improvement after receiving radiation therapy and they either had positive response to radiation therapy (66.7%) or remain stable disease. Progression free interval and overall survival were significant longer in RT group when compared with non-RT group. Acute effects and late effects were self-limited and manageable in most of our patients. Progression free interval and overall survival in non-RT-chemo group was not significant different from RT group but offered some benefit when compared with those patients that only received palliative oral medications. This result may indicate that if radiation therapy is not an amenable option, traditional chemotherapy can still offer some benefit to canine nasal tumor patients.

Duo to the limitations aforementioned of present study, larger case number prospective study with standard operation procedure is encouraged to further demonstrate the benefit of clinical outcome from this radiation treatment strategy, combination of different treatment modalities, and advanced radiation therapy techniques in canine nasal tumor patients.

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Tables

Table 1. VCOG-CTCAE v1.1 grading system, 2011.

	I	II	III	IV	V
Anorexia	Coaxing or dietary change required to maintain appetite.	Oral intake altered (≤3 days) without significant weight loss; oral nutritional supplements/appetite stimulants may be indicated.	Of >3 days duration; associated with significant weight loss (≥10%) or malnutrition; IV fluids, tube feeding or force feeding indicated.	Life-threatening consequences; TPN indicated; >5 days duration.	Death
Lethargy	Mild lethargy over baseline; diminished activity from pre-disease level, but able to function as an acceptable pet.	Moderate lethargy causing some difficulty with performing ADL; ambulatory only to the point of eating, sleeping and consistently defecating and urinating in acceptable areas.	Compromised, severely restricted in ADL; unable to confine urinations and defecation to acceptable areas; will consume food if offered in place.	Disabled, must be force fed and helped to perform ADL.	Death
Neutropenia	1500/μL to <LLN	1000-1499/μL	500-999/μL	<500/μL	Death
Vomit	<3 episodes in 24 h, medical intervention not indicated.	3-10 episodes in 24 h; <5 episodes/day for ≤48 h; parenteral fluids (IV or SC) indicated ≤48 h; medications indicated.	Multiple episodes >48 h and IV fluids or PPN/TPN indicated >48 h.	Life-threatening (e.g. hemodynamic collapse).	Death

LLN: lower limit of normal.

ADL: activities of daily living (eating, sleeping, defecating and urinating).

(Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events (VCOG-CTCAE) Following Chemotherapy or Biological Antineoplastic Therapy in Dogs and Cats V1.1., 2011)

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Table 2. VRTOG Acute Radiation Morbidity Scoring Scheme.

Organ/Tissue	**0**	**1**	**2**	**3**
Skin/hair	no change over baseline	erythema, dry desquamation, alopecia/epilation	patchy moist desquamation without edema	confluent moist desquamation with edema and/or ulceration, necrosis, hemorrhage
Mucus membrane	no change over baseline	injection without mucositis	patchy mucositis with patient seemingly painfree	confluent fibrinous mucositis necessitating analgesia, ulceration, hemorrhage, necrosis
Eye	no change over baseline	mild conjunctivitis and/or scleral injection	KCS requiring artificial tears, moderate conjunctivitis or iritis necessitating therapy	severe keratitis with corneal ulceration and/or loss of vision, glaucoma

(LaDue and Klein, 2001)

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Table 3. VRTOG Late Radiation Morbidity Scoring Scheme.

Organ/Tissue	**0**	**1**	**2**	**3**
Skin/hair	none	alopecia, hyperpigmentation, leukotrichia	asymptomatic induration (fibrosis)	severe induration causing physical impairment, necrosis
CNS	none	mild neurologic signs not necessitating more than prednisone therapy	neurologic signs necessitating more than prednisone therapy	seizures, paralysis, coma
Eye	none	asymptomatic cataracts, KCS	symptomatic cataracts. keratitis, corneal ulceration, minor retinopathy, mild to moderate glaucoma	panophthalmitis, blindness, severe glaucoma, retinal detachment
Bone	none	pain on palpation	radiographic changes	necrosis

(LaDue and Klein, 2001)

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Table 4. Modified Adams' stage system.

Stage	Criteria
I	Confined to one nasal passage, paranasal sinus or frontal sinus, with no bone involvement beyond turbinates.
II	Any bony involvement (beyond turbinates), but with no evidence of orbit/subcutaneous/submucosal mass.
III	Orbit involved or nasopharyngeal or subcutaneous or submucosal mass.
IV	Tumor causing lysis of the cribriform plate.

(Adams et al., 2009)

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Table 5. Summary of chemotherapy adverse effect.

	Anorexia Grade (n)	**Lethargy** Grade (n)	**Hematology** Grade (n)	**GI** Grade (n)
Non-RT group (n=9)	I (4)	I (3)	I (1), II (1)	I (2)
RT group (n=6)	I (2), II (1)	I (2), II (1)	I (2)	I (2)

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Table 6. Summary of radiation therapy adverse effect.

	Acute				Late		
	Skin Grade (n)	**Mucus membrane** Grade (n)	**Ocular** Grade (n)	**Skin/hair** Grade (n)	**CNS** Grade (n)	**Ocular** Grade (n)	**Bone** Grade (n)
RT group (n=15)	I (12), II (1)	I (7), II (4)	I (9), II (1), III (1)	I (14)	0	I (7), II (1), III (1)	0

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Table 7. Summary the variables evaluated for clinical improvement in non-RT group.

	n	P
Clinical stage [a]		0.987
II	2	
III	4	
IV	9	
Histopathology [b]		0.323
Carcinoma/adenocarcinoma	14	
Others	3	
Histopathology [b]		0.059
SCC	7	
Others	10	
Duration of clinical signs [bc]		0.772
≤ 45 days	9	
> 45 days	8	
Treatment		0.162
Chemotherapy	9	
Palliative oral medication	8	
No treatment	3	

[a] Exclude 2 patients without staging and 3 patients without treatment.

[b] Exclude 3 patients without treatment.

[c] Median duration of clinical signs is 45 days.

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Table 8. Summary the variables evaluated for PFI in non-RT group.

	n	PFI, median (range)	P
Clinical stage [b]			0.298, 0.176, 0.893 [a]
II	2	162 (0-324)	
III	5	0 (0-186)	
IV	11	0 (0-205)	
Histopathology			0.899
Carcinoma/adenocarcinoma	16	0 (0-324)	
Others	4	6 (0-186)	
Histopathology			0.923
SCC	7	0 (0-324)	
Others	13	0 (0-205)	
Duration of clinical signs [c]			0.059
≤ 41.5 days	10	0 (0-52)	
> 41.5 days	10	8.5 (0-324)	
Treatment			0.073, 0.106, 0.361 [d]
Chemotherapy	9	17 (0-324)	
Palliative oral medication	8	0 (0-52)	
No treatment	3	0 (0)	

[a] II to III, II to IV, and III to IV, respectively.

[b] Exclude 2 patients without staging.

[c] Median duration of clinical signs is 41.5 days.

[d] Chemotherapy to Palliative oral medication, chemotherapy to no treatment, and no treatment to palliative oral medication, respectively

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Table 9. Summary the variables evaluated for OS in non-RT group.

	n	OS, median (range)	P
Clinical stage [b]			0.765, 0.132, 0.352 [a]
II	2	296.5 (192-401)	
III	5	135 (38-579)	
IV	11	59 (13-290)	
Histopathology			0.321
Carcinoma/adenocarcinoma	16	130.5 (7-401)	
Others	4	140.5 (38-579)	
Histopathology			0.446
SCC	7	126 (7-401)	
Others	13	135 (37-579)	
Duration of clinical signs [c]			0.065
≤ 41.5 days	10	74 (13-257)	
> 41.5 days	10	211.5 (7-579)	
Treatment			0.006, 0.002, 0.746 [d]
Chemotherapy	9	215 (59-579)	
Palliative oral medication	8	38.5 (7-257)	
No treatment	3	38 (38-108)	

[a] II to III, II to IV, and III to IV, respectively.

[b] Exclude 2 patients without staging.

[c] Median duration of clinical signs is 41.5 days.

[d] Chemotherapy to Palliative oral medication, chemotherapy to no treatment, and no treatment to palliative oral medication, respectively

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Table 10. Summary the variables evaluated for treatment response in RT group.

	n	P
Clinical stage		0.435
I	1	
II	1	
III	6	
IV	7	
Histopathology [a]		0.211
Carcinoma/adenocarcinoma	11	
Others	3	
Duration of clinical signs [b]		0.87
≤ 95 days	9	
> 95 days	6	
Treatment		0.87
Chemotherapy	6	
No chemotherapy	9	

[a] Exclude 1 patient without histopathologic diagnosis.

[b] Median duration of clinical signs is 95 days.

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Table 11. Summary the variables evaluated for PFI in RT group.

	n	PFI, median (range)	P
Clinical stage [b]			0.052 [a]
I	1	200	
II	1	1426	
III	5	212 (145-1020)	
IV	6	81.5 (23-308)	
Histopathology [bd]			0.822
Carcinoma/adenocarcinoma	10	154.5 (23-1426)	
Others	2	260 (212-308)	
Duration of clinical signs [bc]			0.422
≤ 95 days	8	174.5 (23-1426)	
> 95 days	5	160 (75-308)	
Treatment [b]			0.324
Chemotherapy	6	200 (23-1426)	
No chemotherapy	7	154.5 (75-308)	

[a] III to IV.

[b] Exclude 2 patients expired not related to tumor and before tumor progression.

[c] Median duration of clinical signs is 95 days.

[d] Exclude 1 patient that did not have histopathologic diagnosis.

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Table 12. Summary the variables evaluated for OS in RT group.

	n	OS, median (range)	P
Clinical stage [b]			0.102 [a]
I	1	329	
II	1	1619	
III	4	635.5 (296-1020)	
IV	6	356.5 (80-485)	
Histopathology [bd]			0.729
Carcinoma/adenocarcinoma	9	372 (90-1619)	
Others	2	383.5 (372-395)	
Duration of clinical signs [bc]			0.497
≤ 95 days	8	407 (80-1619)	
> 95 days	4	372 (341-395)	
Treatment [b]			0.414
Chemotherapy	5	372 (296-485)	
No chemotherapy	7	372 (80-1619)	

[a] III to IV.

[b] Exclude 3 patients expired not related to tumor.

[c] Median duration of clinical signs is 95 days.

[d] Exclude 1 patient that did not have histopathologic diagnosis.

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Table 13. Comparison of patient characteristics in RT group and non-RT group.

	RT group	Non-RT group	P
Age (years)			0.587
Median (range)	10 (6-13)	10 (7-13)	
Sex			0.934
Male	10 (66.7%)	13 (65%)	
Female	5 (33.3%)	7 (35%)	
Body weight (kg)			0.074
Median (range)	13.1 (2.36- 32.8)	15.5 (3.42-46.5)	
Histopathologic diagnosis			0.959
Carcinoma/adenocarcinoma	11 (73.3%)	16 (80%)	
Others	3 (20%)	4 (20%)	
Clinical signs			
Sneezing	8 (53.3%)	9 (45%)	0.681
Nasal discharge	8 (53.3%)	13 (65%)	0.564
Epistaxis	11 (73.3%)	14 (70%)	0.882
Facial deformity	4 (26.7%)	11 (55%)	0.158
Duration of clinical signs (days)			0.012
Median (range)	95 (10-283)	41.5 (3-148)	
Stage			0.486
I	1 (6.7%)	0	
II	1 (6.7%)	2 (10%)	
III	6 (40%)	5 (25%)	
IV	7 (46.6%)	11 (55%)	
Chemotherapy	6 (40%)	9 (45%)	0.805

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Table 14. Univariable analysis of PFI.

		PFI		
	N	HR	95%CI	P
Age (years)		0.975	0.470-2.022	0.946
≤ 10 years old	20			
> 10 years old	13			
Sex		0.772	0.359-1.661	0.508
Male	22			
Female	11			
Histopathologic diagnosis		0.990	0.399-2.455	0.982
Carcinoma/adenocarcinoma	26			
Others	6			
Sneezing		1.030	0.507-2.090	0.936
No	16			
Yes	17			
Nasal discharge		1.049	0.504-2.183	0.899
No	13			
Yes	20			
Epistaxis		0.755	0.350-1.628	0.473
No	10			
Yes	23			
Facial deformity		3.414	1.431-8.145	0.006
No	18			
Yes	15			
Stage		2.620	1.158-5.927	0.021
I-III	14			
IV	17			
Treatment		0.309	0.138-0.689	0.004
No radiation therapy	20			
Radiation therapy	13			
Treatment		0.954	0.461-1.973	0.898
No chemotherapy	18			
Chemotherapy	15			

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Table 15. Univariable analysis of OS.

		OS		
	N	HR	95%CI	P
Age (years)		1.270	0.610-2.644	0.524
≤ 10 years old	19			
> 10 years old	13			
Sex		0.793	0.336-1.719	0.556
Male	21			
Female	11			
Histopathologic diagnosis		0.923	0.372-2.288	0.863
Carcinoma/adenocarcinoma	25			
Others	6			
Sneezing		1.029	0.5-2.121	0.937
No	15			
Yes	17			
Nasal discharge		1.091	0.515-2.309	0.821
No	12			
Yes	20			
Epistaxis		0.554	0.250-1.230	0.147
No	9			
Yes	23			
Facial deformity		3.239	1.470-7.140	0.004
No	18			
Yes	14			
Stage		2.835	1.231-6.531	0.014
I-III	13			
IV	17			
Treatment		0.273	0.119-0.624	0.002
No radiation therapy	20			
Radiation therapy	12			
Treatment		0.763	0.364-1.599	0.474
No chemotherapy	18			
Chemotherapy	14			

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Table 16. Multivariable analysis of PFI.

		PFI		
	N	HR	95%CI	P
Facial deformity		3.741	1.330-10.524	0.012
No	16			
Yes	15			
Stage		1.871	0.791-4.423	0.154
I-III	14			
IV	17			
Treatment		0.347	0.148-0.810	0.014
No radiation therapy	18			
Radiation therapy	13			

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Table 17. Multivariable analysis of OS.

		OS		
	N	HR	95%CI	P
Facial deformity		2.028	0.743-5.532	0.167
No	16			
Yes	14			
Stage		2.450	0.881-6.812	0.086
I-III	13			
IV	17			
Treatment		0.259	0.102-0.661	0.005
No radiation therapy	18			
Radiation therapy	12			

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Table 18. Compare with other intensity modulated radiation therapy studies.

Author	n	RT type	Dose per fraction (Gy)	Total dose (Gy)	BED_3 (Gy)	BED_{10} (Gy)	MST (day)	1 year survive %	2 year survive %
(Lawrence et al., 2010)	31	Tomo/6 MV LA	4.2, daily	42	100.8	59.64	420	NA	NA
(Hunley et al., 2010)	5	IMRT	3, MWF	54	108	70.2	446	50	25
	7		3, MWF	63	126	81.9			
This study	15	4 MV or 6 MV IMRT	8 (7-15), weekly	35 (30-42.5)	117.3	59.5	372	58.3	25

NA: no analysis
Tomo: tomotherapy
LA: linear accelerator
IMRT: intensity modulated radiation therapy
MWF: Monday-Wednesday-Friday
BED_3: biologically effective dose, $\alpha/\beta=3$
BED_{10}: biologically effective dose, $\alpha/\beta=10$

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Table 19. Compare with other hypofractionated radiation therapy studies.

Author	**n**	**RT type**	**Dose per fraction (Gy)** median (range)	**Total dose (Gy)** median (range)	**BED_3 (Gy)**	**BED_{10} (Gy)**	**MST (day)**	**1 year survive %**	**2 year survive %**
(Mellanby et al., 2002)	56	4 MV LA	9	36	144	68.4	212	45	15
(Gieger et al., 2008)	48	Co, LA	8 (4-10)	24 (16-40)	88	43.2	146	25	9
(Buchholz et al., 2009)	38	6 MV LA	6 (3-8)	32 (24-30)	96	51.2	303	NA	NA
(Belshaw et al., 2011)	42	4 MV LA	9 (8.5-9)	34-36	144	68.4	201	NA	NA
(Maruo et al., 2011)	63	4 MV LA	8 (5-10)	32 (10-40)	117.3	57.6	197	25	8
(Fujiwara et al., 2013)	33	4 MV LA	8 (6-10)	32 (16.2-32.4)	117.3	57.6	512	62.4	43.4
This study	15	4 MV or 6 MV IMRT	8 (7-15)	35 (30-42.5)	117.3	59.5	372	58.3	25

NA: no analysis
Tomo: tomotherapy
LA: linear accelerator
IMRT: intensity modulated radiation therapy
MWF: Monday-Wednesday-Friday
BED_3: biologically effective dose, $\alpha/\beta=3$
BED_{10}: biologically effective dose, $\alpha/\beta=10$

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Figures

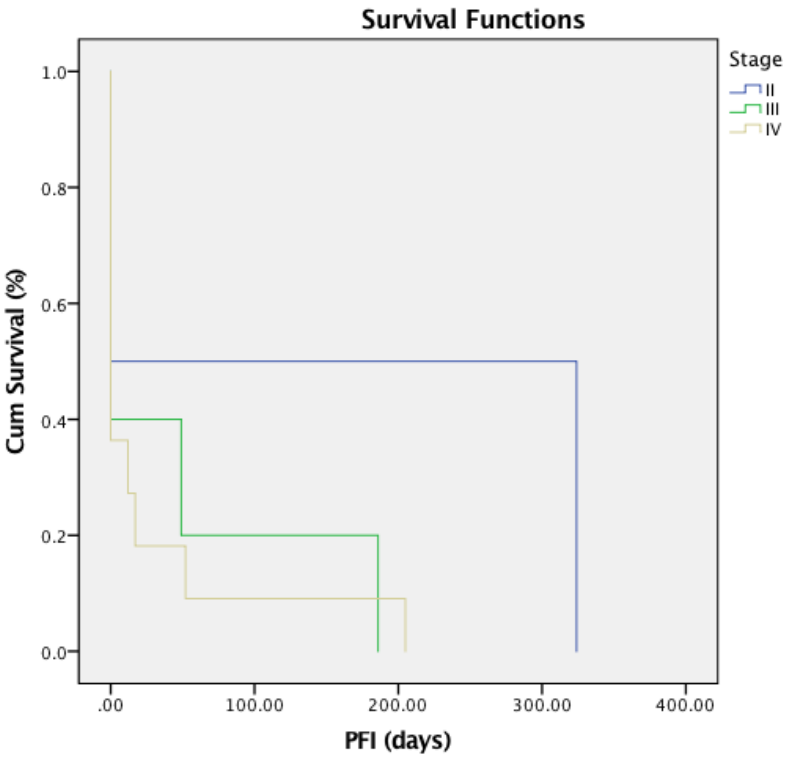

Fig. 1. Kaplan-Meier curves comparing PFI for different stage in non-RT group.

No significant difference between stage II (n=2), III (n=5), and IV (n=11). P=0.298, 0.176, 0.893 (II to III, II to IV, and III to IV, respectively)

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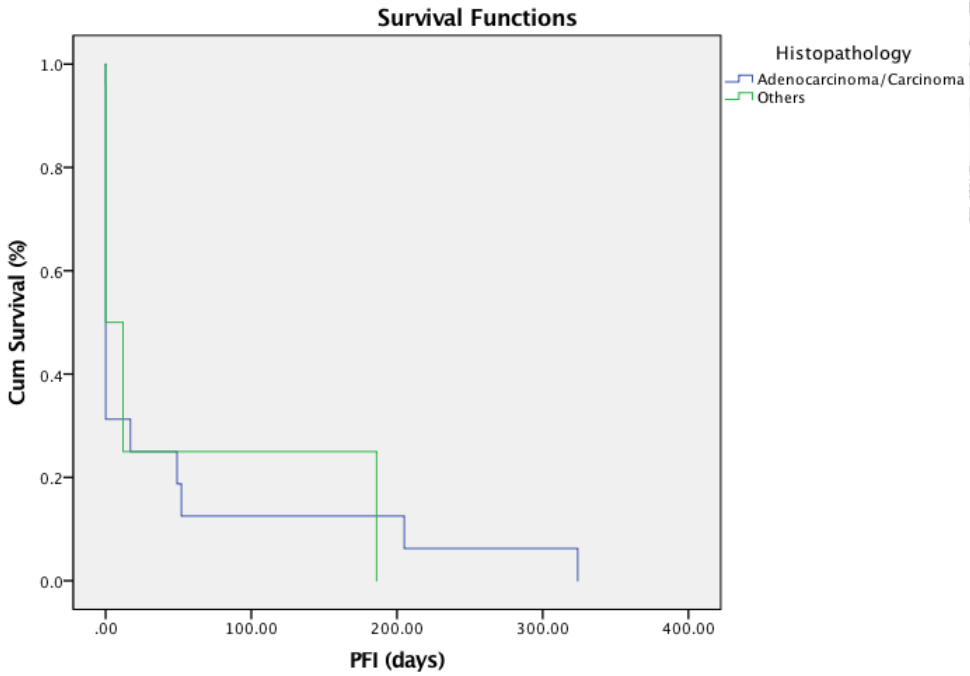

Fig. 2. Kaplan-Meier curves comparing PFI of different histopathologic diagnosis of adenocarcinoma/carcinoma or others in non-RT group.

No significant difference between adenocarcinoma/carcinoma (n=16) and others histopathologic diagnosis (n=4). P=0.899

doi: 10.6342/NTU201602570

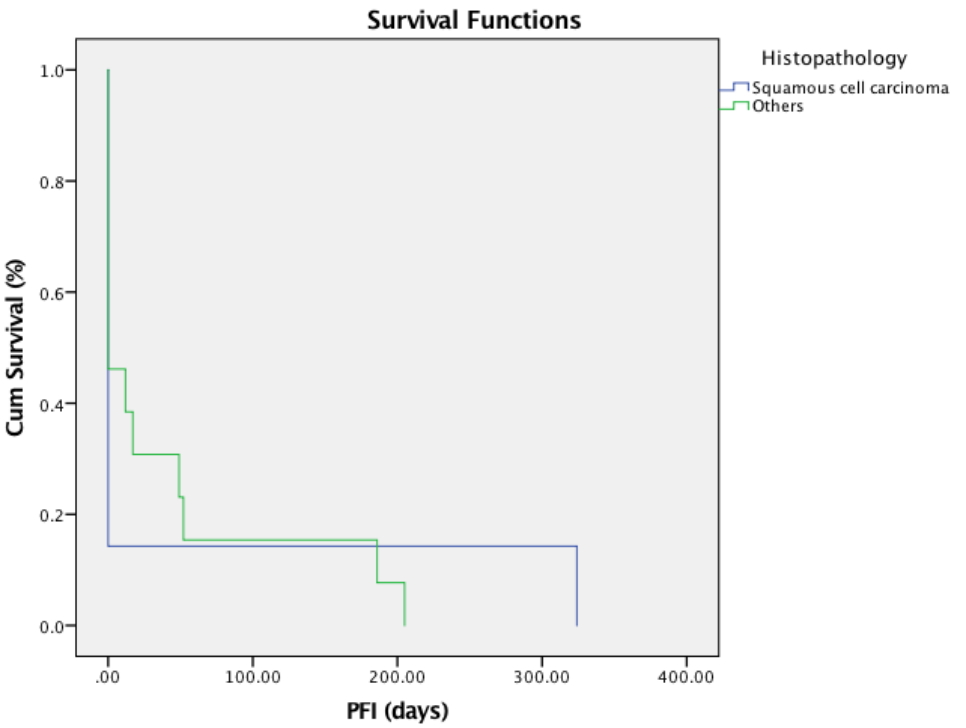

Fig. 3. Kaplan-Meier curves comparing PFI of different histopathologic diagnosis of squamous cell carcinoma or others in non-RT group.

No significant difference between squamous cell carcinoma (n=7) and others histopathologic diagnosis (n=13). P=0.923

doi: 10.6342/NTU201602570

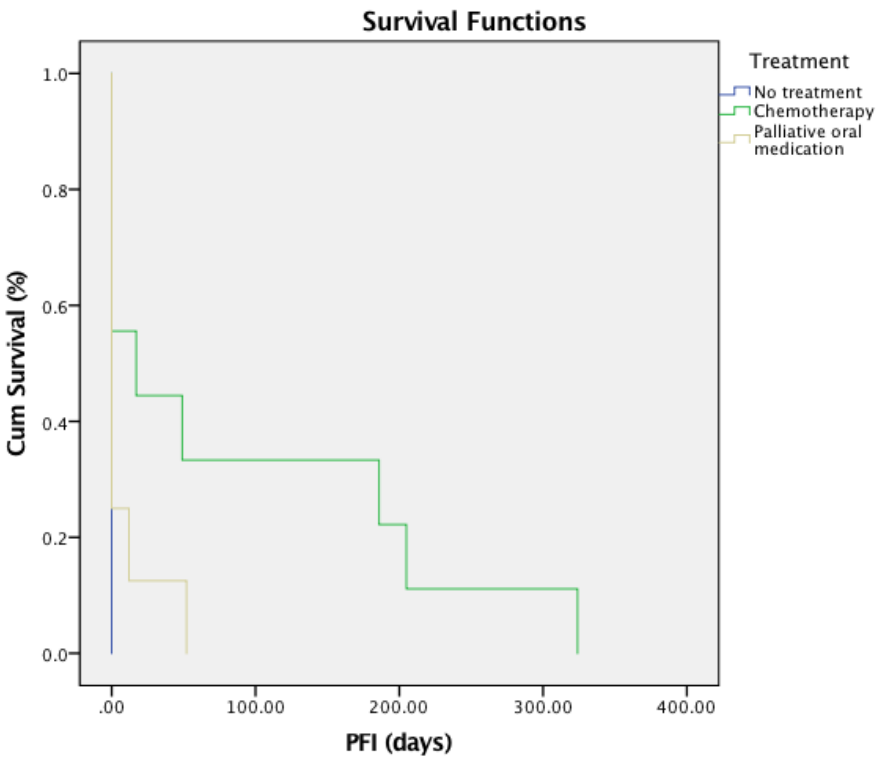

Fig. 4. Kaplan-Meier curves comparing PFI of receiving different treatments in non-RT group.

No significant difference between no treatment (n=3), chemotherapy (n=9) and palliative oral medication (n=8). P=0.073, 0.106, 0.361 (Chemotherapy to palliative oral medication, chemotherapy to no treatment, and no treatment to palliative oral medication, respectively)

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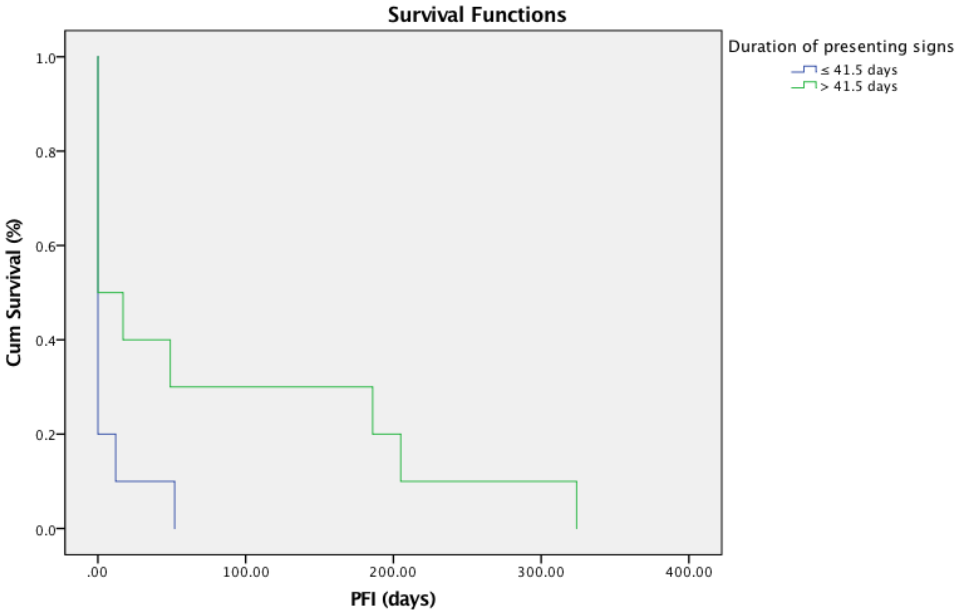

Fig. 5. Kaplan-Meier curves comparing PFI of different duration of presenting signs in non-RT group.

There was no significant difference in PFI of non-RT patients that duration of presenting signs ≤ 41.5 days (n=10) and >41.5 days (n=10). P=0.059

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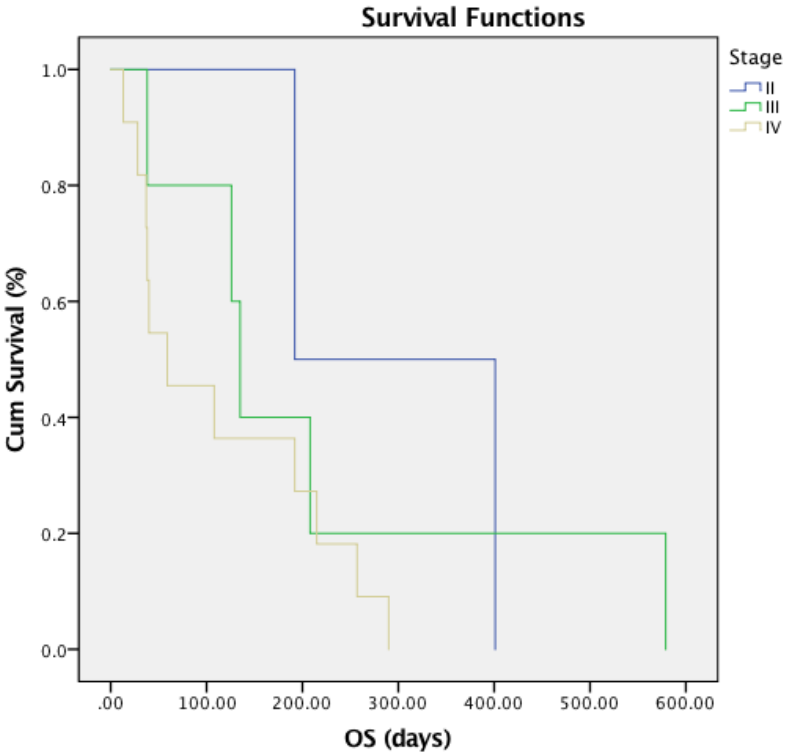

Fig. 6. Kaplan-Meier curves comparing OS for different stage in non-RT group.

No significant difference between stage II (n=2), III (n=5), and IV (n=11). P=0.765, 0.132, 0.352 (II to III, II to IV, and III to IV, respectively)

doi: 10.6342/NTU201602570

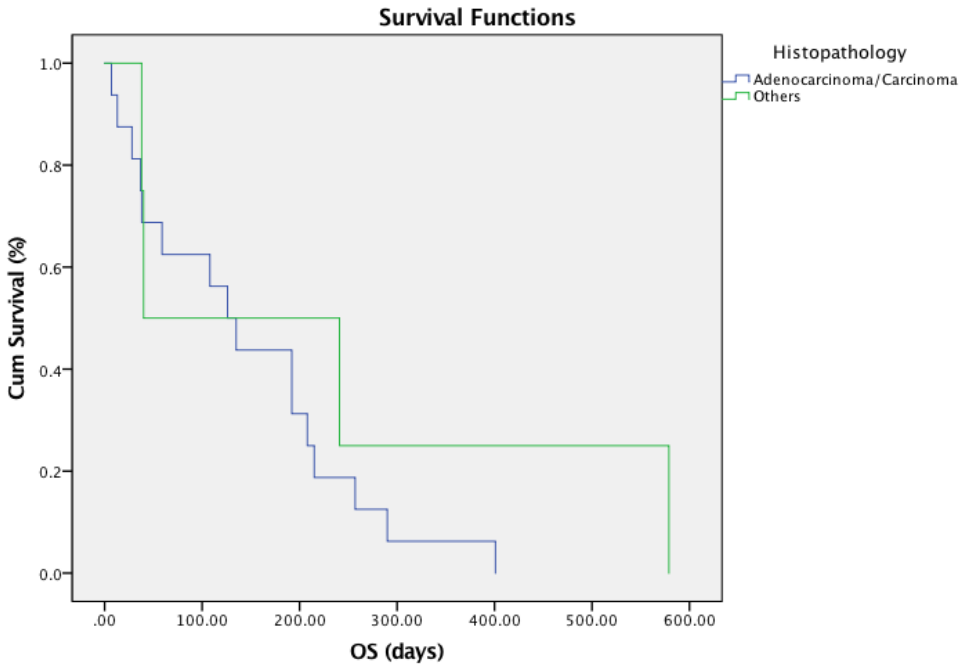

Fig. 7. Kaplan-Meier curves comparing OS of different histopathologic diagnosis of carcinoma/adenocarcinoma or others in non-RT group.

No significant difference between adenocarcinoma/carcinoma (n=16) and others histopathologic diagnosis (n=4). P=0.321

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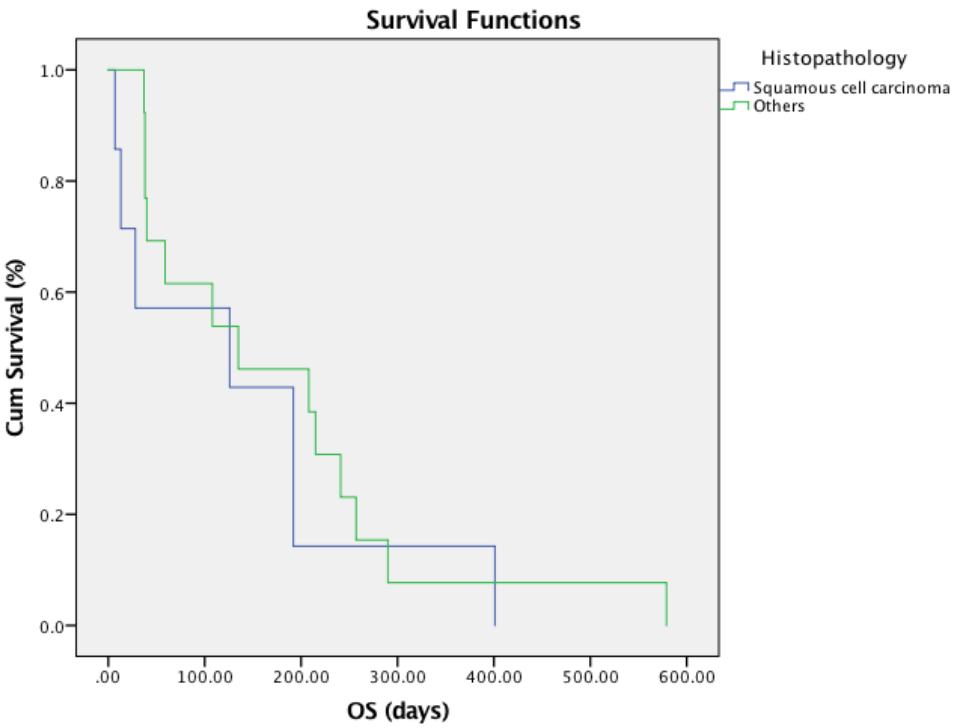

Fig. 8. Kaplan-Meier curves comparing OS of different histopathologic diagnosis of squamous cell carcinoma or others in non-RT group.

No significant difference between squamous cell carcinoma (n=7) and others histopathologic diagnosis (n=13). P=0.446

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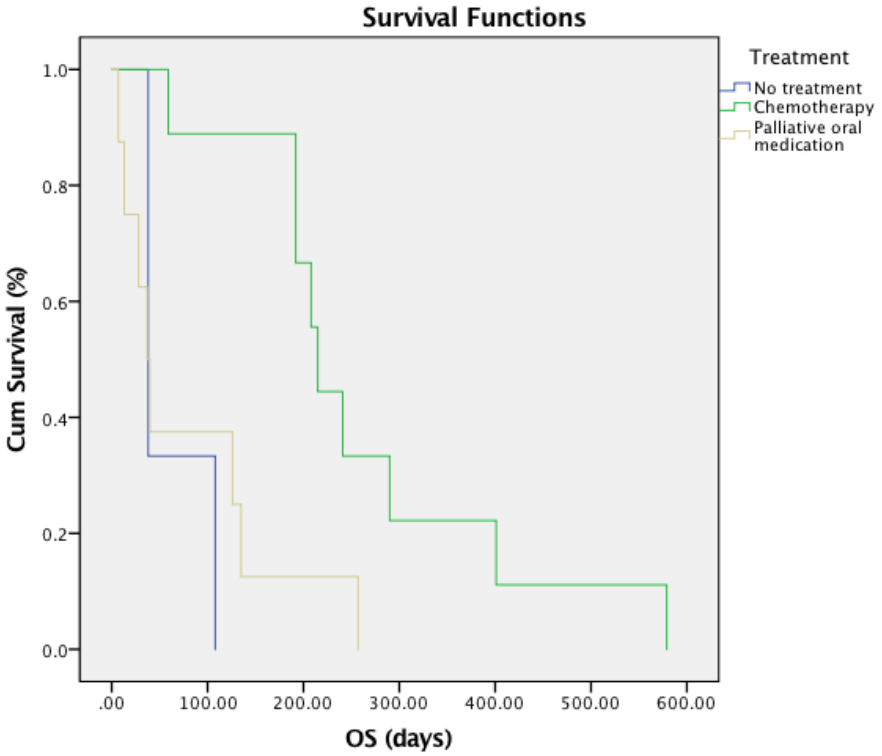

Fig. 9. Kaplan-Meier curves comparing OS of receiving different treatments in non-RT group.

There was significant difference in patients received chemotherapy (n=9) versus palliative oral medication (n=8), P=0.006 and versus no treatment (n=3), P=0.002. Compared palliative oral medication with no treatment, there was no significant difference and P=0.746.

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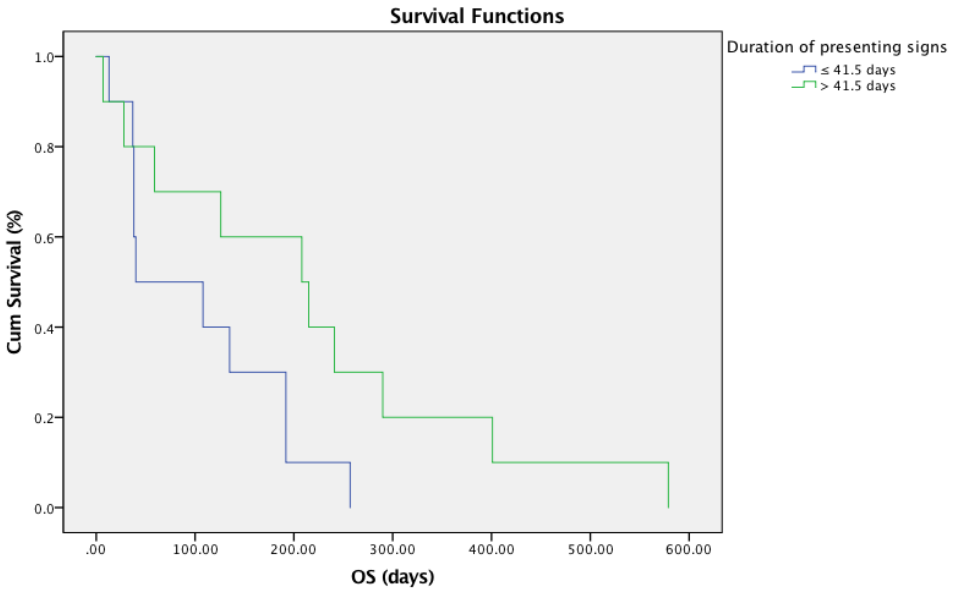

Fig. 10. Kaplan-Meier curves comparing OS of different duration of presenting signs in non-RT group.

There was no significant difference in OS of patients that duration of presenting signs ≤ 41.5 days (n=10) and >41.5 days (n=10). P=0.065

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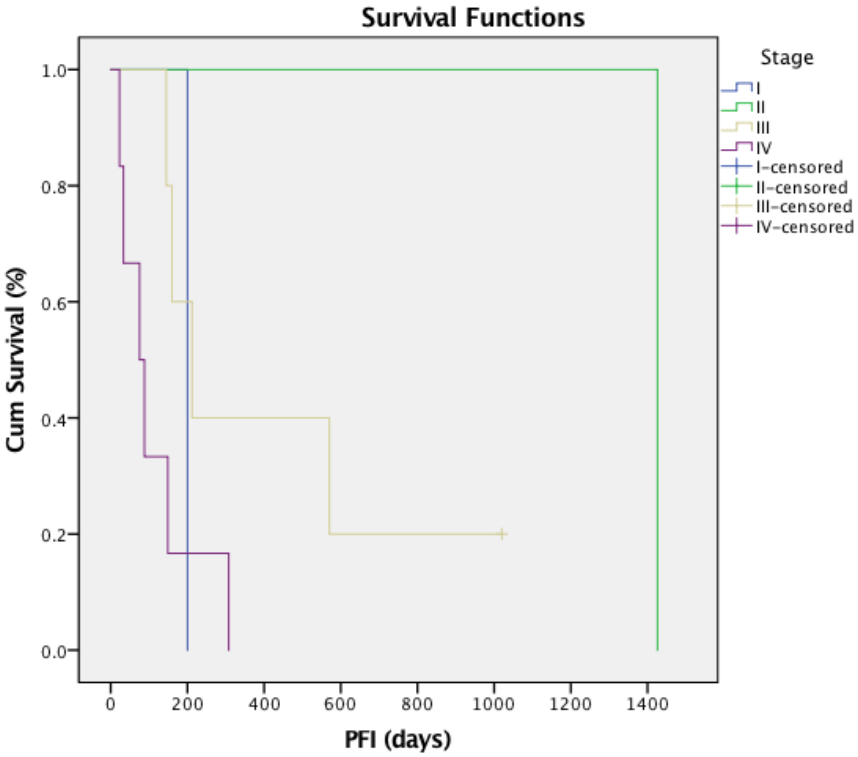

Fig. 11. Kaplan-Meier curves comparing PFI for different stage in RT group.

No significant difference between clinical stages in PFI. The result that nearly achieved significant difference was when compared clinical stage III with stage IV, and the P value was 0.052. Stage I, II, III, IV (n=1, 1, 5, 6, respectively)

doi: 10.6342/NTU201602570

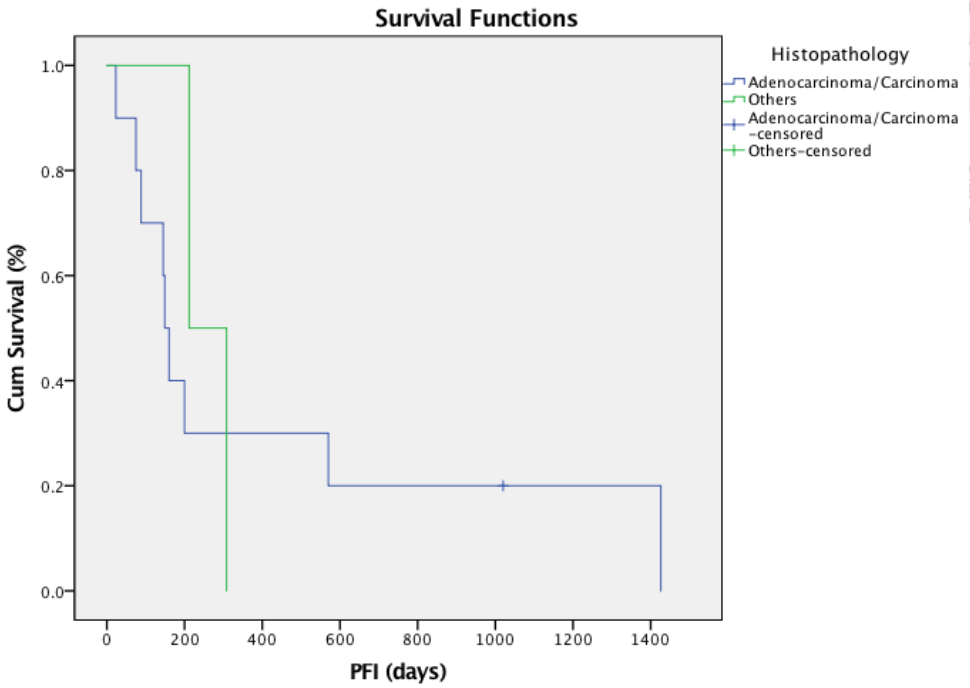

Fig. 12. Kaplan-Meier curves comparing PFI of different histopathologic diagnosis of carcinoma/adenocarcinoma or others in RT group.

No significant difference between adenocarcinoma/carcinoma (n=10) and others histopathologic diagnosis (n=2). P=0.822

doi: 10.6342/NTU201602570

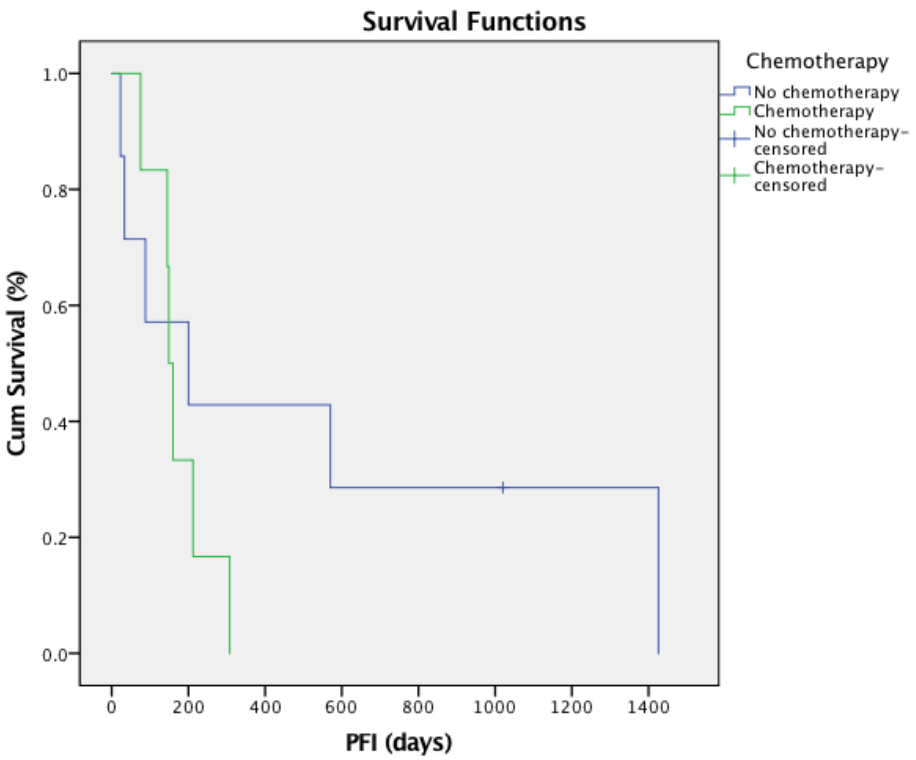

Fig. 13. Kaplan-Meier curves comparing PFI of receiving chemotherapy or not in RT group.

No significant difference between chemotherapy (n=6) and no chemotherapy (n=7). P=0.324.

doi: 10.6342/NTU201602570

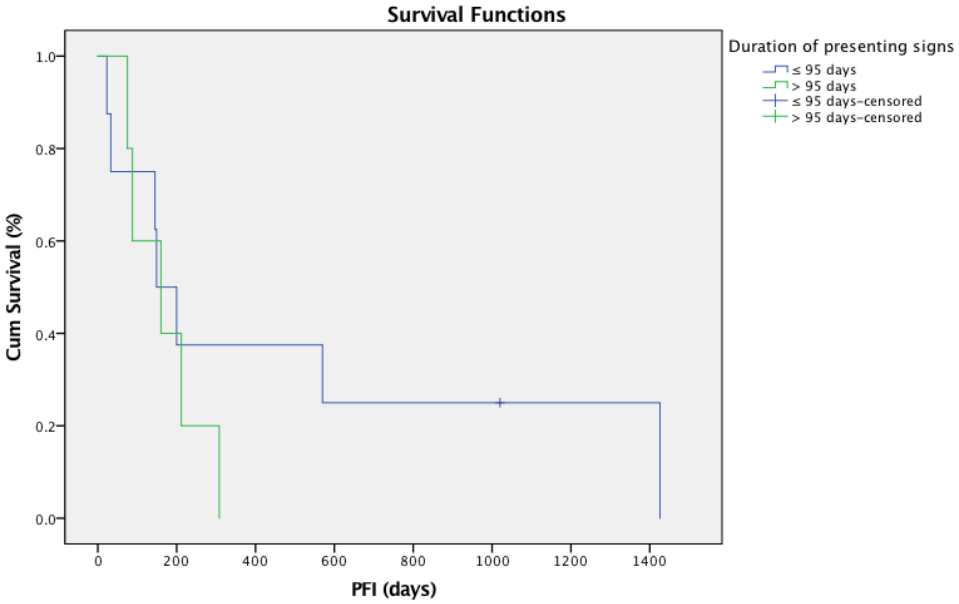

Fig. 14. Kaplan-Meier curves comparing PFI of different duration of presenting signs in RT group.

There was no significant difference in PFI of patients that duration of presenting signs ≤ 95 days (n=8) and >95 days (n=5). P=0.422

doi: 10.6342/NTU201602570

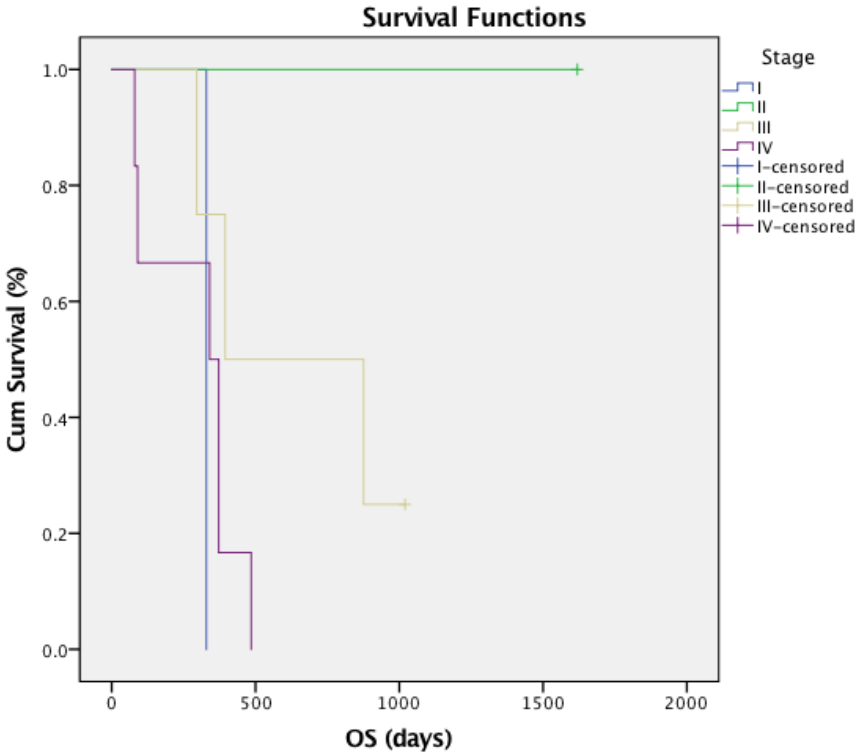

Fig. 15. Kaplan-Meier curves comparing OS for different stage in RT group.

No significant difference between clinical stages in OS. The result that nearly achieved significant difference was when compared clinical stage III with stage IV, and the P value was 0.102. Stage I, II, III, IV (n=1, 1, 4, 6, respectively)

doi: 10.6342/NTU201602570

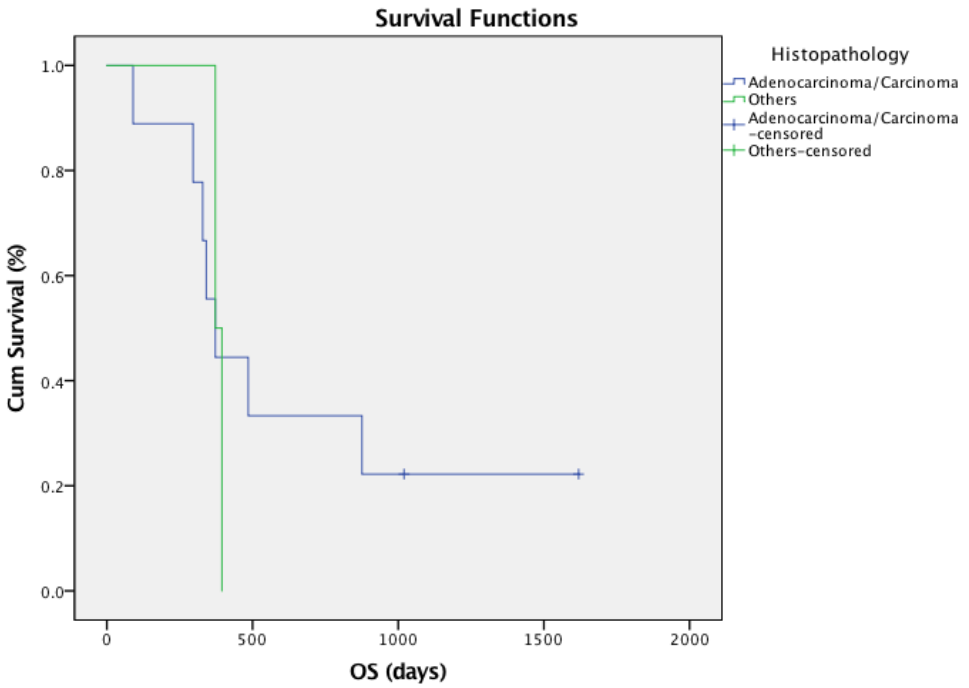

Fig. 16. Kaplan-Meier curves comparing OS of different histopathologic diagnosis of adenocarcinoma/carcinoma or others in RT group.

No significant difference between adenocarcinoma/carcinoma (n=9) and others histopathologic diagnosis (n=2). P=0.729

doi: 10.6342/NTU201602570

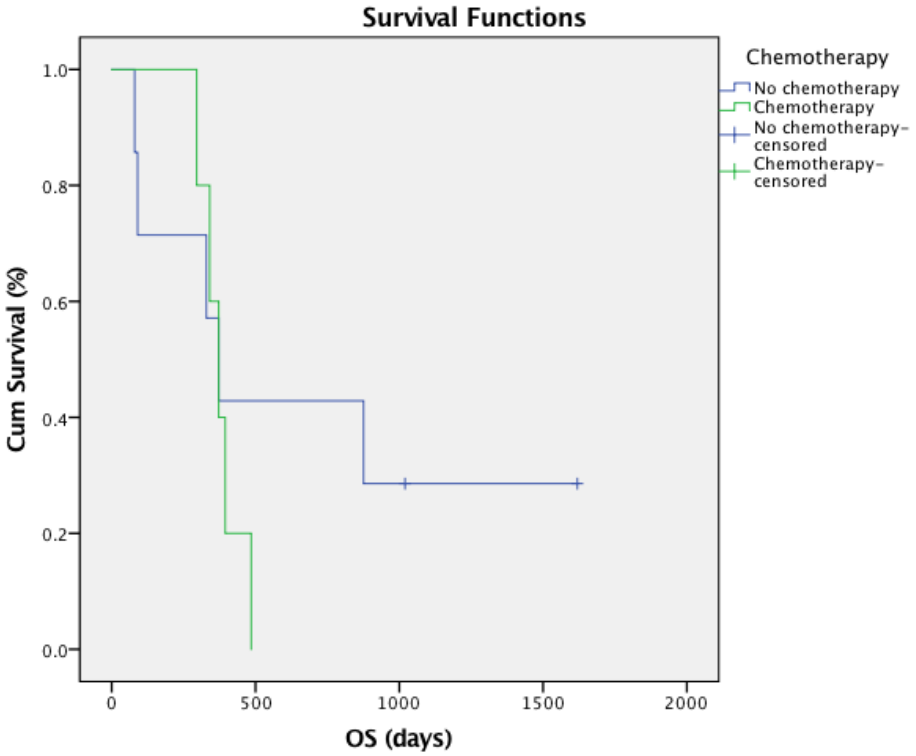

Fig. 17. Kaplan-Meier curves comparing OS of receiving chemotherapy or not in RT group.

No significant difference between chemotherapy (n=5) and no chemotherapy (n=7). P=0.414.

doi: 10.6342/NTU201602570

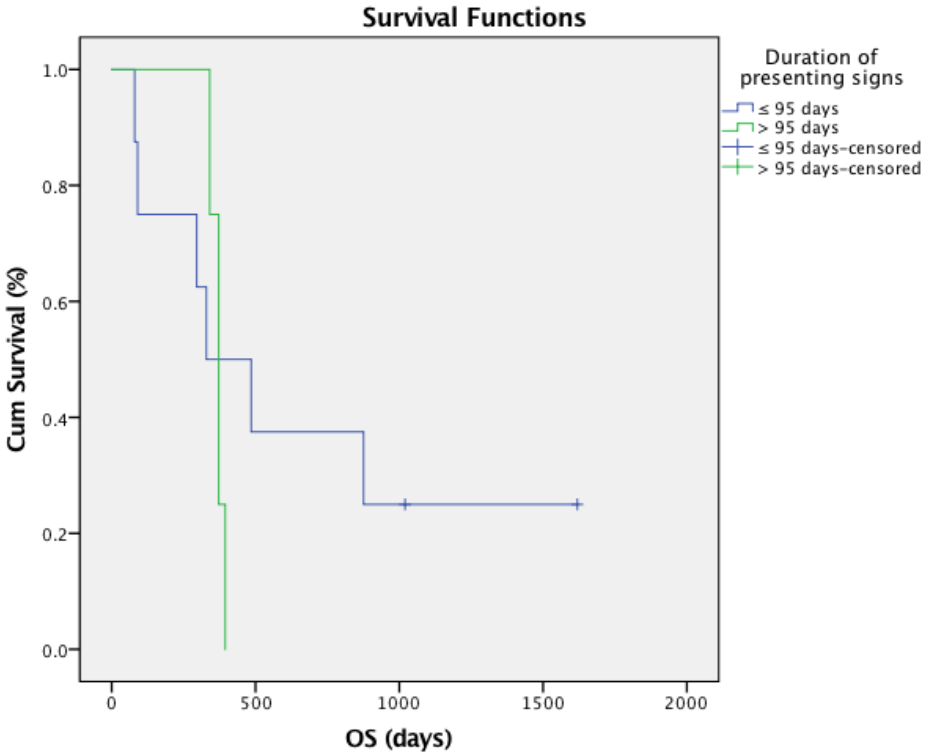

Fig. 18. Kaplan-Meier curves comparing OS of different duration of presenting signs in RT group.

There was no significant difference in OS of patients that duration of presenting signs ≤ 95 days (n=8) and >95 days (n=4). P=0.497

doi: 10.6342/NTU201602570

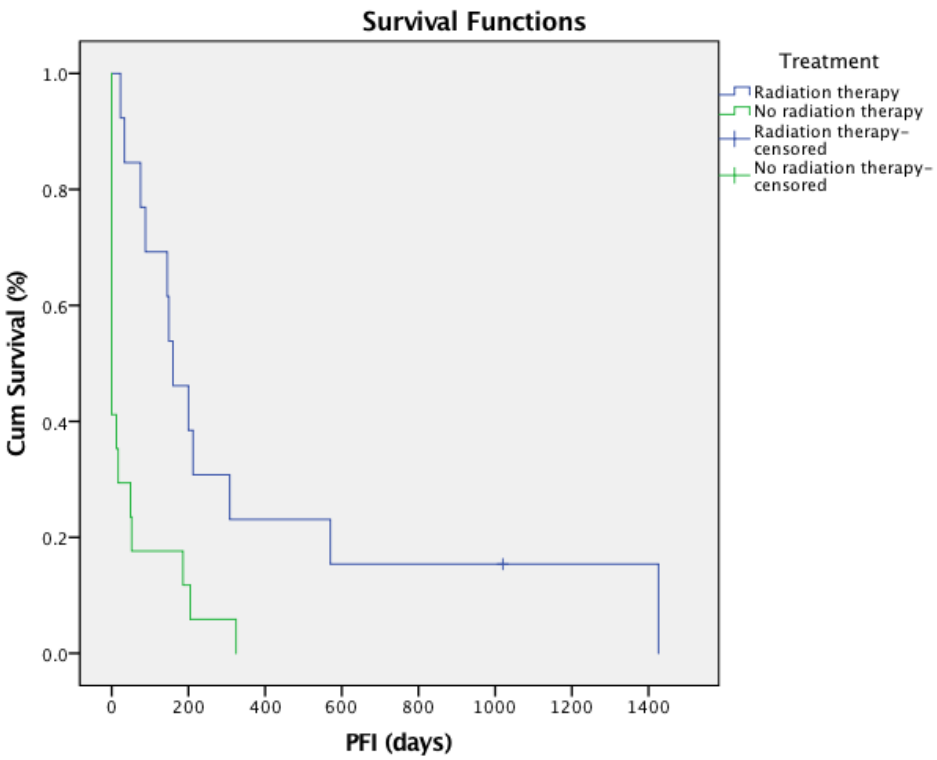

Fig. 19. Kaplan-Meier curves comparing PFI of RT group and non-RT group.

When compared PFI in RT (n=13) and non-RT (n=17) group patients through Kaplan-Meier curve, there was a significant difference. (P=0.002)

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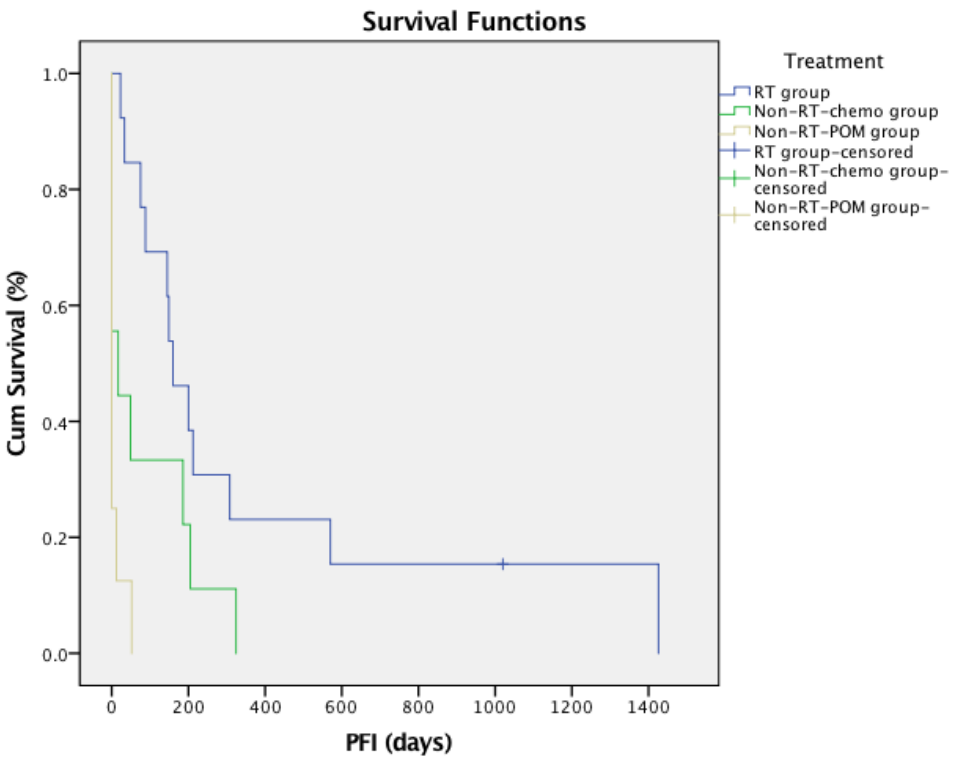

Fig. 20. Kaplan-Meier curves comparing PFI of RT group (n=13), non-RT-chemo group (n=9), and non-RT-POM group (n=8).

The only significant different obtained between RT group and two subgroups was RT group versus non-RT-POM group (P=0.00001). Other comparisons such as RT group versus non-RT-chemo group and non-RT-chemo group versus non-RT-POM group though were close to but did not reach statistically significant (P=0.06 and 0.073, respectively).

doi: 10.6342/NTU201602570

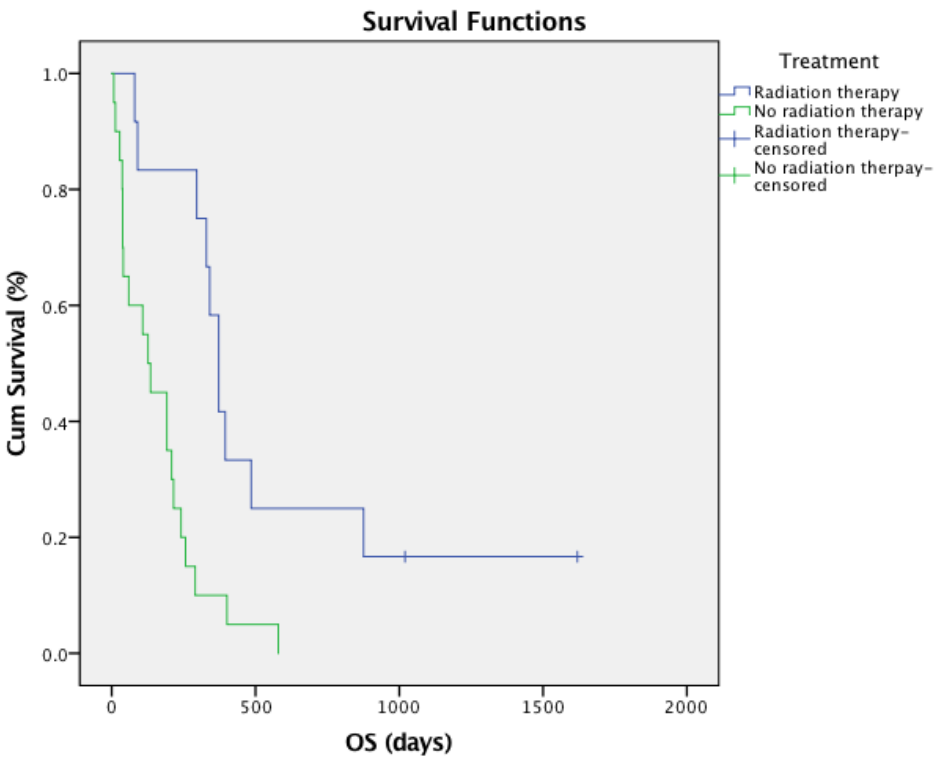

Fig. 21. Kaplan-Meier curves comparing OS of RT group and non-RT group.

When compared OS in RT (n=12) and non-RT (n=20) group patients through Kaplan-Meier curve, there was a significant difference. (P=0.001)

doi: 10.6342/NTU201602570

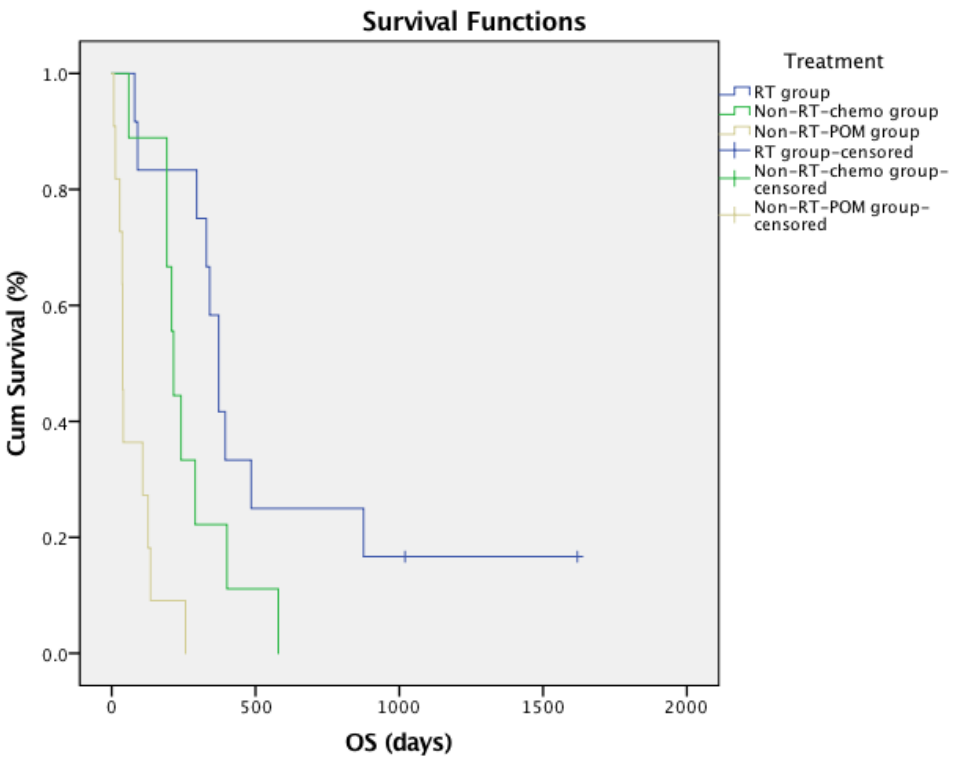

Fig. 22. Kaplan-Meier curves comparing OS of RT group (n=12), non-RT-chemo group (n=9), and non-RT-POM group (n=11).

Significant difference obtained when RT group versus non-RT-POM group (P=0.00002) and non-RT-chemo group versus non-RT-POM group (P=0.002). RT group versus non-RT-chemo group though was close to but did not reach statistically significant (P=0.058).

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