# SURGICO-THERAPEUTIC MANAGEMENT OF ORAL TUMOURS IN DOG WITH REFERENCE TO LASER

## THESIS

*Submitted*

*in partial fulfillment of the requirements for the Degree of*

## MASTER OF VETERINARY SCIENCE

**IN**

## VETERINARY SURGERY & RADIOLOGY

**BY**

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**MAHARASHTRA ANIMAL AND FISHERY SCIENCES**

**UNIVERSITY, NAGPUR - 440 001**

**(INDIA)**

**2021**

## DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled "**SURGICO-THERAPEUTIC MANAGEMENT OF ORAL TUMOURS IN DOG WITH REFERENCE TO LASER"** or part thereof has not been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any university or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

**Signature**

**Date: 06/04/2021**

**Place: Nagpur**

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(Dr. S. B. Akhare)
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**AMBIKA ARVIND** has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled, "**SURGICO-THERAPEUTIC MANAGEMENT OF ORAL TUMOURS IN DOG WITH REFERENCE TO LASER"** submitted by her is the result of research work and is sufficient to warrant its presentation to the examination in the subject of **Veterinary Surgery and Radiology** for the award of **Master of Veterinary Science** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other university.

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# CERTIFICATE

This is to certify that the thesis entitled, "**SURGICO-THERAPEUTIC MANAGEMENT OF ORAL TUMOURS IN DOGS WITH REFERENCE TO LASER"** submitted by **AMBIKA ARVIND** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **Master of Veterinary Science** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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***Alfred North Whitehead***

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Place: Nagpur **(Ambika Arvind)**

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| & | : | And |
| @ | : | At the rate of |
| b. wt. | : | Body weight |
| BSA | : | Body Surface Area |
| Cm | : | Centimeter |
| $Cm^3$ | : | Cubic centimeter |
| Cumm | : | Cubic milli meter |
| DLC | : | Differential Leucocyte Count |
| *et al.* | : | et alii /alia, And others |
| EDTA | : | Ethylene Diamine Tetra Acetic Acid |
| Fig. | : | Figure |
| Gr | : | Group |
| Hb | : | Haemoglobin |
| TNM | : | Tumour Nodes Metastases |
| i.e. | : | id est/that is |
| inj. | : | Injection |
| i.m. | : | Intra muscular |
| i.v. | : | Intra venous |
| S/C | : | Sub cutaneous |
| Kg | : | Kilogram |
| Ltd. Co. | : | Limited Company |
| M:F | : | Male: Female |
| Mg | : | Milligram |
| mg/dL | : | Milligram per deciliter |
| ml | : | Milliliter |
| viz. | : | for example |
| ng/dL | | Nanogram per deciliter |
| No. | : | Number |
| PCV | : | Packed Cell Volume |
| % | : | Per cent |

| | | |
|---|---|---|
| ± | : | Plus Minus |
| ® | : | Registered |
| S.D. | : | Standard Deviation |
| S.E. | : | Standard Error |
| C.D. | : | Critical Difference |
| TVCC | : | Teaching Veterinary Clinical Complex |
| TEC | : | Total Erythrocyte Count |
| PCV | : | Packed Cell Volume |
| TLC | : | Total Leucocyte Count |
| RBC | : | Red Blood Cell |
| WBC | : | White Blood Cells |
| BUN | : | Blood Urea Nitrogen |
| SGOT | : | Serum Glutamic Oxaloacetic Transaminase |
| SGPT | : | Serum Glutamic Pyruvic Transaminase |
| SAP | : | Serum Alkaline Phosphatase |
| etc. | : | Etcetera |
| µ | : | Micron |
| µg | : | Microgram |
| µl | : | Microlitrer |
| L | : | Litre |
| Sr. No. | : | Serial Number |
| IU | : | International Unit |
| > | : | Greater than |
| < | : | Lesser than |

# INTRODUCTION

Neoplasia or neoplasm is an unwanted growth of tissue that is a result of a rapid, uncontrolled division of cells which is not under physiologic control. Typically these cells are non responsive to physiological regulatory control and perform no specific function. These cells act as parasites and derive nourishment from the same host, they are growing in and subsequently cause harm (Vegad, 1995).

Tumours have been mentioned in earlier texts from ancient civilizations, in humans and animals. Cancer in animals was recognized and documented as early as 1600 B.C. in Egypt. Hippocrates, used the word Karkinos, meaning crab, to describe tumours and is thus the origin of the word cancer. Since a long time, discoveries were found to be similar in human and Veterinary Oncology. The discovery of the microscope in 1590 was fundamental to the study of cancers and could help to differentiate, describe and document them.

Oncology is the study of tumours. Classifying tumours as benign and malignant is an important aspect of this. A benign tumour is one which does not metastasize, remains localized, can be removed by surgery relatively easily and often does not cause death, unless it interferes with the functioning of an organ that performs a vital function. Malignant neoplasms while growing invade adjacent tissues, metastasize and often lead to death (Vegad, 1995).

Cancer is one of the major reasons for mortality in dogs, thus making it a disease of particular significance. In a study based in the UK, cancer accounted for 27% of all deaths in purebred dogs (Adams *et al.,* 2010). In another study conducted in Berlin, necropsy results showed that 24.08% of all dogs died of cancer (Walter and Schwegler, 1992).

Oral neoplasms are the fourth most common neoplasm observed in dogs, following skin and soft tissue, mammary and haematopoetic tumours. They account for about 6% of all tumours in dogs. Oral tumours may be benign or malignant, both occurring with almost the same frequency. Benign oral tumours are epulids, ameloblastoma, viral papillomatosis and hyperplasia of gingival. The most common malignant tumours are malignant melanoma, squamous cell carcinoma (SCC) and fibrosarcoma (FSA). Occuring with a lesser frequency are osteosarcoma (OSA), chondrosarcoma, osteochondrosarcoma, myxosarcoma, haemangiosarcoma, lymphoma, mast cell tumour (MCT), and transmissible venereal tumour (Duncan and Lascelles, 2011).

Epulids include fibromas, neoplastic tumours and non-neoplastic odontogenic tumours. They often present as a swelling of the gingiva at junction with teeth. Tumour made of cells of an ameloblast origin cause canine acanthomatous ameloblastoma (DeBowes, 2010).

Melanoma is a non odontogenic, malignant neoplasm usually occurring in dark dogs, which is locally aggressive and often metastasizes (Holmstrom, 2013). It is the most common malignant tumour found in the oral cavity of dogs (30-40%) followed by fibrosarcoma and osteosarcoma. Osteosarcoma is seen typically in large breed dogs (Verheart, 2010).

A patient suffering from an oral tumour, at presentation shows varied clinical signs such as anorexia, halitosis, dysphagia, facial swelling, haemoptysis, sneezing, dyspnoea, change in the sound of the bark, pawing at the face, weight loss and dullness (Dhaliwal, 2010). A physical examination further reveals an oral mass either attached to the gum and oral structures, or appearing to invade surrounding tissues. The consistency of the mass may range from soft and fleshy, to firm, hard and in some cases may even appear calcified.

Oral tumours are often not noticed by many owners, till the animal shows considerable discomfort or illness. Diagnosis involves physical examination, palpation of regional lymph node, which shows changes in case of metastases, radiographic examination or visualization of the area of head, neck and thorax using Computed Tomography or Magnetic Resonance Imaging (Dhaliwal, 2010).

Since, benign oral tumours occur as frequently as malignant tumours, and because distinguishing the type of tumour just by physical appearance is not possible, performing a biopsy is the choice of diagnosis in such cases (Duncan and Lascelles, 2011). In dogs, epulides are often a reactive lesion, however many are still neoplastic or locally invasive, thus making it necessary to always conduct a biopsy (Verstraete, *et al.*, 1992).

Treatment modalities for oral tumours typically consist of surgery, cryosurgery, chemotherapy, radiotherapy, hyperthermia, immunotherapy, and photodynamic therapy, either alone or in combination (Head *et al*., 2008). One of the most effective treatments for oral tumours is surgical resection of the mass, excised along with sufficient margin of normal tissue surrounding the growth. Many oral tumours involve bone as well and thus surgical margins must be achieved in the bone as well. In certain cases mandibulectomy or maxillectomy may be warranted and such techniques have been documented too (Withrow and Holmberg, 1983).

Conventional methods of excision of the tumour using a scalpel, often results in excessive haemorrhage and the control of the same proves difficult. Approaching the base of the tumour for excision, within the oral cavity, is also challenging while using a scalpel. These limitations of conventional surgery can be overcome by LASER surgery.

LASER stands for Light Amplification by Stimulated Emission of Radiation. In this, basically light of a particular wavelength is collimated and a high intensity narrow beam is produced. Oral and maxillo-facial surgeries assisted by Laser have been in existence since the mid 1960’s (Gaspar, 1994).

LASERs commonly used in oral surgery are the $CO_2$ laser, Er:YAG laser, KTP laser and the Nd:YAG laser. The effect shown by laser on a specific tissue depends on the wavelength of light that is reflected, scattered or absorbed by the tissue. Various types of lasers emit light of different wavelengths which are absorbed differently by different tissue constituents such as water and haemoglobin (called as biological chromatophores), thus these have different clinical uses. The wavelength of light produced by $CO_2$ laser is 10600 nm and that produced by Nd:YAG laser is 1064 nm (Neukam and Stelzle, 2010)

Diode lasers are better absorbed by haemoglobin, having a shorter wavelength, hence improving haemostasis, particularly of larger blood vessels. Diode lasers of wavelength of 980 nm are used more commonly by Veterinarians. They are more economical, efficient and compact (Sullins, 2002).

Oral cancer is treated typically by complete surgical excision, where possible, which is followed by multimodal management or adjunctive therapy to prevent recurrence or metastasis. This includes: radiation therapy, chemotherapy, and immunotherapy (Duncan and Lascelles, 2011).

Chemotherapy is a modality of treatment where antineoplastic drugs are employed to kill the rapidly multiplying cancer cells. It is used in case of malignant tumours and helps to control its metastasis. Chemotherapy is recommended as an adjuvant to surgical excision and as a stand alone therapy, it does not seem to have much of a benefit. Chemotherapy has been indicated in malignant melanomas due to the tendency of the tumour to metastasize (Duncan and Lascelles, 2011). In a study, including dogs with oral malignant melanomas, assessed the use of Carboplatin as chemotherapy along with surgery, and it was found that the time taken by the tumour to recur was delayed, increasing survival time (Brockley *et al.*, 2013). The commonly used drugs in therapy of oral tumours are Carboplatin, Cisplatin, 5-Fluorouracil, Paclitaxel, Docetaxel and Hydroxyurea. In case of carcinomas, Doxorubicin and Cyclophosphamide are commonly used as well.

Antioxidant therapy has also been advocated in the treatment of oral cancers. Antioxidants interact with free radicals produced in the body and neutralize them, preventing them from causing damage. Increased oxidative stress has been reported in canine cancer patients, which was recognized due to an increased activity of antioxidants like Catalase and Super oxide dismutase (SOD) in tumour patients. A study conducted by Plavec *et al.* (2008) reported a significant increase in SOD activity in patients suffering from malignant melanoma, mast cell tumor, multicentric lymphoma and oral fibrosarcoma. Common antioxidants are beta-carotene, lycopene, vitamins C, E, and A and other substances. Thus, antioxidants may slow and prevent cancer development.

Immunotherapy is the use of the host's immune system against the cancer cells invading the body. The body's immune system has an important role in the outcome of cancer by regulating it (La-Beck *et al.*, 2015). Immunotherapy has thus evolved as a strategy to manage canine oral tumours, particularly malignant melanoma (Almela and Anson, 2019). These target either the hosts' innate or adaptive immune system. Immune therapies that have been employed in the management of these tumours are monoclonal antibodies, gene therapy, cell therapy, lymphokine activated killer cell therapy, tumour vaccines and immunotherapy activated by bacteria. Immune- modulatory treatments such as use of Cox-2 inhibitors and Colony Stimulating factors have also been undertaken.

Oral tumours are an intimidating disease in terms of prognosis and survival time of the animal. As pet and pet-parent relations have changed over the years to a family like bond, cancer has become a daunting disease. The main aim of the treatment of any cancer is to prolong survivability of the animal and improve the quality of life for as long as the animal is alive. Keeping in view all the above mentioned facts, the study on "Surgico-therapeutic management of oral tumours in dog with special reference to LASER" was planned with the following objectives:

1) To record prevalence of oral tumours in dogs.
2) To evaluate the efficacy of LASER surgery and chemotherapy as a therapeutic regimen for oral tumours in dog.

# REVIEW OF LITERATURE

## 2.1 Oral tumours and their incidence

**Dorn and Priester (1976)** performed an epidemiological study on oro-pharyngeal tumours in 4 species of animals, with 84% of cases recorded in dogs only. According to this study the most common oral tumour in dogs was melanoma; fibrosarcoma and squamous cell carcinoma were less frequently noted. Older male dogs were at higher risk of developing melanoma and fibrosarcoma as compared to females. High risk breeds noted in this study were Golden retrievers, Boxers, Cocker spaniels, Weimaraner and German shorthaired pointer.

**Vos and van der Gaag (1987)** reviewed biopsy and necropsy files of canine and feline oropharyngeal tumours. They stated that 62.8% of canine tumours were malignant. Most common benign tumour was epulis (70.5%) and malignant tumours were malignant melanoma (27.9%), squamous cell carcinoma (27.9%) and fibrosarcoma (22.7%). Male predisposition was seen in cases of certain tumours like malignant melanoma, fibrosarcoma and squamous cell carcinoma of tonsils. The animals presented with oral tumours were mostly old dogs above 8 years of age. Mixed breed dogs showed 10% of all oral tumours. Breed predisposition was also noted with German Shepherd dogs showing a predisposition to SCC. In case of malignant melanomas the breeds predisposed were Cocker Spaniel, Dachshund and Poodles. Epulis was seen more in Boxers. The most common location of oral tumours was the gingiva followed by lips, buccal mucosa, tonsils and tongue.

**Kosovsky *et al.* (1991)** conducted a study including 142 dogs with oral tumours. No sex predilection was noted. Mean age at presentation was 9.9 years, with majority of the dogs being older than 12 years. Younger dogs showed a better survivability rate. Mean age of occurrence and type of oral tumour in dogs were: oral melanomas (26.1%) at 12.2 years, osteosarcomas (14.1%) at 10.3 years, fibrosarcomas (13.4%) and ameloblastomas (29.6%) at 9.1 years and squamous cell carcinomas (16.9%) at 8.1 years. They observed that the dogs with ameloblastoma and squamous cell carcinoma had significantly better survival periods than the rest.

**Wallace *et al.* (1992)** conducted a study comprising 69 dogs that underwent hemimaxillectomy as treatment of oral tumours. In their study, out of the total dogs affected 61% were males and 39% females. Median age of affected dogs was 10 years. Tumour sizes were recorded and 10% were less than 2 cm in diameter, 72% were 2-4 cm and 18% were more than 4 cm. Based on the location they were categorized as rostral (40%), involving incisors and premolars, caudal (54%), involving the caudal premolars and molars and 6% involved the entire maxilla. The types of tumours detected in this study were melanomas (33%), ameloblastoma (26%), fibrosarcoma (22%), squamous cell carcinoma (10%) and osteosarcoma (9%).

**Felizzola *et al.* (1999)** conducted a study on oral tumours involving 130 dogs admitted to the Veterinary Hospital of the School of Veterinary Medicine, University of Sao Paulo. As per this study males (57.69%) were affected more than female dogs (42.31%). Mean age at presentation was 7 to 12.83 years. Breeds commonly presented were mongrels (30.76%), German Shepherd dogs (17.69%), Boxer (7.70%), Pekingese and Poodle (5.33% each). Common clinical signs observed were foul breath, bleeding from oral cavity, loss of teeth and in one dog, muscle atrophy was noted. Common site of neoplastic growths within the mouth was the gums, which was the most common location followed by palate, lips and mouth floor.

**Boria *et al.* (2004)** in a study evaluating the dose of Cisplatin combined with Piroxicam in treatment of oral malignant melanoma and squamous cell carcinoma, found a female predisposition, with 13 females and 7 male dogs suffering from these tumours. Mean age of presentation in dogs was 11.6 years. The tumours were most often found on mandible, followed by maxilla, tonsils and tongue.

**Bergman (2007)** stated that melanomas were the most frequent malignant tumour in the oral cavity of dogs. The author also stated that it was a highly invasive and metastatic tumour. It is a tumour that occurs in old dogs with no sex predilection.

They occur within the oral cavity, on the gingiva, lips, tongue and hard palate in a decreasing order. Most melanomas are pigmented with amelanotic melanomas occurring rarely.

**Head *et al.* (2008)** published a study on tumours of the gastrointestinal tract. Malignant melanoma, squamous cell carcinoma and fibrosarcoma were the most common malignant neoplasms found in the oral cavity and pharynx of the animal.

**Roza *et al.* (2009)** conducted a study on clinical cases presented with oral neoplasms. They reported 64.3% malignant tumours and 35.7% were benign tumours. Benign tumours commonly encountered were epulides (35.7%), types being acanthomatous and ossifying epulides. Squamous Cell Carcinoma (42.8%) was the most common malignant neoplasm, followed by melanoma, haemangiosarcoma and histiocytoma each at 7.1% of total cases. Senile dogs (> 7.3 years) represented 57.14% of all the dogs presented with oral tumours. Most common location of the tumour was gingiva, accounting for 40% of all cases, followed by mandible (20%) and then oral mucosa (10%), tongue (10%) and frontal bone (10%). The authors found equal gender presentation with oral neoplasms during the study.

**Bronden *et al.* (2009)** conducted a study on head and neck tumours of dogs from different regions of Denmark. They studied a total of 1768 cases and found 7.2% of head and neck tumours. The occurrence of these tumours was near equal in both males and females. Majority of the head neck tumours were reported to be of the oral cavity i.e 46% out of which 51% were malignant tumours, 29% benign and 20% unknown type. Based on the study, Oral Malignant Melanomas were the second most common malignancy of the head and neck region, of which metastases were noted in 38% of the cases. Three dogs suffering from epulis were also included in the study, representing about 2% of all head and neck tumours.

**Duncan and Lascelles (2011)** stated that the fourth most common type of neoplasms in dogs are oral neoplasms. They account for 6% of all tumours in dogs. They are presented as both malignant and benign neoplasms. The most common malignant oral neoplasms that occur in dogs, in decreasing order, are malignant melanoma, squamous cell carcinoma, fibrosarcoma followed by others like osteosarcoma, chondrosarcoma, lymphoma, mast cell tumour and transmissible venereal tumour, among many others. Benign neoplasms are of the epulis group, ameloblastoma and viral papillomatosis. Non neoplastic gingival hyperplasia also occurs commonly in dogs.

**Brockley *et al.* (2013)** conducted a retrospective study on malignant melanomas in 63 dogs of which 46% were diagnosed with oral malignant melanomas. The study included 36 males and 27 females, with a median age of 10 years. Mixed breed dogs represented 29% of the total and purebred dogs 71% including the following breeds: Staffordshire Bull terriers (5), poodles (4), Rottweilers (3) and Golden Retrievers (3).

**Simons (2015)** conducted a retrospective study of oral tumour cases presented to the small animal clinic, Utrecht University. It was found that Acanthomatous Ameloblastoma was the most common (19.1%), followed by Fibrosarcoma (16.4%), Squamous cell carcinoma (15.6%), and then malignant melanomas(11.8%) being fourth most common. These findings were unusual since malignant melanomas were found to be the most common in several studies. The breeds that were most often presented were Golden Retriever, Labrador Retriever, German Shepherd, Rottweiler, Jack Russel Terrier and Boxer. The study also revealed that mostly males were diagnosed with oral tumours, they however attributed it with the higher number of male dogs presented to the clinic.

**Goldschmidt *et al.* (2017)** stated that canine acanthomatous ameloblastoma is the most common odontogenic tumour. The tumour occurrence was noted at a mean age of 9.44 years. Predisposition based on sex was not noted. A higher prevalence in Golden Retrievers (12.26%), Cocker Spaniels (4.17%), Labrador Retrievers (13.26%) and mixed breed dogs (27.65%) was noted. They attributed the higher prevalence in these breeds, to the increased number of dogs belonging to these breeds in the general hospital population. Based on this, they also stated that mixed breed dogs showed lower risk of development of CAA. They observed that the most common location of CAA was the rostral mandible, followed by caudal mandible, rostral maxilla and caudal maxilla.

**Kate (2018)** stated that tumours were majorly found in the older (> 8 years) dogs. Majority of the dogs presented with tumours were male and of a non-descript breed.

**Wingo (2018)** documented a retrospective study based on the histopathological samples from a private practice in Arizona specializing in veterinary dentistry and oral surgery. Histopathology of biopsy samples collected from cats and dogs were reviewed. A total of 403 samples were collected from dogs, with a mean age of 9.3 years, of which 202 were from females and 201 from males. The predominant breeds were mixed breeds (18%), Labrador retrievers (8%) and golden retrievers (5.7%). Based on the histopathology reports 30% were malignant neoplasms, 34% odontogenic tumours and 28% were of an inflammatory origin. This particular study reported canine squamous cell carcinoma (7%) as the most common neoplasia in the oral cavity of dogs.

**War *et al.* (2018)** reported 5 cases of oral tumours that were presented to the veterinary polyclinic IVRI. This report included 1 case of acanthomatous ameloblastoma, 2 cases of odontogenic fibroma and 2 cases of malignant melanoma. The clinical signs exhibited by the dogs were bleeding from the oral cavity, dysphagia, halitosis, tooth displacement, facial deformity among others. Acanthomatous ameloblastoma was reported in 10 year old female Spitz. Oral malignant melanoma was reported in a 9 year old spitz and Great Dane which was 8 years of age, both male.

**Kshirsagar (2019)** stated that majority of dogs discovered with tumours were male, older dogs. Major pool of dogs in the study with superficial tumours were non-descript or cross breed dogs.

**Mikiewicz *et al.* (2019)** conducted a study to evaluate various oral lesions in 486 animals (146 cats and 340 dogs), presented as oral tumours. They were to analyze neoplastic lesions (benign or malignant), inflammatory and hyperplastic lesions. In dogs, lesions were found with similar frequencies in sexes, 51.47% males and 48.53% in females. Crossbred (63.53%) dogs were most commonly presented followed by Labrador retrievers (4.41%), German Shepherd dog (3.5%) and Boxer (3.24%), among others. The distribution of lesions was such: 24.12% dogs had hyperplastic lesions, 29.11% were benign tumours, 32.06% were malignant tumours and 14.7% inflammatory lesions. Among benign tumours the common types were peripheral odontogenic fibroma (65.66%) and acanthomatous ameloblastoma (8.08%); whereas, amongst malignant tumours, oral malignant melanoma (35.78%) was most frequent followed by squamous cell carcinoma, fibrosarcoma and osteosarcoma being the more commonly found oral malignancies.

**Cray *et al.* (2020)** assessed the demographics of dogs and cats with oral tumours based on information collected from the veterinary medical database for the period between January 1996 to December 2017. He stated that 6-7% and 3% of all canine and feline neoplasms are of the oral cavity, respectively. A total of 1810 dogs were included in their study. Incidence of oral tumours in dogs was 0.5% or 4.9 per 1000 cases of dogs presented with an ailment. Median age at presentation was 10 years in dogs. Both males and females developed oral tumours with equal frequency. Of these, malignant tumours (53.6%) were most frequent, followed by benign tumours (25.4%) and in 21.1% cases went undiagnosed. Breeds commonly presented with oral tumours were mixed breed dogs (26%), Labrador Retrievers (13%), Golden Retrievers (11.1%) and boxers (3.2%). The most common location of oral tumours in dogs was the gingiva (24.4%) and the lip (14.1%).

## 2.2 Diagnosis and Clinical Staging

**Kosovsky *et al.* (1991)** performed partial mandibulectomy in 142 dogs with oral tumours. Radiographs of the skull were obtained in 136 cases and osteolysis was recorded in 63.2% cases, particularly high rate of osteolysis was noted in case of osteosarcomas (90%), ameloblastomas (71.4%) and squamous cell carcinoma (66.67%). However, this did not affect the survival time. Thus, osteolysis did not serve as a factor in tumour staging. Dogs with larger tumour size showed lower survival time, however this observation was statistically insignificant.

**Felizzola *et al.* (1999)** on a study conducted in 130 dogs with oral tumours discussed the prevalence, diagnosis, prognosis and treatment. Animals were subjected to a thorough physical examination, head and chest thoracic radiograph to detect associated bone lysis or metastasis if any. Osteolysis was found in 31.54% of malignant neoplasms; 1.54% of benign non-odontogenic neoplasms and 17.69% of benign odontogenic neoplasms. In 34.5% malignant neoplasms and 6.15% benign odontogenic neoplasms ulcers were observed. Necrosis was seen in 14.61% of malignant neoplasms and 0.77% of benign odontogenic neoplasms. Benign non-odontogenic neoplasms showed no ulceration or necrosis. All dogs underwent surgical excision and histopathological examination, of tumour samples thus obtained, was performed. Based on TNM classification, benign non odontogenic tumours were mostly stage I, while odontogenic tumours mostly belonged to stage II classification. Malignant tumours most often were of stage III. On histopathological examination the neoplasms were categorized into benign non odontogenic, benign odontogenic and malignant neoplasm. Among benign odontogenic neoplasms were odontogenic fibroma (75.38%), ossifying fibroma (10%), odontoma (1.54%). Benign non odontogenic neoplasms were Fibroma (1.54%), Plasmacytoma (0.77%), Pilomatrixoma (0.77%), Melanoma (33.85%), among several others.

**Grier and Mayer (2007)** discussed the TNM classification of oral tumours by WHO with respect to carcinomas in canines (Table 2.1**)**:

**Table 2.1: TNM classification of Oral tumours**

| **T- Primary tumour** | |
|---|---|
| **$T_1$** | Tumour diameter < 2cm |
| **$T_{1a}$** | No invasion of bone |
| **$T_{1b}$** | Evidence of bone invasion |
| **$T_2$** | Tumour diameter between 2-4cm |
| **$T_{2a}$** | No bone invasion |
| **$T_{2b}$** | Evidence of bone invasion |
| **$T_3$** | Tumour diameter greater than 4cm |
| **$T_{3a}$** | No bone invasion |
| **$T_{3b}$** | Evidence of bone invasion |
| **N- Regional Lymph Nodes** | |
| **$N_0$** | No regional lymph node involvement |
| **$N_1$** | Movable ipsilateral lymph nodes |
| **$N_{1a}$** | No metastasis to the lymph node |
| **$N_{1b}$** | Evidence of metastasis to lymph node |
| **$N_2$** | Movable contralateral lymph nodes |
| **$N_{2a}$** | No metastasis to the lymph node |
| **$N_{2b}$** | Evidence of metastasis to lymph node |
| **$N_3$** | Lymph nodes are fixed |
| **M - Distant Metastasis** | |
| **$M_0$** | No evidence of distant metastasis |
| **$M_1$** | Distant Metastasis present |

**Bergman (2007)** discussed the WHO TNM staging system for oral melanoma in dog. Primary tumour (T) was classified as: $T_1$ (tumour size <2cm), $T_2$ (tumour size between 2-4cm), $T_3$ (tumour size >4cm). Involvement of regional lymph nodes (N) is classified as $N_0$ (no regions lymph node involvement), $N_1$ (Cytologic evidence of lymph node involvement), $N_2$ (fixed nodes). The tumour is classified into 2 based on evidence of distant metastasis (M): $M_0$ (no evidence of distance metastasis), $M_1$ (evidence of distant metastasis). Stage I includes $T_1$ $N_0$ $M_0$, stage II includes $T_2$ $N_0$ $M_{0,}$ stage III includes $T_2$ $N_1$ $M_0$ or $T_3$ $N_0$ $M_{0\,,}$ stage IV includes $T_{1\text{-}3}$ $N_{0,1}$ $M_{1.}$

**Liptak and Withrow (2007)** stated that in case of animals with oral tumours, determining tumour type and clinical staging is essential. Biopsy is required for diagnosis and clinical staging is done based on size of tumour and extent of metastases. The common sites of metastases were the lungs and regional lymph nodes. Impression smears and aspiration cytology may not prove to be an effective mode of diagnosis due to the presence of necrosis and inflammatory tissue at the site, as is often seen in the case of oral tumours. Punch or wedge biopsies are preferred over the previous methods and helps categorize the tumours into malignant and benign.

**Mickelson *et al.* (2020)** conducted a study aimed at detecting metastasis of tumour to the palatine tonsils in dogs. Out of all the samples collected from tonsils, 56% showed neoplastic changes. Metastasis was detected in 41(4.6%) cases, of which malignant melanoma (25) and carcinoma (10) were most frequently identified. Squamous cell carcinoma (55%), lymphoma (17%) and melanoma (12%) were the most frequent primary tumours of the palatine tonsils. No lymphatic drainage was identified to the tonsils and the route of metastatic spread was suspected to be haematogenous. The authors stated that thorough examination of the oral cavity, including the palatine tonsils is a must in cases of oral tumours and melanoma, so as to diagnose metastasis.

## 2.3 Treatment for Oral Tumours

**Kosovsky *et al.* (1991)** performed partial mandibulectomy to treat oral neoplasms in 142 dogs. This procedure was recommended for malignant or large tumours. It is generally a well tolerated procedure, with few postoperative complications with rapid return to mastication in such dogs. A few cases however did require placement of pharyngostomy tubes. The results of this study indicated that partial mandibulectomy were effective in treating and prolonging survival in case of ameloblastoma and squamous cell carcinoma. Dogs with melanomas, osteosarcomas and fibrosarcomas showed a high rate of local recurrence and distant metastases indicating the need for adjuvant therapy for improving survival rate.

**Wallace *et al.* (1992)** performed maxillectomies, to treat maxillary tumours in dogs. In this, based on the location of the tumour on the maxilla, hemimaxillectomy was performed; however the type of surgery did not affect prognosis. In one case of squamous cell carcinoma, with lymph node metastasis, the dog underwent chemotherapy using Cyclophosphamide, Chlorambucil and Doxorubicin. In another dog with oral melanoma that had metastasized to the bone marrow, treatment using Doxorubicin was attempted; however the dog did not survive the chemotherapy. In a dog with local recurrence of fibrosarcoma, maxillary nasal resection was performed. The dog also underwent radiation post surgery The dog survived for 2 years post surgery but was euthanized due to recurrence of the tumour. Hemimaxillectomy is a well tolerated procedure, with lesser complications. It is effective in decreasing the chance of recurrence of ameloblastoma, acanthomatous epulis and squamous cell carcinoma, where it also decreased the risk of metastasis. Melanomas were not effectively treated by hemimaxillectomy. Metastasis was reduced or prevented in case of Fibrosarcoma and Osteosarcoma by hemimaxillectomy however there was a high probability of local tumour recurrence post this procedure. Adjuvant chemotherapy and radiation therapy was required for complete treatment.

**Bergman (2007)** discussed various therapeutic modalities employed in the treatment of melanomas. Surgical excision for local tumour control was advantageous due to its speed and reduced cost. Staging was recommended so as to consider wider margins of excision, and deciding the need for further therapy. Radiation therapy was further used in cases where complete excision was not possible and in those cases with local lymph node metastasis. Chemotherapies using Carboplatin, Cisplatin and Piroxicam were reported to be more effective as compared to other agents like Melphalan, Doxorubicin, Dacarbazine. However, the overall response rate to chemotherapy was low. Studies have revealed that human melanomas are very resistant to chemotherapy. The researcher attempted immunotherapy using tumour vaccines, liposomal immunostimulators, bacterial superantigen with colony stimulating factors and DNA vaccinations. However, production has been very expensive and difficult. DNA vaccines are relatively more practical to produce. The author discussed the development, use and efficacy of tyrosinase DNA vaccine, which has shown promising results.

**Head *et al.* (2008)** discussed the therapeutic modalities used in treatment of head and neck tumours. Surgery, radiation therapy, chemotherapy, immunotherapy, photodynamic therapy, cryosurgery, hyperthermia were the modalities recorded. They also stated that chemotherapy as the sole therapeutic regime had no particular significance and it was best performed in conjunction to or following surgical intervention to reduce chance of recurrence, and prolong survival.

**Frazier *et al.* (2012)** published a study involving 29 dogs with oral fibrosarcoma. Of these, 21 dogs underwent only surgical excision of the tumour, while 8 dogs underwent both surgery and radiation therapy. This study showed better results for dogs treated using surgical excision as compared to previous studies. Results from radiation were unclear due to the lesser subjects studied. Authors suggested surgical excision with wide margins, when possible, as a treatment for dogs with oral fibrosarcoma.

**Coyle *et al.* (2015)** reviewed studies on 51 dogs that underwent mandibulectomy as treatment for osteosarcoma. Of the 51 dogs reviewed, 21 dogs were also treated by chemotherapy. Archived tumour samples were evaluated for mitotic index (MI) and tumour grade. They observed affections in 52% females and 48% males during their study. Distribution of commonly presented breeds was as follows: mixed breed dogs (24%), Rottweiler (14%), Labrador Retriever (12%) and Golden Retriever (8%).

**Ottnod *et al.* (2013)** retrospectively studied the use of Oncept vaccine as an adjunct therapy following local control of the tumour. Records from 45 dogs were included in the study. The study did not find the vaccine to have a major advantage as an adjective treatment, since there was no significant improvement in the median survival time, progression free survival or disease free interval following the use of the vaccine. The author also stated that the use of such a costly and ineffective modality would give false hope to the owner, presenting an ethical dilemma.

**Farcas *et al.* (2014)** in a review article, stated that oral and maxillofacial osteosarcoma progress less rapidly than appendicular osteosarcoma. Wide, clean surgical margins are necessary for better prognosis and loco-regional control. Standard therapy included surgical excision and adjuvant chemotherapy and radiotherapy.

**Gardner *et al.* (2015)** carried out a retrospective study involving 65 dogs diagnosed with oral fibrosarcoma. Sex distribution in the study showed female dogs at 41.54% and male dogs at 58.46% of the total pool. Common breeds of dogs presented were Golden Retrievers (33.84%), Labrador Retrievers (13.84%), German Shepherd dogs (7.69%). Dogs were treated with surgery (radical or conservative) with or without post-operative radiation therapy. Based on the study, dogs treated with both surgery and radiation therapy had the longest median survival times.

**Biller *et al.* (2016)** published the AAHA oncology guidelines for dogs and cats. They mentioned that oral malignant melanomas show a high rate of metastases

(80% of all cases) and recommended staging with the help of radiographs, mandibular lymph node biopsy and if possible CT and MRI to facilitate surgery planning. Surgical excision is the first treatment of choice along with mandibulectomy or maxillectomy if required. If incomplete resection was suspected then radiation therapy was recommended. Systemic treatment including chemotherapy using Carboplatin or immunotherapy using xenogeneic DNA vaccine was advised. Squamous cell carcinoma of oral cavity was also included in the guidelines. These tumours are locally aggressive. Staging in a similar manner as mentioned for oral melanoma was mentioned along with a skull radiograph. Treatment regime included surgery, radiation therapy and systemic treatment using Carboplatin, mitoxantrone based chemotherapy, Toceranib phosphate, NSAIDs and metronomic chemotherapy, however none of these were proven to benefit survival. The estimated median survival time in such cases was 6 months. The use of carboplatin, cisplatin and doxorubicin for osteosarcoma was also mentioned. The guideline also mentions immunotherapy using a DNA vaccine designed for veterinary patients suffering from canine oral melanoma specifically for stage II and III, following control of local tumour.

**Elliott *et al.* (2016)** conducted a retrospective study based on records of 33 dogs with oral mucosal mast cell tumours. These were rare and more aggressive in nature as compared to mast cell tumours involving the skin. Labrador retrievers and golden retrievers appeared to be the breeds predisposed to mast cell tumours. At the time the tumour diagnosis itself, prognosis was found to be poor, considering that 55% of the dogs from the study showed lymph node metastasis of the tumour. The dogs underwent many treatments, most of them receiving multimodal therapy. The different therapies were surgery, radiotherapy and chemotherapy using vinblastine and prednisolone, and in some cases lomustine as well. Few were treated using masitinib and toceranib. About half the number of dogs were euthanized because of local progression of tumour in the study period. Rate of distant organ metastasis was very low. Neoadjuvant chemotherapy was administered to few dogs so as to reduce the size of the primary tumour before attempting local control, so as to be able to obtain clean surgical margins at the time of excision. The response rates for the same were good, with 22% dogs showing a complete response. They opined that mitotic index could be be used as an indicator for prognosis when the tumour could not be graded. The author suggests that though aggressive, with appropriate treatment dogs may show remission in this case.

**Simcic *et al.* (2020)** conducted a study on non tonsillar squamous cell carcinoma of oral cavity of twelve dogs. Therapy typically included surgery and radiotherapy. The authors assessed the effectiveness of electrochemotherapy as a therapeutic procedure for these tumours, which attempted at improving the distribution of chemotherapeutic drugs to the tumour. Dogs were treated using electrochemotherapy and Bleomycin administered intravenously. Four dogs showed complete remission, one showed a partial response. Toxic effects associated with electrochemotherapy were fewer and recovery was faster.

### 2.3.1 Antioxidant therapy

**Noda and Wakasugi (2001)** stated that the cancer patients have been shown to have increased activity of enzymes such as superoxide dismutase and catalase, which is indicative of oxidative stress being present in such patients. Oxidative stress has also been linked to resistance of patients to treatments such as chemotherapy and radiation therapy.

**Marquez *et al.* (2016)** studied anti-oxidant and anti-inflammatory properties of Ocoxin oral solution in the prevention of liver metastases of colo-rectal cancers in mice. Based on the study, metastasis of colo-rectal cancer to the liver was reduced after Ocoxin therapy. The solution showed effect by increasing apoptosis of cancer cells, decreasing mitotic activity of cancerous cells, fibroblast migration to tumour and inhibited expression of inflammatory mediators locally.

**Ramos *et al.* (2021)** studied the efficacy of Ocoxin-Viusid in human patients with prostate cancer, used as a supportive treatment. Epigallocatechin gallate, present in green tea extract has anticancer properties. It inhibits tissue necrosis factor, potentiates nuclear factor κ-light-chain-enhancer of activated B cells and inhibits cyclooxygenase 2 expression. It also acts as an immunomodulator promoting interleukin 12 and interferon production. It also inhibits tumour invasion, metastasis and angiogenesis. The supplement showed reduction in pain and prostate symptoms. It generated better clinical and humoral response in patients. The supplement was believed to increase tolerance to treatment, improve quality of life of patients, improve patients' nutritional status and also delayed the progression of the disease.

### 2.3.2 Laser excision

**Fisher *et al.* (1983)** studied the difference in wound healing after conventional excision and $CO_2$ laser excision of buccal mucosa in dogs. There was lesser damage to adjacent tissues in case of laser excision. Laser causes vaporization of the intracellular fluid and disintegration of cellular structure. This type of cellular destruction may not release inflammatory chemical mediators, as a result of which the extent of inflammation in laser wounds was much lesser than in conventional surgery. There was minimal wound contraction and myofibroblasts across the wound. Distribution of myofibroblasts were irregular and lacked orientation in laser wounds while in surgical excision they were arranged parallel to the wound surface. Following laser tissue excision there was also lesser collagen formation, delayed and irregular reepithelialization.

**Guerry *et al.* (1986)** published a review involving 51 people suffering from oral squamous cell carcinoma, treated using $CO_2$ laser via microdirect pharyngoscopy. The author described the various advantages of $CO_2$ laser such as haemorrhage control, better field of visualization, precise removal, increased margins can be achieved since tumour cells have been vaporised, disintegrated and not allowed to spread. As compared to electrocautery, laser did not damage vessels and nerve supply to the wound as much.

**Katzir (1991)** described types of Laser and its use in medicine. The use of high power lasers and modes of lasers suitable for incision of tissues including skin was stated. Performing incision using continuous wave mode caused damage to adjacent structures and thus the author recommended the use of pulse wave mode, which performs ablation of tissue more effectively with lesser damage.

**Gaspar (1994)** described the use of high power lasers in oral and maxillofacial surgeries. The researcher stated that $CO_2$ laser has excellent haemostatic effect and preserved the tissue adjacent to the tissue well, due to these properties it has applicability in oral surgery. There were more advantages such as minimal tissue scarring, undisturbed wound healing and reduced pain and edema following surgery. The author also stated that it is suitable for treatment of epulis, lingual carcinomas, gingival hyperplasia among many others. Nd:YAG laser provides deep coagulation and is used in surgeries where high vascularity of tissue is expected or when large vessels are supplying the structure to be removed.

**Romanos and Nentwig (1999).** conducted a study to evaluate healing following surgery of oral cavity using a 980 nm diode laser. Twenty-two patients with various oral affections were studied. Both continuous wave and pulsed modes were used. The authors found that precision of incision while using a diode laser was much more as compared to the other lasers. The coagulation rendered by the diode laser was comparable to Nd:YAG laser and cutting effect similar to $CO_2$ laser. They also stated that due to the coagulation properties of diode laser, sutures were not required and surgery was quick. They recommended the use of diode laser in daily practice.

**Zeira *et al.* (2004)** performed a study to evaluate effectiveness of carbon dioxide laser surgery as compared to conventional surgery in the treatment of oral tumours in 16 dogs. Carbon dioxide laser offered the advantage of precision and predictable cutting since the beam intensity could be controlled, sealing of nerves and lymphatic vessels as well as vessel cauterization. Seven types of tumours were identified: epulides, melanomas, squamous cell carcinomas, fibrosarcoma, fibroma, adamantinoma and lymphoma. LASER was used for excision of all the tumours. There was minimal haemorrhage, post-operative pain and discomfort. Surgical time was much lesser than would be required for conventional excision. No infection was reported even in cases where antibiotics were not used and no tissue reaction was noted in any dog. Rapid healing within 5-9 days post surgery was noted. In the 12 months of follow up, one case of local tumour recurrence and 3 cases of local lymph node metastases were noted. The one disadvantage noted was the need for evacuation of smoke produced by CO2 laser, which is difficult to do within the oral cavity.

**Maiorana and Salina (2006)** used super-pulsed diode laser in various surgical procedures involving oral cavity including an epulis. All patients were administered local anaesthesia prior to procedure. No antibiotic prophylaxis was advised and only an antibiotic mouth rinse was prescribed. Healing happened without major inflammation and complications. In case of epulis, the surgical wound healed completely at 40 days, without any shrinking of tissue. The advantages offered were precision cutting, haemostasis, minimum damage to adjacent tissues, minimal post-operative pain and swelling.

**Deppe and Horch (2007)** authored a review article on application of laser in oral surgery. They stated that the $CO_2$ preserved tissue components and function without any ill effects. However, use of the same for precancerous lesions caused higher risk of recurrence. Thus, in such cases the use of diode or Nd-YAG lasers was recommended.

**Fornaini *et al.* (2007)** conducted a clinical study involving the use of 980 nm diode, 810 nm diode and 1064 nm Nd-YAG laser for soft tissue surgery related to orthodontic procedures.  Surgery was performed using only topical anaesthetics. Laser was a good alternative aid for orthodontic procedures with reduced time for surgery, negligible intra-operative bleeding. Sutures were not required following surgery. All these advantages made the use of lasers convenient in young patients.

**Dunié-Mérigot *et al.* (2010)** published a study on soft palate resection in 60 brachycephalic dogs using laser and electrocautery. The palatoplasty was performed using $CO_2$ laser, diode laser and electrocautery and results were compared. The outcome was favourable in majority of cases from all groups. However, $CO_2$ laser showed best results when compared and also showed the best haemostasis.

**Neukam and Stelzle (2010)** discussed the use of laser in the treatment of oral and maxillofacial tumours. Radical excision of tumour with clean margins of 5-15cm was the standard therapy for malignant tumours and surgical nucleation for benign tumours. However, in the head region the extent of radical excision was limited due to fear of distorting function and cosmetic appearance. Different types of lasers were used in oral surgeries. Wavelength of laser determines the effect it had on tissue. Varying wavelengths of light are absorbed by the different tissue constituents and thereby affecting the energy deposited in the tissue. Lasers based on their wavelength and absorption of light by the different chromatophores, like haemoglobin and water were used accordingly for oral surgery. Carbon dioxide and Er-YAG lasers showed low tissue penetration and promoted healing as they were absorbed mostly by water. They showed effective cutting of both hard and soft tissue. Nd:YAG lasers showed more tissue penetration as they were absorbed by both water and haemoglobin. They showed higher tendency to damage and coagulate surrounding tissue and were recommended in cases where haemorrhage was expected. Potassium titanyl phosphate lasers were well suited for haemostasis and used often for coagulation. They were of particular use in highly vascular tissue such as the tongue. Laser surgery offered many advantages over conventional surgery such as haemostatic effect, reduced inflammation, oedema and pain especially post oral laser surgery. The wounds healed by secondary intention and suturing was not needed post tumour excision. There was reduced cicatrization and tissue contracture post laser surgery, thus reduced tissue contracture. Disadvantage of laser was thermal damage to surrounding tissue. This may cause delay in healing during the initial days post surgery. However, complete wound healing was noted 4 weeks post surgery, similar to scalpel excision. It was difficult to assess the depth of penetration of laser. This may lead to inadvertent damage of functional and essential structures such as blood vessels and nerves, affecting function and aesthetics.

**Azma and Safavi (2013)** discussed the use of diode laser in surgery of soft tissues of oral cavity. It was employed as an aid in surgery due to ease of application, improved haemostasis and lack of requirement of sutures. They further stated that the healing was better, reduced edema and minimal scarring.

**Aldelaimi and Khalil (2015)** published a paper on maxillofacial surgery using 980nm diode laser. They performed the procedures using analgesic sedative and topical anaesthetic. The patients reported no intra-operative pain. Haemorrhage during surgery was negligible, giving a clear field of view and no sutures were required following surgery. Mild edema was noted in the first 3 days post surgery which was resolved. There was a complete return to function within 4 weeks in all cases. There were no complications or infection and minimal scar tissue formation. Thus, diode laser served as an excellent alternative to conventional surgery.

**De Lorenzi *et al.* (2015)** treated epiglottis chondrosarcoma in a dog by performing epiglottectomy using 980 nm diode laser at 10W power, with an optical fibre of diameter 400 µm using continuous wave mode. Complications related to tissue heating such as scar formation and delayed healing were expected, however, the advantages such as improved haemostasis, less tissue manipulation and improved visibility, encouraged the author to use intraoral diode laser for the procedure. The author expressed that diode laser also has a precise focus point, cuts sharply and evaporates with reduced tissue heating.

**Derikvand *et al.* (2016)** described the use of 980 nm diode laser in various dentistry cases in humans. Although the same procedures could have been done by conventional surgery, the authors chose laser due to the advantages it offered like reduced haemorrhage and improved field of vision during surgery and less pain post surgery. Post operatively the patients did not develop an infection. Based on these findings the authors recommend the use of diode laser in place of conventional surgery for oral soft tissue surgery.

**Igna *et al.* (2016)** described three cases of dogs with oral malignant melanoma subjected to laser excision of tumour, following recurrence after previous surgery. Excision was performed using 980 nm diode laser, at a power of 10 W with 400 µm diameter optical fibre. Surgery was performed comfortably with easy access to tissue and minimal haemorrhage. Following laser surgery, there was local redness and the wound was sore on palpation for up to 2 days post surgery, however, no alteration of masticatory function was exhibited. Wound healing occurred by 9$^{th}$ day in all three cases. Dogs were reevaluated monthly for the first three months after surgery then again at 6 months and 12 months from surgery. At the time the animal underwent laser excision all the cases were in stage I as per TNM classification. At re-evaluation of the dogs one year post surgery no recurrence or metastases was noted.

### 2.3.3 Chemotherapy

**Rassnick *et al.* (2001)** conducted a retrospective study including 27 dogs with malignant melanoma occurring at various locations in the body. Mean age of these dogs was 11 years, and of these 17 dogs were male and 10 female. Carboplatin was administered at a dose rate of 350 mg/m$^2$ for dogs weighing more than 15 kg and 300 mg/m$^2$ for dogs weighing less than 15 kg. Dosing interval was 21 days. Response to treatment was rated as complete response where there was 100% reduction in tumour size, partial response with 50-100% reduction, stable disease when there was less than 50 % reduction or no reduction at all and progressive disease in case there was a growth in tumour size by more than 25%. Overall response rate was 28%. One dog had complete remission post treatment, 24 % of dogs showed partial response, 36% had stable disease and progressive disease each. Gastrointestinal toxicosis was exhibited by 5 dogs, where the dogs showed vomiting, diarrhoea and anorexia. One dog had to be euthanized due to severe gastroenteritis. They observed the significant relation between body weight and gastroenteritis, in that the lesser the body weight the more the risk of developing gastrointestinal toxicosis. This was because doses were calculated based on BSA which meant that small dogs received more drug per kilogram of body weight. For the same reason smaller dogs show better response to carboplatin treatment than larger dogs. However, appropriate dosage adjustments needed to be made so as to reduce the toxic effects of the drugs.

**Schmidt *et al.* (2001)** studied the efficacy of Piroxicam as treatment for canine oral squamous cell carcinoma. Total 17 dogs were studied and Piroxicam was administered at 0.3 mg/kg PO, once a day till signs of toxicosis developed, progressive disease or death of the dog occurred. Following treatment 3 dogs showed remission (1 complete and 2 partial) that is 18% remission rate and 5 dogs (29%) showed stable disease. When compared to treatment using other cytotoxic agents these results were favourable. In addition to anti-tumour activity the use of Piroxicam may also improve quality of life through analgesic and anti-inflammatory properties. During the study, only 1 dog developed signs of gastrointestinal toxicosis which was treated by Misoprostol addition.

**Boria *et al.* (2004)** studied combined therapy involving Cisplatin and piroxicam for the treatment of squamous cell carcinoma and malignant melanoma of oral cavity in 9 and 11 dogs, respectively. Piroxicam was administered per orally at 0.3 mg/kg, once a day, starting 5 days before Cisplatin administration. The maximum tolerated dose of Cisplatin was determined to be 50 $mg/m^2$ when used with Piroxicam. Cisplatin was administered in 20 minute period at 3 weekly intervals. Diuresis was initiated 4 hours before and 2 hours after cisplatin treatment using 0.9% NaCl saline solution administered intravenously. Butorphanol at 0.4 mg/kg was antiemetic selected. The conclusion of this study was that Cisplatin and Piroxicam combination had anti-tumour activity primarily against squamous cell carcinoma in dogs. It was a viable treatment option when renal function is closely monitored for signs of toxicity in cases of oral malignant melanoma and squamous cell carcinoma in dogs.

**Murphy *et al.* (2005)** published a study on dogs with oral malignant melanoma. None of the dogs chosen had pulmonary metastasis of the tumour. The dogs received radiotherapy with or without chemotherapy using Carboplatin. Based on this study there was no added advantage of Carboplatin therapy over radiation used alone.

**Brockley *et al.* (2013)** published a retrospective study of 63 dogs with malignant melanomas located in the oral cavity, digits or skin to evaluate the efficacy of carboplatin therapy on survival of such dogs either following excision or as the primary therapy. The carboplatin dosing was 300 $mg/m^2$ repeated every 21 days with an average of 4-6 cycles or based on how it was responded to and tolerated by dogs. A median survival rate of patient with oral melanoma was 389 days. Survival of dogs with oral melanoma did not increase with adjuvant carboplatin therapy. However, carboplatin was well tolerated in dogs and the survival time increased in the dogs that responded to chemotherapy.

**Dank *et al.* (2014)** published a paper on the use of Carboplatin as an adjuvant therapy for oral malignant melanoma in dogs following surgical excision. The study included 17 dogs with oral malignant melanomas. Carboplatin *cis*-diammine or 1, 1-cyclobutane-dicarboxylatol platinum is an analogue of cisplatin, and a second generation platinum compound. Carboplatin therapy was started following radiation therapy or surgery, at a median time range of 11 days. Of these 11 dogs received Carboplatin at 300 $mg/m^2$, 4 dogs at dose of 250-300 $mg/m^2$, and 2 dogs weighing less than 15 kg at dose of 150-250 $mg/m^2$. Carboplatin was administered at an interval of 21 days and each dog was scheduled to receive four doses at least. However, the range of doses varied from 2-11 doses. Neutropenia associated with Carboplatin developed in a total of 7 dogs. In these dogs the dosage of Carboplatin was reduced by 25%.  The therapy was completed in 10 dogs and stopped in 7 because of advanced disease. Following treatment 11 dogs relapsed. Three dogs died prior to a relapse. Following chemotherapy, the median progression free survival rate in dogs that underwent surgery and chemotherapy was 210 days and for dogs receiving radiotherapy as well, was 291 days. Overall median survival time was 440 days. Of 17 dogs, 10 dogs died as a result of the tumour. Dogs with oral malignant melanoma have a poor long term prognosis, despite adjuvant treatment with carboplatin. The study shows that dogs receiving radiation therapy following surgery showed lower local recurrence of the tumour. The authors recorded that in cases where radiation therapy was not affordable, surgery and carboplatin therapy may be an alternative for treating dogs with oral malignant melanoma.

**Woodruff *et al.* (2019)** published a study including 29 cases of intranasal tumour in dogs treated by alternately employing Doxorubicin and Carboplatin as chemotherapeutic agents along with Piroxicam. Associated side effects following chemotherapy were gastrointestinal and haematological disturbances. Neutropenia was reported following Carboplatin therapy which however resolved with time. DIC was reported in one case following Carboplatin therapy. At least one adverse effect of the treatment was reported in 69% of dogs. Besides these, the author claims that the chemotherapeutic regime was mostly tolerated by all dogs. Dogs died due to progression of primary tumour and not due to distant metastases. Chemotherapy can be considered the choice of treatment particularly in cases where radiation therapy is not possible, especially due to economical considerations and in view of accessibility.

### 2.4 Haematological and Biochemical Parameters

**Medway *et al.* (1969)** described a decrease in the haemoglobin value due to the loss of blood that occurs during surgical excision of highly vascular malignant neoplasms.

**Coles (1986)** reported a decrease in total erythrocyte count and haemoglobin as a result of blood loss that occurs during surgical excision of highly vascularised tumours.

**Dobson and Gorman (1994)** discussed bone marrow suppression as the most common side effect of chemotherapy which was reflected in the haematological study as significant leukopenia and neutropenia.

**Oberhoff *et al.* (1998)** stated that anemia is a common side effect of platinum based chemotherapy, in study on the effect of human erythropoietin following chemotherapy.

**Ginel *et al.* (2002)** found an increase in serum alkaline phosphatase following administration of glucocorticoids, for varying durations. Based on the drug administered the period for return to baseline SAP levels ranged from 1 weeks to 4 weeks following administration.

**Wilson *et al.* (2004)** studied the effects of various anaesthetic protocols on splenic size. They found a significant reduction in haematocrit during surgery following Ketamine and Diazepam induction, and splenic size was maximum. They suggested that the decrease in haematocrit could be a result of sequestration in sites such as the liver, skin and muscle, as well as the spleen.

**Edelman and Rupard (2006)** stated that thrombocytopenia was the major dose limiting toxicity of Carboplatin therapy in humans. Mild nausea and vomition were recorded. Nephrotoxicity and Neurotoxicity were fewer in comparison.

**Chun (2007)** stated that Carboplatin showed myelosuppressive action, leading to Neutropenia and thrombocytopenia. In dogs, the neutrophil and platelet nadir was reached by day 11 to 14, following Carboplatin administration. The authors advice to evaluate the haematological parameters (both CBC and platelet count) on day 14, at least in dogs undergoing Carboplatin therapy.

**Townsend (2007)** discussed Carboplatin as a chemotherapeutic agent and described adverse effects such as myelosuppression leading to anemia, leukopenia, thrombocytopenia, gastrointestinal toxicity, elevation of liver enzymes and nephrotoxicity.

**Dobson *et al.* (2008)** mentioned that Carboplatin is platinum based chemotherapeutic compound that has lesser nephrotoxic effects, less toxic effects in gastrointestinal tract and lesser potency as compared to Cisplatin. It shows similar action against osteosarcoma in dogs as Cisplatin. It is also employed in the treatment of malignant melanomas and carcinomas in dogs. It is to be used with caution in dogs with previous renal disease. Myelosuppression is a dose-limiting toxicity of Carboplatin with neutrophil and platelet nadir developing by day 14 following Carboplatin therapy. Mild nephrotoxicity has been recorded in dogs and cats. Hepatotoxic effects have been recorded in humans.

**MacDonald (2009)** found that the most common adverse effect of chemotherapy was bone marrow suppression which manifests as Neutropenia, thrombocytopenia and anaemia. Anaemia was however uncommon and of a mild to moderate grade.

**Benjamin (2010)** stated that in malignant neoplasms a decrease in total erythrocyte count, leukocytes, neutrophils and eosinophils could be observed due to bone marrow replacement. The author also stated that following and during surgery anaemia can be noted due to surgical and anaesthetic stress.

**Sharma *et al*. (2010)** noted that the adverse events following chemotherapy were decreased total leukocyte count, total erythrocyte count and platelets. They also noted neutropenia, lymphocytosis and monocytosis as a result of the same.

**LiverTox (2012)** stated that as a chemotherapeutic agent Carboplatin had milder adverse effects than Cisplatin. The author also stated that transient increase in SGOT levels were observed in some of the patients undergoing Carboplatin therapy. However it was found that clinical signs of acute liver damage was rare.

**Cohen *et al.* (2013)** stated that leukocytosis could be a sign of stress response as well as a sign of infection. Leukocytosis post procedure was a common occurrence. Neutrophilia was often concurrent with the leukocytosis.

**Ehrhart *et al.* (2013)** stated that the Neutropenia was dose-limiting toxicity of Carboplatin. Carboplatin could be used as a single drug in the treatment of Osteosarcoma in dogs and the drug was well tolerated by dogs during the study.

**Geyikoglu *et al*. (2017)** discussed the action of Oleuropein against oxidative injury and renal failure associated with Cisplatin chemotherapy. They stated that following Cisplatin therapy there was an elevation in serum blood urea nitrogen and creatinine.

**Papich (2016)** stated that Carboplatin has myelosuppressive activity leading to anaemia, leukopenia, neutropenia and thrombocytopenia. Carboplatin is also mildly nephrotoxic, comparably lesser than the other platinum drug Cisplatin.

**Kate (2018)** in a study on tumours found a decrease in haemoglobin following surgical excision of tumours and attributed the same to stress related to surgery and anaesthesia.

**Ramalingam *et al.* (2018)** stated that Carboplatin was tolerated much better than Cisplatin by most subjects. Carboplatin shows less gastrointestinal and renal toxicosis as compared to Cisplatin, however severe thrombocytopenia occurred more frequently with Carboplatin therapy.

**Rothmann *et al.* (1985)** stated that Platinum compounds particularly Cisplatin caused acute tubular necrosis of tubular cells of kidney, as the major adverse effect of the drug is Nephrotoxicity. The authors also stated that possible causes of anaemia associated with such therapy was decreased Erythropoietin production, damage to haematopoietic stem cells and increased erythrocyte destruction.

**Skeith *et al.* (2020)** found that post-operative thrombocytopenia has numerous causes such as haemodilution, increased platelet consumption in the period after surgery. However thrombocytopenia occurring later could indicate infection of bacterial or fungal origin.

## 2.5 Complications

**Kosovsky *et al.* (1991)** performed partial mandibulectomies, to treat oral tumours in 142 dogs. Few complications were noted immediately post surgery. Return to masticatory function and distortion of cosmetic appearance was determined by mandibulectomy procedure and dog breed. Dogs that underwent bilateral rostral mandibulectomy, suffered from hanging and protrusion of tongue from oral cavity. Suture dehiscence was noted in overall 11 dogs. Some were allowed to heal via secondary intention and some resutured. In most dogs with partial hemimandibulectomy instability of the mandible was reported.

**Wallace *et al.* (1992)** treated maxillary tumours by surgical hemimaxillectomy in 69 dogs. Post operative complications observed were anaemia due to intraoperative haemorrhage in 6 dogs, which resolved over a week post surgery. Symptoms of pain were exhibited by the animal such as pawing of the face and crying. These symptoms were managed by pain medication. Dehiscence of the surgical site was seen in 5 animals, resulting in oronasal fistula. These required surgical closure of the wound.

**Dank *et al.* (2014)** published a retrospective study involving 17 dogs subjected to chemotherapy using carboplatin as a treatment regime for oral malignant melanoma. Seven dogs developed carboplatin associated neutropenia. One dog had thrombocytopenia, 1 showed signs of renal toxicity and 4 developed gastrointestinal toxicity.

## 2.6 Prognosis and Survivability

**Wallace *et al.* (1992)** included only maxillary tumours in dogs during their study and found that osteolysis was caused by both benign and malignant oral tumours. However, this had no significant effect on survival time of the dog. The survival time was mostly determined by the type of tumour. Dogs with melanomas showed lower survival rate and time as compared to dogs with ameloblastoma and squamous cell carcinoma. Post surgical treatment by hemimaxillectomy, 18 dogs with hemi-maxillectomy showed median disease free interval of 21.5 months, and survival rate of 72%, one year from surgery. Two dogs were euthanized on tumour recurrence and one dogs showed local recurrence and metastases 2 years after surgery. Dogs with squamous cell carcinoma had a median survival time of 19.2 months and 1 year survival rate of 57%. 23 dogs with malignant melanoma had a 27% one year survival rate and median survival time of 9.1 months. Local recurrence was noted in almost half the dogs as well as pulmonary metastasis in 26%. Twelve dogs died or were euthanized due to progressive stage of disease. Dogs with fibrosarcoma had median survival time of 12.2 months and a 1 year survival rate of 21%. Of these 8 dogs died or underwent euthanasia due to advanced disease. In case of osteosarcoma, the dogs showed a median survival time of 4.6 months, and 1 year survival rate of 17%. Out of 6 dogs that were affected by osteosarcoma 5 were euthanized or died due to advanced disease. Based on this study survival time was significantly affected only by the age of the dog, tumour type and wide surgical margins. Younger dogs survived longer than old dogs. Dogs with oral ameloblastoma or carcinoma have a better prognosis than dogs with sarcomas.

**Bergman (2007)** stated that primary tumour size is an important factor in determining the prognosis of oral melanoma. However, this is not accurate since the tumour size is not standardized according to breed and patient size. Other prognostic factors are incomplete surgical margins, location of tumour in oral cavity, mitotic index which is greater than 3 and osteolysis.

**Mestrinho *et al.* (2017)** conducted a study involving 13 dogs suffering from squamous cell carcinoma of the oral cavity and evaluating multiple factors in relation with the prognosis of the disease. Dogs were subjected to complete surgical excision with wide margin. Survival time was not affected by age, location or tumour size. High grade tumours resulted in lower survival times of the dogs. This study also evaluated proliferating cell nuclear antigen (PCNA), which is a marker of cell proliferation indicative of potential malignancy of the tumour. Those dogs with a PCNA labelling index of greater than 65% showed lower median survival times.

**Wingo (2018)** based on a retrospective study conducted by the author based of the histopathological diagnosis from a private veterinary dentistry and oral surgery practice, found that squamous cell carcinoma was seen as the most common oral malignancy in the said practice. Metastatic rate of rostral oral SCC was 20% while that of tonsillar and caudal lingual was 73%.

# MATERIALS AND METHODS

The study was undertaken on fifteen clinical cases of tumours of the oral cavity, in dogs presented to the Teaching Veterinary Clinical Complex, Nagpur Veterinary College, Nagpur during the December 2019 to January 2021. Data regarding incidence of oral tumours were obtained from the cases recorded at Teaching Veterinary Clinical Complex, N.V.C, Nagpur from December 2019 to January 2021.

## 3.1 Selection of Animals and Design of Study

The selected dogs were subjected to detailed general clinical, haematological and serum biochemical examinations on the first day of treatment and subsequently as per the plan. Complete anamnesis was collected in all dogs. The prevalence of oral tumours was recorded, based on data collected from the Teaching Veterinary Clinical Complex, Nagpur Veterinary College, Nagpur for the study period.

The oral tumours were excised using LASER and variably, dogs were subjected to adjunct therapy.

## 3.2 Prevalence

All the clinical cases reported at TVCC, NVC, Nagpur were screened for oral masses. The observations on breed, age, sex, tumour location and appearance were recorded. The data, thus collected was evaluated to study the prevalence of oral tumours in dogs brought to the TVCC.

## 3.3 Clinical Examination

The selected dogs were subjected to a thorough examination and the collected data was recorded.

### 3.3.1 Examination of tumour

Observations regarding the appearance of the tumour, such as colour and ulceration were noted. Location of the tumour: gingiva, tonsils, lips, tongue, buccal mucosa, labial mucosa, soft palate, hard palate, was also recorded in all the cases during the study.

### 3.3.2 Haematological parameters

In all animals, two ml of blood was collected from the Cephalic or Saphenous vein in EDTA (Ethylene Diamine Tetra-acetic Acid) vacutainer tubes and mixed well by a rolling motion. A Complete Blood Count (CBC) was performed on 0, $7^{th}$ and $14^{th}$ day of laser surgery and of all cycles of chemotherapy, using an automated Veterinary Haematology Analyser (Model: ABX Micros ESV 60, Company: HORIBA ABX SAS). Haemoglobin (gm/dL), Packed Cell Volume (%), Total Erythrocyte Count (million/cumm), Total Leucocyte Count (thousand/cumm), Differential Leukocyte Count (%) and Platelet count (lakhs/cumm) were studied.

### 3.3.3 Serum biochemical parameters

Blood (4ml) was collected into a clot activator tube on 0, $7^{th}$ and $14^{th}$ day interval of laser surgery and of all cycles of chemotherapy. Tube was allowed to rest for 15 minutes after which it was centrifuged at 3000 RPM for 10 minutes. The separated serum was used for estimation of Serum Alkaline Phosphatase (IU/L), Serum Glutamate Oxaloacetate Transaminase (IU/L), Serum Glutamate Pyruvate Transaminase (IU/L), Blood Urea Nitrogen (mg/dL) and Serum Creatinine (mg/dL) by using Semi-auto Analyser (model: STAR 21) using standard diagnostic kits.

## 3.4 Histopathological Examination of Tumours

Samples collected from the excised tumour were preserved in 10% buffered formalin. After tissue processing, paraffin embedded blocks of tissue were prepared. Thin sections were prepared from the block. The sections were then mounted onto slides and stained using Haematoxylin and Eosin (H&E) stain and histomorphological features of the tumour were recorded to identify the type of tumour.

### 3.5 Tumour Recurrence

In all cases, the recurrence of tumour was assessed till 60$^{th}$ post-operative day by visual observation and palpation at the site.

### 3.6 Equipment

The surgical LASER used in this study was the GaAlAs diode LASER of VELAS series, manufactured by Gigaalaser. Technical Specifications are:

**Table 3.1: Technical Specifications of GaAlAs diode LASER used**

| | |
|---|---|
| Wavelength | 980nm |
| Maximum power | 30W / 60W |
| Operation mode (used) | Continuous wave, single or repeat pulse |
| Transmission system (Fibres) | 200 um ,400 um and 600 um fibers with SMA905 connector |
| Pilot beam | Red diode laser of 635nm, power < 5mW |
| Control mode | True Colour Touch Screen |
| Voltage/ Current rating | 110/240 VAC, 5A, 50/60 Hz |
| Weight | 13.4 kg |

Other details of the LASER unit used -

Components:

- LASER surgical unit’s front panel (Plate 3.1):
  - Colour Touch screen with controls for laser
  - Key lock switch
  - Emergency switch
  - Laser aperture/ optical fibre receptacle
- Back panel (Plate 3.2):
  - Power switch (on/off)
  - Power entry module
  - Foot switch connector
  - Door interlock
  - Main fuse switch
- Optical fibre (Plate 3.3)
- Hand pieces (Plate 3.4)
- Foot switch (Plate 3.5)

**3.7 Treatment**

During the study, all dogs were subjected to surgical excision of the tumour using diode LASER. The dogs were kept off feed and water was withheld for 12 hours prior to surgery. Suitable dogs with malignant oral tumour, were subjected to

## Surgical Laser Unit and Instrumentation

**Plate 3.1:** Front panel of GaAlAs surgical laser unit

**Plate 3.2:** Back Panel of Laser Unit

**Plate 3.3:** Laser optical fibre

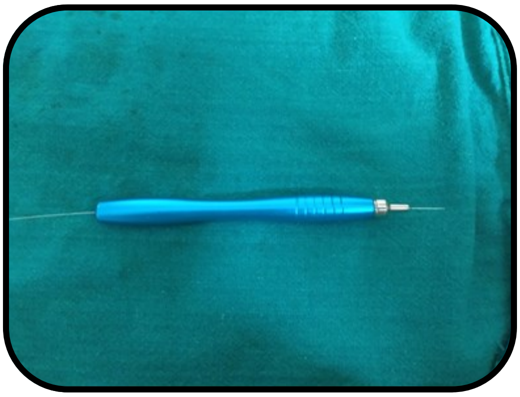

**Plate 3.4:** Handpiece with optical fibre

**Plate 3.5:** Foot Switch Plate

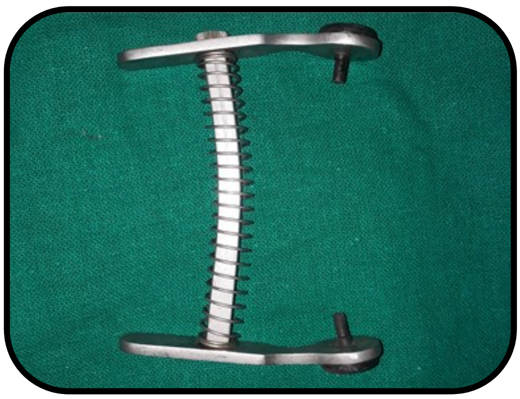

**Plate 3.6:** Gray's mouth gag

adjuvant therapy using antineoplastic chemotherapeutic agents and/or antioxidant therapy, as per the requirement of the case.

### 3.7.1 Premedication and anaesthetic protocol

During the study, prior to sugery all dogs were premedicated using an anticholinergic (Inj. Atropine Sulphate[1] @ 0.04 mg/kg S/C), NSAID (Inj. Meloxicam[2] @ 0.2 mg/kg IM), analgesic (Inj. Tramadol[3] HCl @ 2 mg/kg IM) and an antibiotic injection (combination of Amoxycillin and Cloxacillin[4] @10 mg/kg). Dogs were sedated using Inj. Xylazine Hydrochloride[5] @ 1 mg/kg IM. The dogs during surgery were infused with intravenous crystalloid fluid Normal Saline[6] solution. Anaesthesia was induced using Inj. Ketamine HCl[7] @ 5 mg/kg and Inj. Diazepam[8] @ 0.25 mg/kg, administered through the infusion intravenously.

### 3.7.2 Presurgical preparation

The mouth was kept open after induction of anaesthesia either with the use of a Gray's mouth gag (Plate 3.6) or using sterilized bandages tied behind the canines of both jaws. The oral cavity was prepared antiseptically using, either dilute KMnO4 (1:1000) mouth rinse or 0.2% Chlorhexidine[9] mouth rinse, diluted at 1:1 with sterile water. The dogs were then intubated to ensure and maintain patency of the airway during the surgery.

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[1] Atropine Sulphate - 0.6 mg/ml, Vulcan Laboratories, Kolkata
[2] Melonex- Meloxicam 5 mg/ml, 100 ml, Intas Pharmaceutical Ltd., Ahmedabad (Guj.)
[3] Tramadex - Tramadol 50 mg/ml, 2 ml, Laborate Pharmaceuticals India Ltd., HP
[4] Intamox- D: 3.5g, Amoxicillin- Cloxacillin, Intas Pharmaceutical Ltd., Ahmedabad (Guj.)
[5] Xylaxin- Xylazine Hydrochloride, 20 mg/ml, Indian Immunological Ltd., Mumbai
[6] Normal Saline- 0.9% Normal Saline, Aishwarya Life Sciences, Solan, HP
[7] Ketmin 50- Ketamine Hydrochloride, 50 mg/ml, Themis Medicare Ltd., Haridwar, Uttarakand
[8] Lori- Diazepam, 5mg/ml, Neon Laboratories Ltd., Palghar (Thane), M.S.
[9] Hexidine- Chlorhexidine gluconate 0.2% w/v, ICPA, Andheri, Mumbai

### 3.7.3 Surgical procedure

Once the animal was anaesthetized and asepsis of oral cavity was achieved, the tumour was examined and adherence to the adjacent structures estimated. The laser surgical hand piece with optical fiber passing through it was used analogous to a scalpel. The LASER was operated in Continuous Wave mode and set to a power between 15-20 W and a 2 mm focal spot. The tumour was excised at its base where it was attached (to gingiva, soft palate or buccal mucosa).

The oral cavity was mopped intermittently with sterile gauze so as to clear the field of visualization. Small bleeders were coagulated using the laser tip itself or controlled using pressure with surgical mop. The large blood vessels were ligated using catgut size 1-0 along the way and oral mucosa was sutured using Polyglactin1-0, where required. After tumour excision, dental cleaning was performed and plaque adhered to the teeth was removed. In cases where the periodontal attachments were lost and tooth was loosely fitted in the alveolar socket, teeth extractions were also performed. Following completion of surgery, the oral cavity was thoroughly cleaned and an oral gel containing Tannic Acid and Choline Salicylate[10] was applied at the surgical site. (Plate 3.7 to Plate 3.14)

### 3.7.4 Post operative care

Following surgery all dogs were advised oral antibiotic (Amoxycillin and Potassium Clavulanate[11] @ 15 mg/kg q12h for 7 days), NSAID (Meloxicam[12] @ 0.1 mg/kg q24h for 3 days), analgesic (Tramadol[13] @ 2 mg/kg PO q12h for 3 days). Topically, the owner was instructed to apply the oral gel containing tannic acid and Choline Salicylate for 10 days at the site of excision.

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[10] Oracep gel- 15 ml, Elan Pharma India Pvt. Ltd., Mumbai
[11] Toxomox tablet- 250/500mg, Savavet, Pune, Maharashtra
[12] Inflavet tablet- 2.5 mg, Virbac Animal Health India Pvt. Ltd., Mumbai
[13] Ultrafix T- 50 mg, Corise Healthcare Pvt. Ltd., Thane, Maharashtra

## Laser Excision of Oral Tumour- Surgical Procedure (Plates 2.7 – 2.14 )

**Plate 3.7:** Fibrosarcoma on labial mucosa in a Labrodor retriever

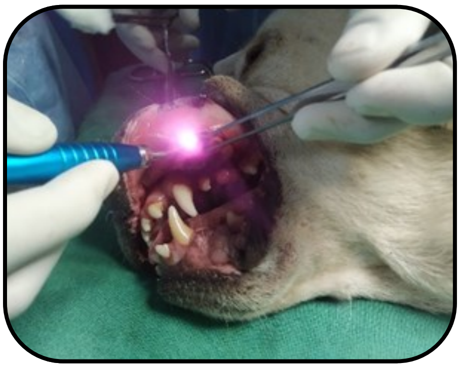

**Plate 3.8:** Dissection of tumour mass using Laser

**Plate 3.9:** Dissection of tumour with minimal haemorrhages

**Plate 3.10:** Removal of dissected mass

**Plate 3.11:** Sealing of small blood vessels using Laser

**Plate 3.12:** Preservation of blood vessel and nerve evident after debulking of tumour mass

**Plates 3.13 and 3.14 :** Suturing of buccal mucosa to cover the dissected cavity

The owners were also instructed to keep the dogs in an Elizabethan Collar, so as to prevent self-mutilation. Dogs with malignant oral neoplasms were advised to be kept on a supplementary antioxidant therapy[14] orally @ 1 ml per 5 kg body weight for 60 days. A regular oral examination was carried out and particularly on 0$^{th}$, 3$^{rd}$, 7$^{th}$, 14$^{th}$ day post-surgery.

### 3.7.5 Complications and recurrence

Complications, if any during and after surgery were recorded. Also, recurrence, if any, was recorded till 60$^{th}$ post-operative day.

### 3.7.6 Chemotherapy

Chemotherapy was used as an adjunctive therapy following laser excision of tumour in case of Malignant Melanoma. Carboplatin *cis*-diammine[15] (1, 1-cyclobutane-dicarboxylatol platinum) was administered as chemotherapeutic drug. The drug was administered slow intravenously @ 150-300 mg/m$^2$ (Dank *et al.,* 2014). The drug was available at a concentration of 10 mg/ml, which was further diluted in 5% Normal Saline and administered over a period of 30-60 minutes, while constantly observing the animal.

Following histopathological confirmation of tumour malignancy, suitable dogs (3) were selected and chemotherapy was started on the 15$^{th}$ day, following surgery. The chemotherapy was given for 4 cycles at the interval of 21 days. The haemato-biochemical parameters were recorded in all dogs on 0, 7$^{th}$ and 14$^{th}$ day of each cycle of chemotherapy. Routine physical examination was also performed to assess response or signs of toxicity.

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[14] Ocoxin - Anti-oxidant supplement, Vivaldis Health and Food Pvt. Ltd., Pune
[15] Carboplatin – Kemocarb 150 mg/15 ml, Fresenius Kabi India Pvt. Ltd., Pune

### 3.8 Statistical Analysis

The data recorded at scheduled time interval after Laser excision of tumour was analyzed by one way ANOVA; whereas, the data recorded after different cycles of chemotherapy was statistically analyzed using two way factorial Completely Randomized Design and discussed using a bar graph (Snedecor and Cochran, 1994).

# RESULTS AND DISCUSSION

The study was conducted on dogs with oral neoplasia, reported to the Teaching Veterinary Clinical Complex, Nagpur Veterinary College, Nagpur . During the study, the prevalence of oral tumours in dogs was recorded and efficacy of surgical excision using LASER, followed by adjuvant chemotherapy as treatment of oral cancer was evaluated. The study was carried out from December 2019 to January 2021. After detailed clinical examination, 15 dogs suffering from both benign and malignant oral neoplasms were included in the study. All dogs first underwent surgical excision of tumour using 980 nm diode LASER, operated under 15-20W power in continuous wave mode. After confirmation of malignant tumour type on the basis of histopathological examination of tumour, three dogs were subjected to Carboplatin chemotherapy and Ocoxin oral liquid was advised as an antioxidant supplement.

The observations on various parameters were recorded in all the dogs for a period of 60 days. The observations recorded during the study are discussed in detail in light of the past work done.

## 4.1 History and Prevalence

Dogs presented with any growth within the oral cavity, first underwent a thorough physical examination, and owners were questioned about history. The common clinical signs observed were haemoptysis, dysphagia, inappetance, excessive salivation and halitosis.

The total number of surgical cases presented to the TVCC between December 2019 and January 2021 were 1732 cases. Total number of animals that underwent surgical procedures were 331. The number of animals that were presented to TVCC with different type of tumours were 138. The prevalence of oral tumours based on all surgical cases was 1.03%. Out of 138 cases of tumours, 13.04% were dogs with oral tumours (n=18). This number is higher than prevalence recorded by different authors such as Duncan and Lascelles (2011) and Cray *et al.* (2020).

Of 18 dogs presented with oral tumours, 15 dogs were selected for this study, based on their suitability and wellbeing to warrant inclusion. Of these 15 dogs, one dog was presented with two oral tumours at the same time, with different gross appearances. For the purpose of estimating prevalence, it will be considered as two tumours. Therefore, the total number of oral tumours included in this study are 16, however number of dogs is considered as 15, so as to avoid error due to repetition.

The different oral tumours presented were recorded in Plates 4.1-4.16. In one case with soft palate tumour, respiratory sounds were evident. Information about age, sex, breed, tumour appearance and location within the oral cavity was collected and compiled in a tabular form (Table 4.1) as below.

**Table 4.1: Prevalence of oral tumours in dogs presented to TVCC**

| Parameter | | Number | Percentage |
|---|---|---|---|
| Age | Less than 8 years | 4 | 26.67% |
| | More than 8 years | 11 | 73.33% |
| Sex | Female | 5 | 33.33% |
| | Male | 10 | 66.67% |
| Breed | Non-Descript / mixed breed | 6 | 40% |
| | Spitz | 2 | 13.33% |
| | Labrador Retriever | 2 | 13.33% |
| | Golden Retriever | 1 | 6.67% |

**Plate 4.7:** D6 - Epulis in a non-descript dog

**Plate 4.8:** D7 - Malignant Melanoma on buccal mucosa in a GSD

**Plate 4.9:** D8 - Acanthomatous Ameloblastoma involving maxilla in a Labrador Retriever

**Plate 4.10:** D9 - Epulis in a Great Dane

**Plate 4.11:** D10 - Acanthomatous ameloblastoma involving the palate in a Spitz

**Plate 4.12:** D11 - Fibrosarcoma involving the palate in a non-descript dog

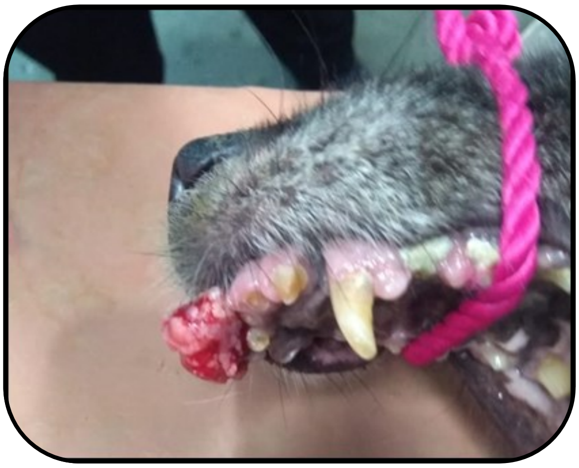


**Plate 4.13:** D12 - Squamous cell carcinoma on gingival border in ND dog

**Plate 4.14:** D13 - Malignant melanoma on buccal mucosa in ND dog

**Plate 4.15:** D14 - Squamous Cell Carcinoma on buccal mucosa in a Golden Retriever

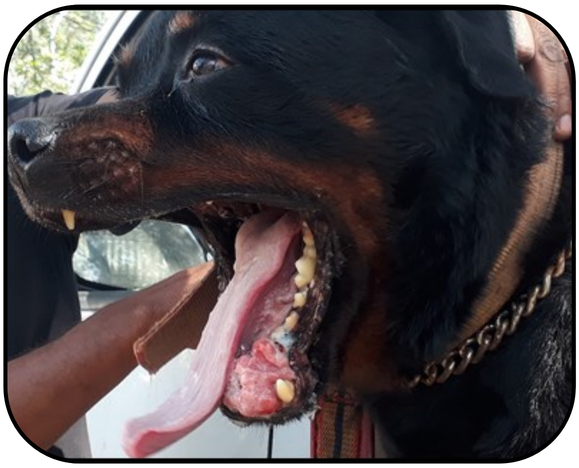


**Plate 4.16:** D15 - Mandibular Acanthomatous Ameloblastoma in a Rottweiler

| | | | |
|---|---|---|---|
| | German Shepherd Dog | 1 | 6.67% |
| | Great Dane | 1 | 6.67% |
| | Boxer | 1 | 6.67% |
| | Rottweiler | 1 | 6.67% |
| Tumour location | Gingiva | 8 | 50% |
| | Palate | 4 | 25% |
| | Buccal mucosa | 3 | 18.75% |
| | Labial mucosa | 1 | 6.25% |
| Tumour appearance | Ulcerative | 10 | 62.5% |
| | Non-ulcerative | 6 | 37.5% |

### 4.1.1 Age

The dogs presented with oral tumours were of the ages ranging from 6 to 15 years, with mean age of 10.43 years and median age of 10 years. Dogs older than 8 years of age formed majority of the pool at 73.33% while dogs younger than 8 years made up 26.67% of total cases. The same is represented in Table 4.1 and Figure 4.1.

These findings are similar to those of Vos and van der Gaag (1987), Kosovsky *et al.* (1991), Wallace *et al.* (1992), Boria *et al.* (2004)**,** Roza *et al.* (2009), Brockley *et al.* (2013), Wingo (2018), Kate (2018), Kshirsagar (2019) and Cray *et al.* (2020).

Increased prevalence of cancer in older dogs has been attributed to number of reasons such as prolonged exposure to carcinogens, decreased immunity with age, hormonal imbalances and collective damage to genes with aging.

### 4.1.2 Sex

A higher number of oral tumour cases were reported in male dogs (66.67%) and much lesser in females (33.33%). The higher tendency of males to develop oral tumours is observed during the study. This data is represented in Table 4.1 and Figure 4.2.

Similar reporting was done by Dorn and Priester (1976), Vos and van der Gaag (1987), Wallace *et al.* (1992), Felizzola *et al.* (1999), Brockley *et al.* (2013), Simons (2015), Kate (2018) and Kshirsagar (2019).

However, Boria *et al.* (2004) reported a greater number of females. Whereas Kosovsky *et al.* (1991), Bergman (2007), Roza *et al.* (2009), Bronden *et al.* (2009), Wingo (2018) and Cray *et al.* (2020) reported no sex predilection in their studies.

The greater percentage of males presented with oral tumours in present investigation could be due to the higher number of male dogs brought to the TVCC. People prefer keeping male dogs rather than female dogs and this could also be attributed as the reason for the present observation.

### 4.1.3 Breed

In the present study, 40% or 6 cases were reported in mixed breed / non-descript dogs. The other breeds presented with oral tumours were Spitz (2), Labrador Retriever (2), Golden Retriever (1), German Shepherd Dog (1), Great Dane (1), Boxer (1), Rottweiler (1). The breed wise distribution is represented in Table 4.1 and Figure 4.3.

These findings were in accordance to studies conducted by Vos and van der Gaag (1987), Felizzola *et al.* (1999) and Brockley *et al.* (2013). Non-descript dogs were most frequently presented to TVCC. This may be the reason why they formed the majority of the population of dogs with oral tumours in this study.

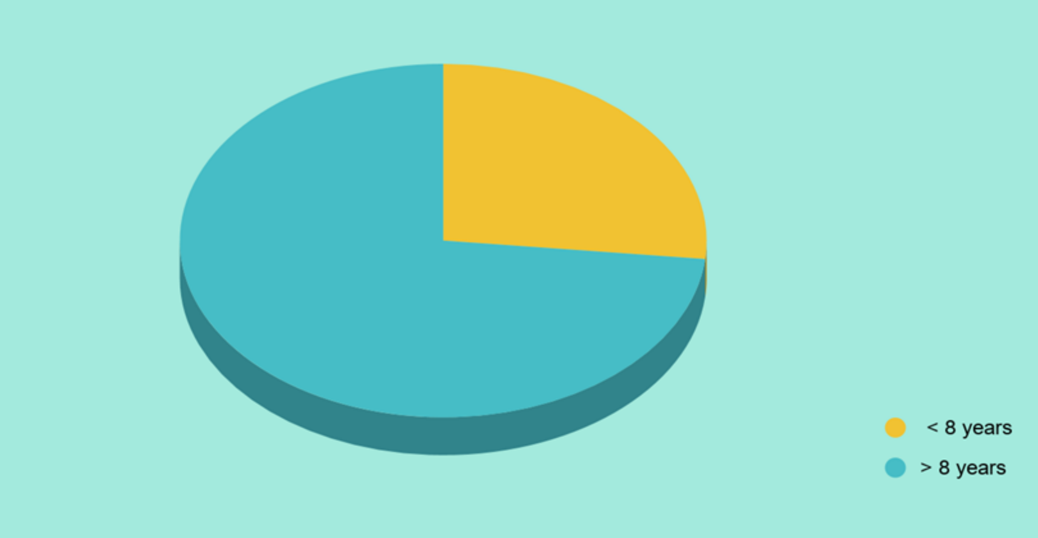


Figure 4.1: Oral tumour incidence in dogs based on age

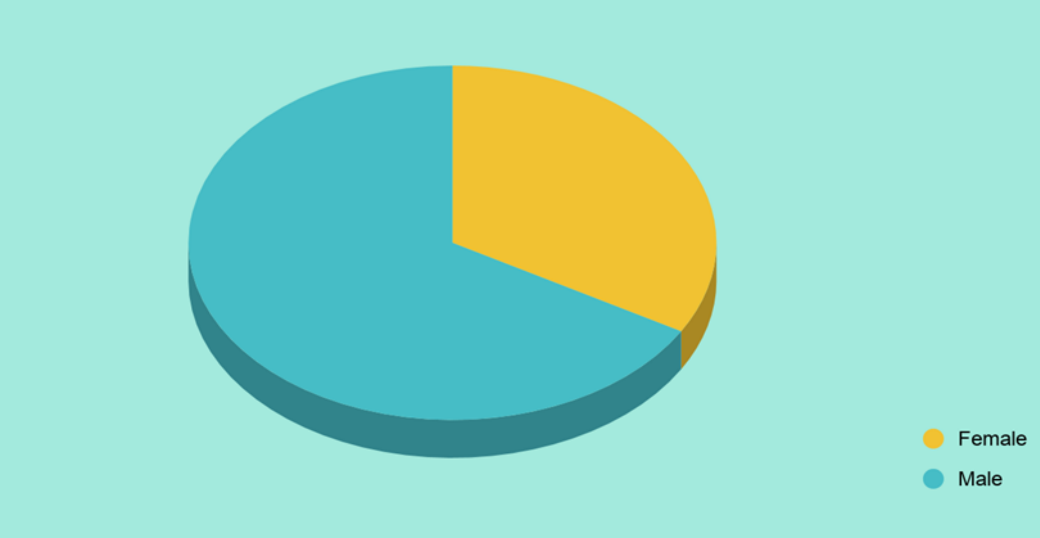


Figure 4.2: Gender based distribution of oral tumours in dogs

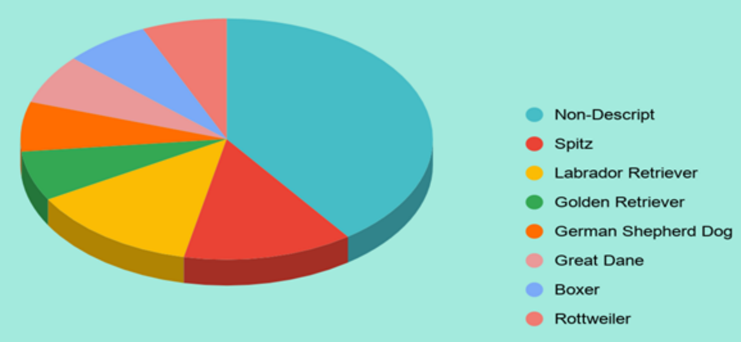



Figure 4.3: Breed wise incidence of oral tumours in dogs presented to TVCC

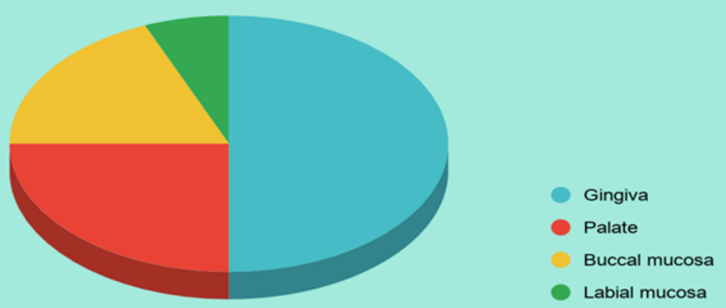



Figure 4.4: Distribution based on location of tumour within oral cavity

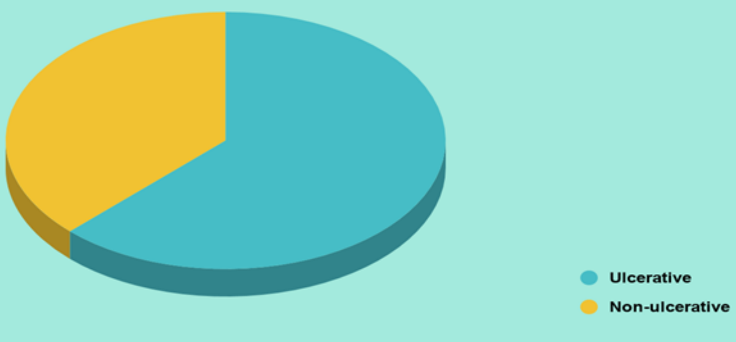



Figure 4.5: Distribution based on visual appearance of oral tumour

### 4.1.4 Tumour location

The most common location of tumour appearance was the gingival border of maxilla or mandible (50%), followed by palate (25%), buccal mucosa (18.75%) and labial mucosa (6.25%). Distribution of cases based on tumour location is represented in Table 4.1 and Figure 4.4.

These findings are in agreement with Vos and van der Gaag (1987), Felizzola *et al.* (1999), Rassnick *et al.* (2001), Boria *et al.* (2004), Roza *et al.* (2009), Bergman (2007) and Cray *et al.* (2020). In the present study, the different locations recorded could not be attributed to any specific reason.

### 4.1.5 Tumour appearance

In the present study, more than 50% oral neoplasms showed ulcerative lesions. Ulcerated neoplasms were found in 10 cases (62.5%) and non ulcerative lesions were found in 6 cases (37.5%). This is represented in Table 4.1 and depicted in Figure 4.5.

It is postulated that due to mastication and constant rubbing of the tumour surface against teeth or feed material, ulceration of oral tumours, is a common occurrence.

## 4.2 Haematological parameters

### 4.2.1 Haemoglobin

Haemoglobin was estimated on the $0^{th}$ day prior to surgery, followed by $7^{th}$ and $14^{th.}$ day. Haemoglobin was also estimated following chemotherapy at similar intervals. The mean Haemoglobin values of all the dogs recorded on different days are represented in Table 4.2 and Figure 4.6.

**Table 4.2: Mean ± SE of haemoglobin gm/dL recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 13.01 ± 0.61 | 12.45 ± 0.64 | 13.29 ± 0.48 |
| Adjuvant Chemotherapy | 03 | 12 | 12.85 ± 0.26[a] | 10.23 ± 0.27[b] | 10.88 ± 0.24[b] |

Different superscripts row-wise wise indicates significance

Mean ± SE haemoglobin value recorded on day 0 following laser excision of tumour was 13.01 ± 0.61 gm/dL. On day 7, the mean haemoglobin value dropped slightly to 12.45 ± 0.64 gm/dL, which again increased by day 14 to 13.29 ± 0.48 gm/dL. The slight decrease in the mean haemoglobin value on 7th day could be attributed to intraoperative haemorrhage as well as stress associated with surgery and anaesthesia. However, there was no statistically significant difference in the mean Haemoglobin values recorded at different time interval.

These findings were in accordance with Medway *et al.* (1969) and Coles (1986) who reported a decrease in Haemoglobin following surgery of highly vascular malignancies. Benjamin (2010), Kate (2018) and Kshirsagar (2019) attributed decreased Haemoglobin to anaesthetic and surgical stress.

The mean haemoglobin value recorded prior to chemotherapy for 3 dogs across 4 cycles was 12.85 ± 0.26 gm/dL. After chemotherapy, on the 7th day the mean haemoglobin value decreased significantly to 10.23 ± 0.27 gm/dL, which increased to 10.88 ± 0.24 gm/dL on the 14th day. There is a statistically significant difference at 5% level of significance (CD= 0.74) in the mean haemoglobin values on the different days. The data accumulated across the 4 cycles of chemotherapy on the various days is depicted in Figure 4.7**.** A decrease was recorded in haemoglobin value on day 7 which typically increased by day 14**,** in each cycle of chemotherapy. There was a significant decline in the mean Haemoglobin with the advancement of chemotherapy.

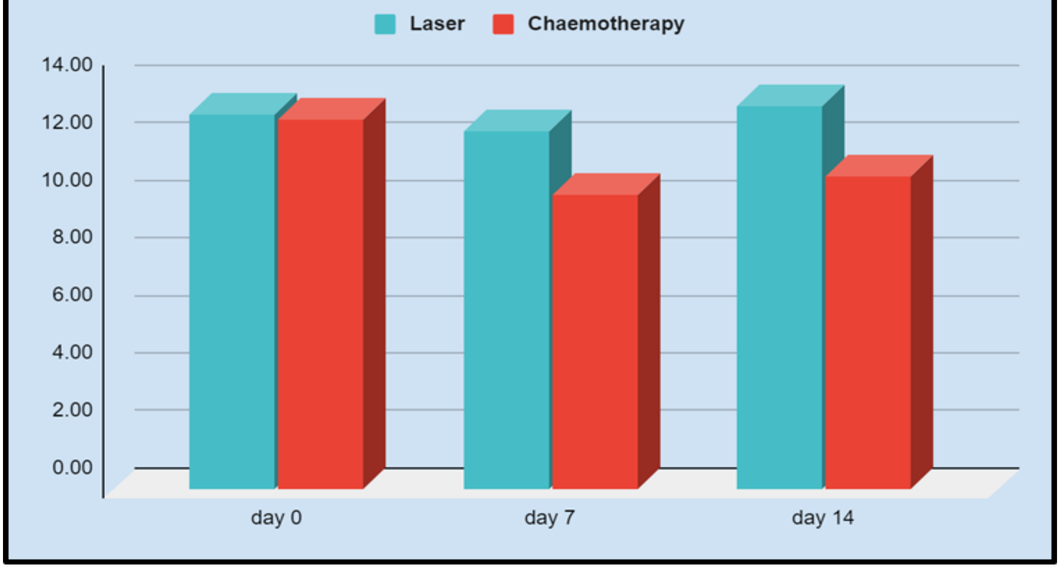



Figure 4.6: Mean Haemoglobin (g/dL) at scheduled intervals for both treatments

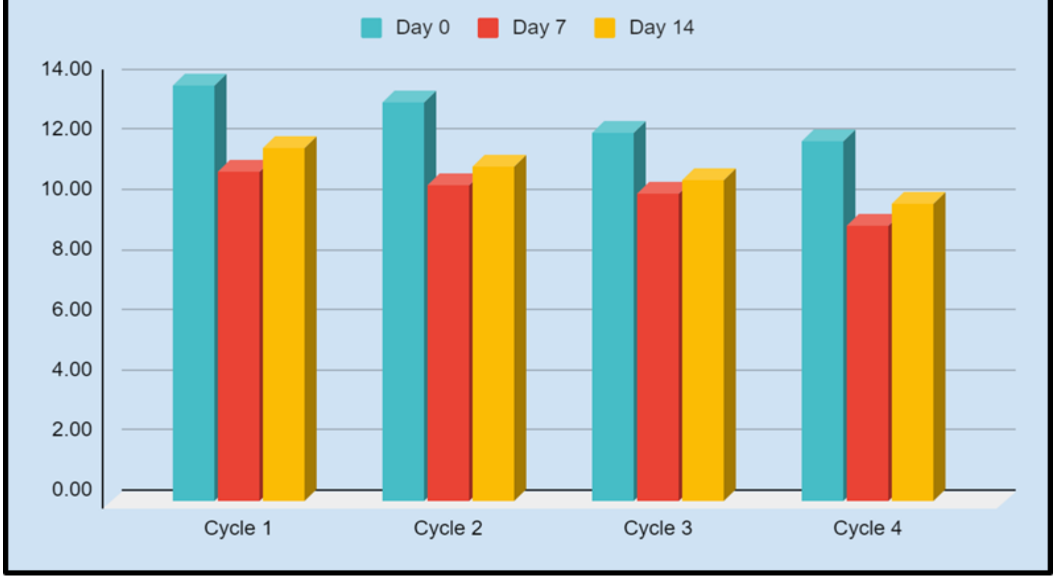



Figure 4.7: Mean Haemoglobin (g/dL) at scheduled intervals during 4 chemotherapy cycles

This could be attributed to the myelosuppression caused by Carboplatin, which suppresses the erythroid progenitor cells in bone marrow (Oberhoff *et al.*, 1998). It could also be associated with the nephrotoxic effect resulting in impairment of erythropoietin producing cells of kidney (Rothmann *et al.*, 1985).Carboplatin associated myelosuppression and nephrotoxicity was mentioned by Rassnick *et al.* (2001), Edelman and Rupard (2006), Chun (2007), Townsend (2007), Brockley *et al.* (2013), Dank *et al.* (2014), Papich (2016) and Woodruff *et al.* (2019).

Thus, the decrease in Haemoglobin in the present study could be attributed to the myelosuppressive and nephrotoxic effects of the chemotherapeutic drug Carboplatin.

### 4.2.2 Total Erythrocyte Count (million/cumm)

Total Erythrocyte Count was estimated on 0, $7^{th}$ and $14^{th}$ day of surgery and chemotherapy. Mean TEC was recorded in all dogs at scheduled intervals and the mean values are presented in Table 4.3 and Figure 4.8.

**Table 4.3: Mean ± SE of Total Erythrocyte Count (million/cumm) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 5.78 ± 0.26 | 5.67 ± 0.26 | 6.01 ± 0.21 |
| Adjuvant Chemotherapy | 03 | 12 | 5.85 ± 0.10[a] | 5.07 ± 0.06[c] | 5.42 ± 0.08[b] |

Different superscripts row-wise wise indicates significance

The mean TEC on day 0 prior to laser excision was 5.78 ± 0.26 millions/cumm. The value thereafter decreased to 5.67 ± 0.26 millions/cumm on day 7. On day 14 the mean TEC value recorded was 6.01 ± 0.21 millions/cumm. The change in the mean TEC values on day 0, 7 and 14 were statistically non-significant. All the values during the study were within the physiological reference range.

The slight decrease in TEC value on day 7 could be attributed to loss of blood during surgery and also because of the stress induced by anaesthesia and surgery.

Similar findings were reported by Medway *et al.* (1969), Coles (1986), Benjamin (2010) and Kshirsagar (2019).

The TEC was recorded on day 0, 7 and 14 in all dogs subjected to chemotherapy during cycle 1 to cycle 4. The haemoglobin value on day 0 was 5.85 ± 0.10 millions/cumm in the dogs given chemotherapy. The value showed decrease in the mean TEC value on $7^{th}$ day to 5.07 ± 0.06 millions/cumm. The mean value of all cycles on $14^{th}$ day was 5.42 ± 0.08 millions/cumm. The critical difference calculated at 5% level of significance was 0.23. There was a statistically significant ($P<0.05$) change in mean TEC values. A similar undulating trend was recorded at the scheduled intervals in each cycle of chemotherapy. A steady decline in the mean TEC value can be observed with advancement of Carboplatin therapy cycles and thus the lowest TEC is recorded following the fourth cycle of chemotherapy for each dog. These trends are presented in Figure 4.9.

Similar effects of chemotherapy were also reported by Edelman and Rupard (2006), Chun (2007), Townsend (2007), Sharma *et al.* (2010) and Papich (2016).

The decrease in TEC, following Carboplatin therapy might be due to the myelosuppressive effects as well as mild nephrotoxicity associated with carboplatin administration.

**4.2.3 Packed Cell Volume (Percent)**

In the present study, Packed Cell Volume was recorded on day 0, 7 and

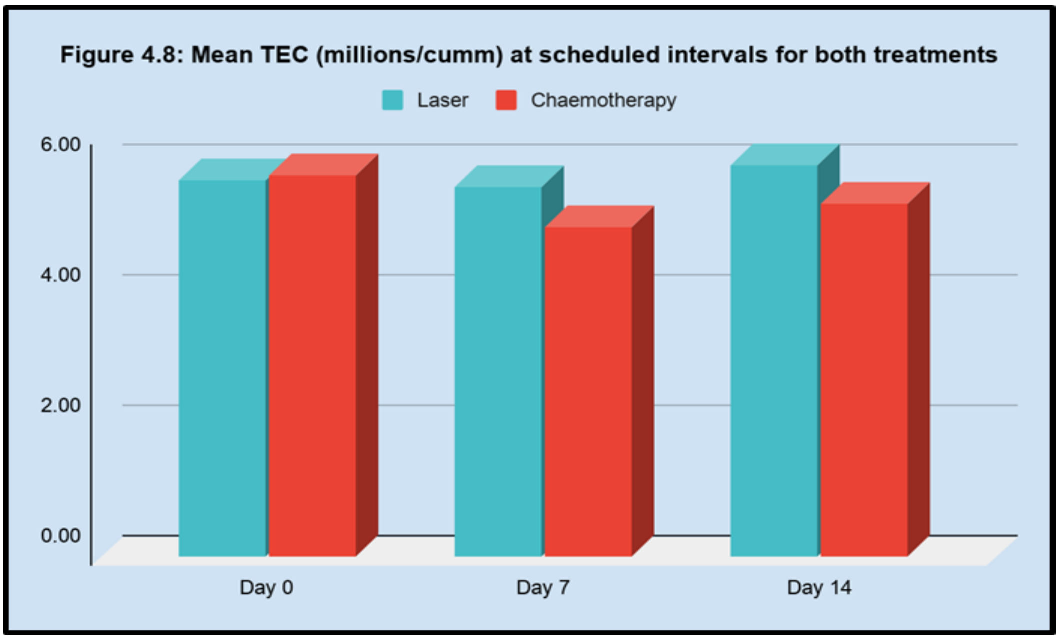


Figure 4.8: Mean TEC (millions/cumm) at scheduled intervals for both treatments
Laser
Chaemotherapy
6.00
4.00
2.00
0.00
Day 0
Day 7
Day 14

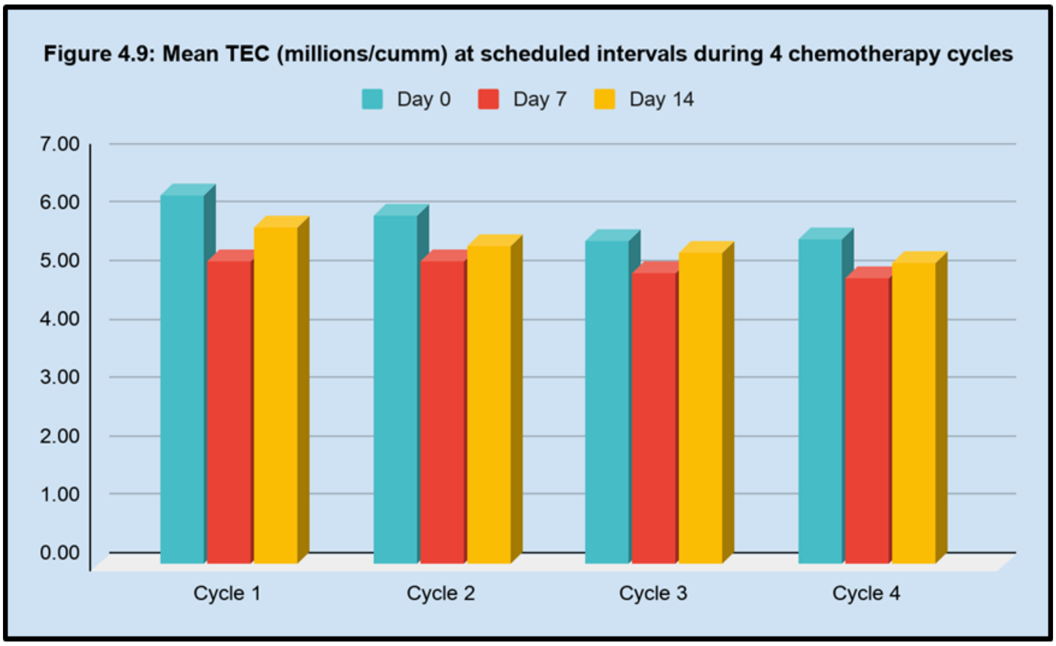


Figure 4.9: Mean TEC (millions/cumm) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Cycle 1
Cycle 2
Cycle 3
Cycle 4

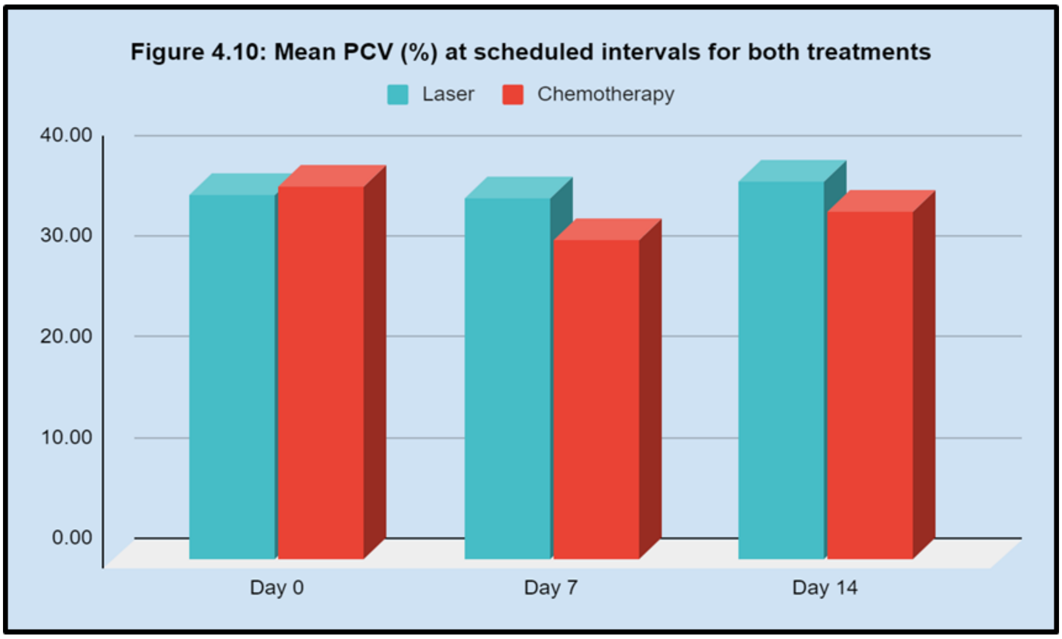


Figure 4.10: Mean PCV (%) at scheduled intervals for both treatments
Laser
Chemotherapy
40.00
30.00
20.00
10.00
0.00
Day 0
Day 7
Day 14

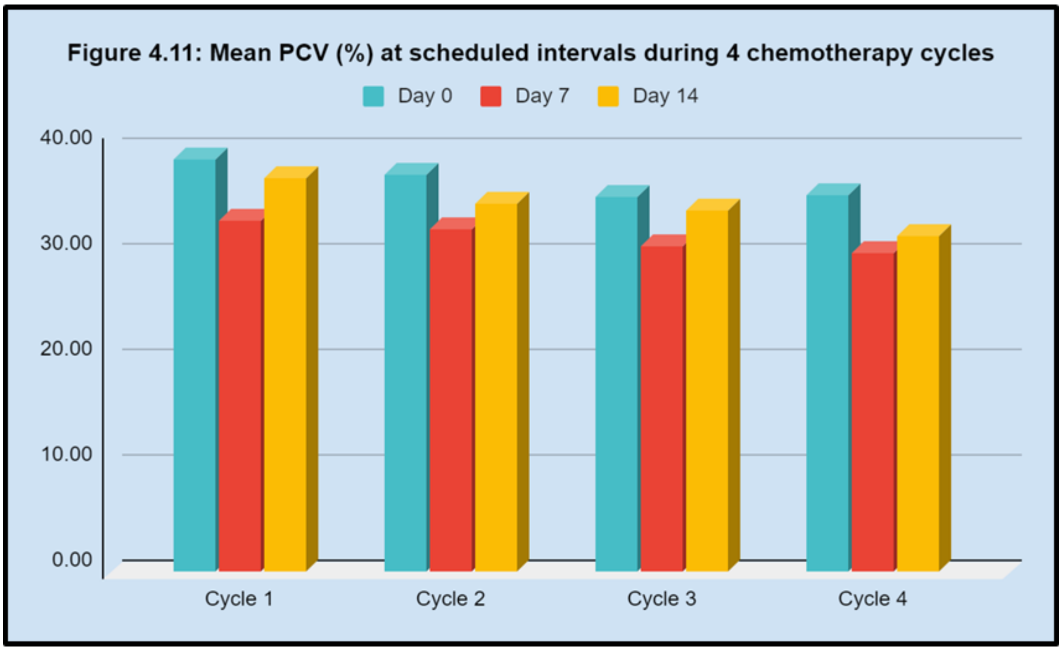


Figure 4.11: Mean PCV (%) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
40.00
30.00
20.00
10.00
0.00
Cycle 1
Cycle 2
Cycle 3
Cycle 4

14 following surgery as well as chemotherapy. The mean value is calculated and presented in Table 4.4 and Figure 4.10.

**Table 4.4: Mean ± SE of Packed Cell Volume (Percent) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 36.19 ± 1.63 | 35.98 ± 1.73 | 37.49 ± 1.29 |
| Adjuvant Chemotherapy | 03 | 12 | $37.02 \pm 0.50^{a}$ | $31.73 \pm 0.51^{c}$ | $34.58 \pm 0.63^{b}$ |

Different superscripts row-wise wise indicates significance

The mean packed cell volume on day 0 prior to laser excision of tumour was 36.19 ± 1.63%. Mean PCV on day 7 was 35.98 ± 1.73% and 37.49 ± 1.29% on day 14. The changes in the mean packed cell volume were non-significant and within normal physiological reference range.

All the recorded values were within the normal physiological range. The changes during the present study are in agreement with the findings of Medway *et al.* (1969), Coles (1986), Benjamin (2010) and Kshirsagar (2019).

The decreased PCV on day 7 could be attributed mainly to surgical stress, as well as anaesthetic stress that would have caused erythrocyte sequestration and thereby a decreased haematocrit, as also stated by Wilson *et al.* (2004).

PCV was recorded at the scheduled intervals during each cycle of chemotherapy. Mean ± SE PCV was calculated for day 0, 7 and 14 across all cycles. On day 0, before chemotherapy, the mean ± SE PCV was 37.02 ± 0.50%. The values on day 7 and day 14 were 31.73 ± 0.51% and 34.58 ± 0.63%, respectively. The critical difference calculated at 5% level of significance was 1.58. The changes noted in mean PCV were statistically significant ($P < 0.05$).

There was a decrease in PCV by the end of the last cycle, as compared to the value prior to the start of chemotherapy. There was a decrease in mean PCV on day 7 which increased by day 14, in all cycles. These changes are graphically depicted in Figure 4.11.

Such effects of chemotherapy have been described by Edelman and Rupard (2006), Chun (2007), Townsend (2007) and Papich (2016).

The decreased PCV following chemotherapy could be explained due to the myelosuppression following Carboplatin therapy and the impaired renal function following chemotherapy.

### 4.2.4 Total Leukocyte Count (thousand/cumm)

Complete Blood Count including Total leukocyte count was conducted on Day 0, 7 and 14 of laser excision of the oral tumour, as well as chemotherapy.

The arithmetic mean of TLC values was calculated for the respective days and the same is depicted in Table 4.5 and Figure 4.12.

**Table 4.5: Mean ± SE of Total Leukocyte Count (thousand/cumm) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 12.75 ± 0.96$^{b}$ | 16.54 ± 0.98$^{a}$ | 13.86 ± 0.85$^{b}$ |
| Adjuvant Chemotherapy | 03 | 12 | 14.44 ± 0.44$^{a}$ | 12.18 ± 0.46$^{b}$ | 10.48 ± 0.33$^{c}$ |

Different superscripts row-wise wise indicates significance

In the present study, the mean TLC on day 0, 7 and 21 of Laser excision of tumour was 12.75 ± 0.96, 16.54 ± 0.98 and 13.86 ± 0.85 thousand/cumm, respectively. The critical difference for the intervals, at 5% level of significance was

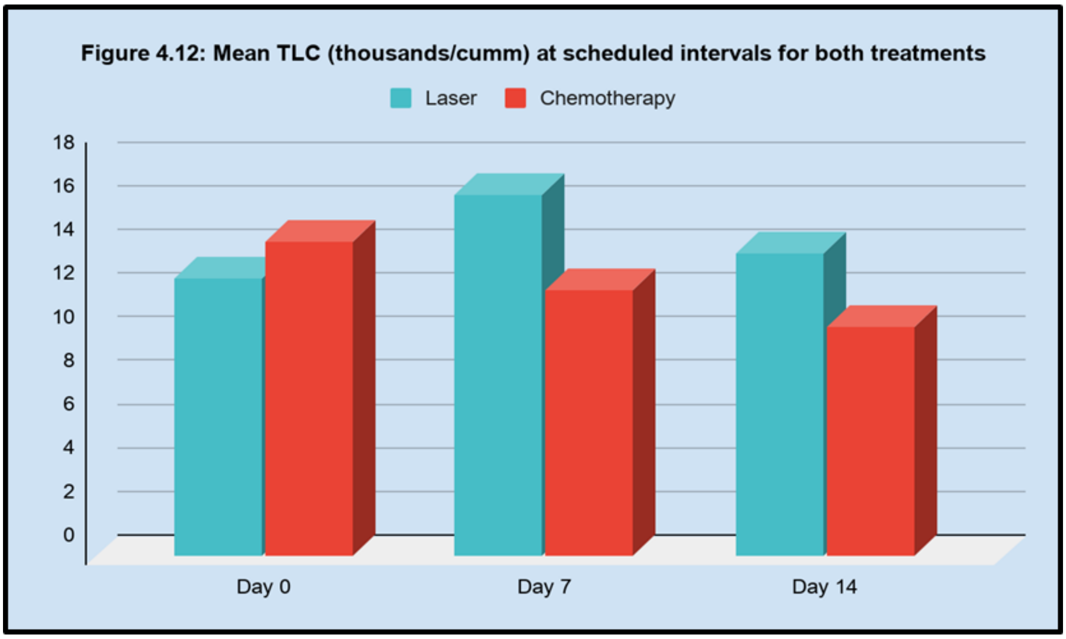


Figure 4.12: Mean TLC (thousands/cumm) at scheduled intervals for both treatments
Laser
Chemotherapy
18
16
14
12
10
8
6
4
2
0
Day 0
Day 7
Day 14

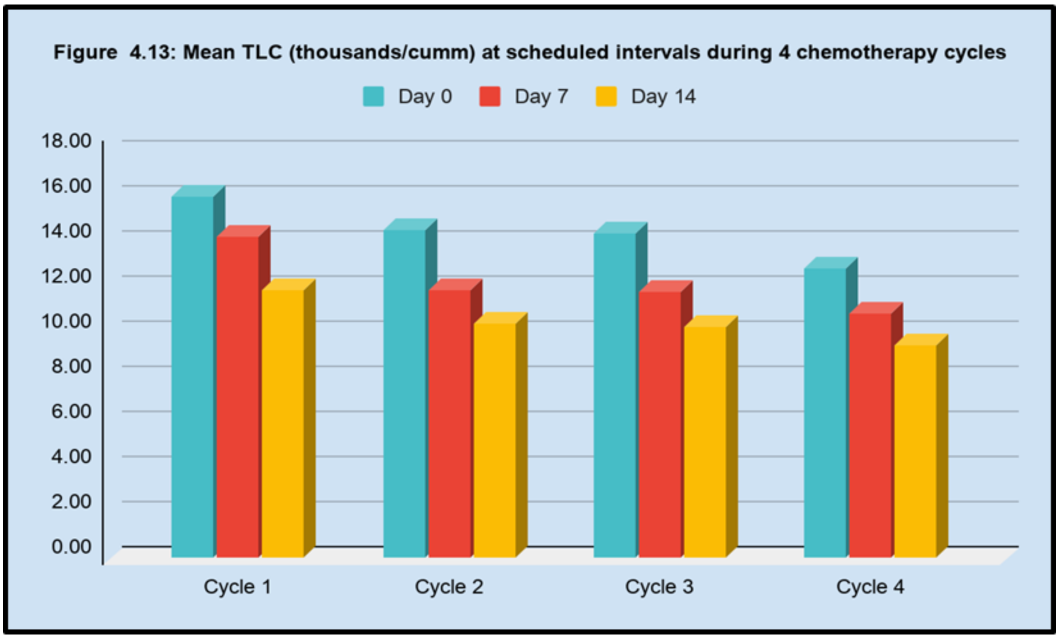


Figure 4.13: Mean TLC (thousands/cumm) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Cycle 1
Cycle 2
Cycle 3
Cycle 4

calculated as 2.665. There was a significant difference ($P < 0.05$) between the mean of the TLC values estimated on day 7, as compared to day 0 and day 14. However, it was within normal physiological range. The mean TLC on $7^{th}$ day was increased, which later decreased on $14^{th}$ day.

Leukocytosis is expected in the early postoperative period and may be a sign of developing infection or as a result of normal surgical stress response, as stated by Cohen *et al.* (2013) and Jung *et al.* (2019).

Leukocyte count was estimated on day 0, 7 and 14 during each cycle of chemotherapy. The mean leukocyte count on day 0, before chemotherapy was 14.44 ± 0.44 thousands/cumm. There was a decrease in the mean TLC by day 7 to 12.18 ± 0.46 thousands/cumm, which further decreased to 10.48 ± 0.33 thousands/cumm by day 14. The changes observed were statistically significant at 5% level of significance (CD = 1.20).

Figure 4.13 represents the decreasing trend observed in each cycle with progression of days following Carboplatin administration. The figure represents that following each cycle there is a decrease in TLC on day 7 which further decreases by day 14, either marginally or significantly. An overall declining trend in TLC is observed with each subsequent chemotherapy cycle. In each dog the TLC recorded at the end of all 4 cycles is significantly lower than that recorded prior to the start of chemotherapy.

Decrease in the total leukocyte count following chemotherapy is expected due to the myelosuppressive effects of cytotoxic chemotherapeutic drugs. Similar effects were stated by Edelman and Rupard (2006), Chun (2007), Townsend (2007), Ehrhart *et al.* (2013) and Papich (2016) in chemotherapy with Carboplatin.

#### 4.2.5 Differential Leukocyte Count

Differential Leukocyte Count was estimated on day 0, 7 and 14 of surgery and chemotherapy. The different counts of Neutrophil, Lymphocytes, Monocyte and Eosinophils were estimated and are expressed in percent. The observations and conclusions are recorded as below.

##### 4.2.5.1 Neutrophil (Percent)

Neutrophil was estimated on day 0, 7 and 14 both before and after Laser surgery and chemotherapy using Carboplatin. Arithmetic mean of all dogs was calculated at different scheduled intervals and represented in Table 4.6 and Figure 4.14.

**Table 4.6: Mean ± SE of Neutrophil (Percent) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | $75.13 \pm 1.84^{b}$ | $81.03 \pm 1.18^{a}$ | $74.98 \pm 1.19^{b}$ |
| Adjuvant Chemotherapy | 03 | 12 | $73.13 \pm 0.71^{a}$ | $66.84 \pm 0.70^{b}$ | $64.15 \pm 1.20^{c}$ |

Different superscripts row-wise wise indicates significance

Mean of percent neutrophils on day 0 before laser excision of tumour was 75.13 ± 1.84%. Mild neutrophilia was observed and the mean percent neutrophils on $7^{th}$ post surgery day was 81.03 ± 1.18%, which resolved and the mean neutrophil count by day 14 was 74.98 ± 1.19%. The critical difference calculated at 5% level of significance was 4.09. There was a statistically significant difference ($P<0.05$) observed between the days, in the mean TLC value. Day 7 value was not within physiological reference range and can be attributed to inflammatory changes or an infection of surgical wound. The Neutrophilia however resolved by day 14 in all cases.

**Figure 4.14: Mean Neutrophil count (%) at scheduled intervals for both treatments**

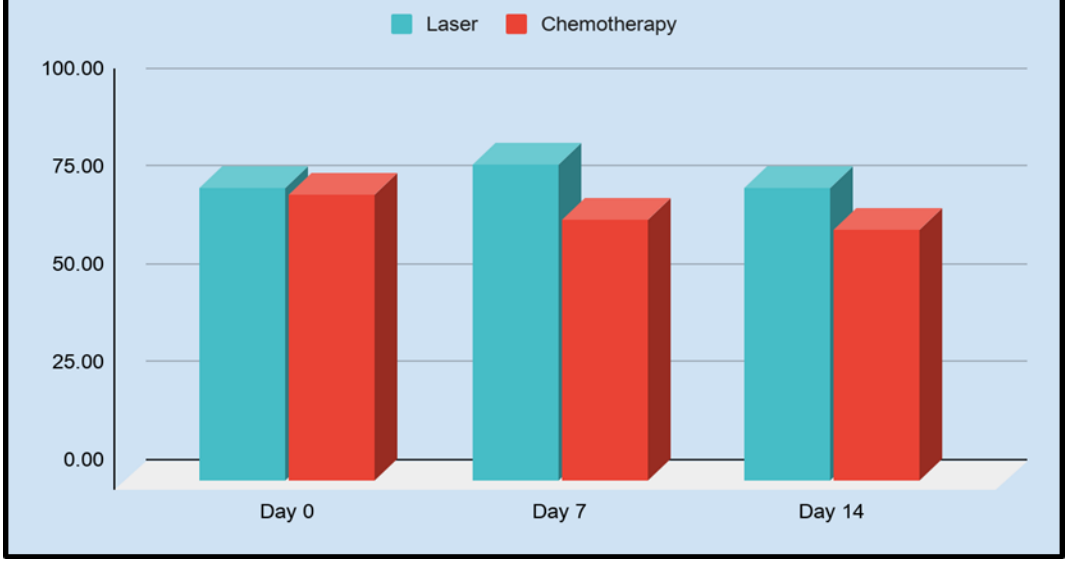
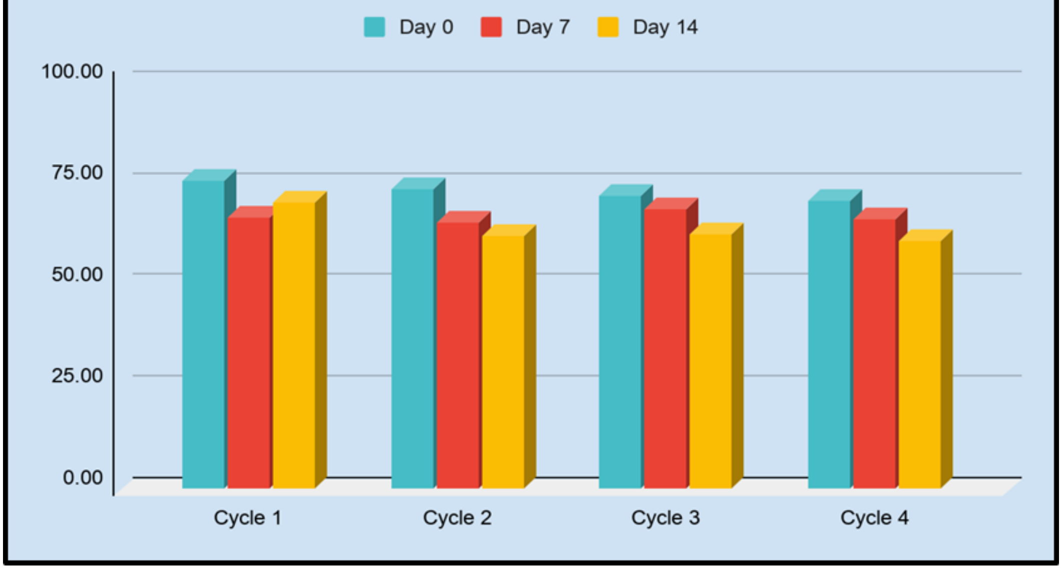



**Figure 4.15: Mean Neutrophil Count (%) at scheduled intervals during 4 chemotherapy cycles**



Neutrophil count was studied on day 0, 7 and 14 of every chemotherapy treatment cycle. The mean values of neutrophil on the various days from the 4 cycles were recorded. The mean Neutrophil count on day 0 was 73.13 ± 0.71%, which was decreased to mean percentage of 66.84 ± 0.70% on day 7. The mean value on day 14 was 64.15 ± 1.20%. The critical difference calculated at 5% level of significance was 2.60. Thus the changes in the mean neutrophil percent were statistically significant ($P < 0.05$).

The mean percentage of neutrophils at different scheduled intervals during the 4 cycles of chemotherapy is depicted in Figure 4.15. Except for the first cycle that showed an undulating trend, all the cycles showed a decreasing trend following each cycle of chemotherapy. A significant decrease in the neutrophil percent was observed by the end of chemotherapy. Mean Neutrophil percent on day 7 was decreased, following chemotherapy, which decreased further by the $14^{th}$ day, in all but the first cycle of chemotherapy.

This trend of neutropenia during the present study could be explained by the myelosuppressive effects of Carboplatin. The neutrophil nadir is usually reached by $10^{th}$ - $14^{th}$ day following chemotherapy, which may in some cases occur by day 21. After the nadir, the neutrophil values usually increase after 1-3 days (MacDonald, 2009).

Neutropenia associated with Carboplatin therapy has been discussed by Edelman and Rupard (2006), Chun (2007), Townsend (2007), Dobson *et al.* (2008), Ehrhart *et al.* (2013) and Papich (2016).

#### 4.2.5.2 Lymphocyte (Percent)

Differential leukocyte count was estimated on 0, $7^{th}$ and $14^{th}$ day of surgery and chemotherapy. Mean of percent lymphocytes was calculated at scheduled intervals and presented in Table 4.7 and Figure 4.16.

**Table 4.7: Mean ± SE of Lymphocyte (Percent) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 16.71 ± 1.37[a] | 12.71 ± 0.97[b] | 16.29 ± 0.82[a] |
| Adjuvant Chemotherapy | 03 | 12 | 16.71 ± 0.63[b] | 22.89 ± 0.83[a] | 25.23 ± 1.22[a] |

Different superscripts row-wise wise indicates significance

Mean percent of lymphocytes on day 0 prior to laser excision of tumour was 16.71 ± 1.37%. The value on 7[th] day was slightly decreased and the value was 12.71 ± 0.97% which increased by 14[th] day to 16.29 ± 0.82%. The critical difference calculated for 5% level of significance is 3.086. The changes in the mean lymphocyte percent are statistically significant ($P<0.05$) at this level. The values are however within physiological reference range.

The decrease observed in percent lymphocytes on 7[th] day could be associated with concurrent increase in neutrophil count due to inflammatory changes following surgery.

Lymphocyte count was recorded at the scheduled intervals during all cycles of chemotherapy. The mean ± SE lymphocyte count was calculated from lymphocyte count estimated on the scheduled 3 days during all 4 cycles of chemotherapy. The lymphocyte count on day 0 was 16.71 ± 0.63%. There was an apparent increase to 22.89 ± 0.83% on day 7. The mean lymphocyte count on day 14 was 25.23 ± 1.22%. The changes were statistically significant at 5% level of significance (CD = 2.67).

Figure 4.17 depicts the trend in percent Lymphocytes across the 4 cycles of chemotherapy with mean lymphocytes calculated for the scheduled days. There appeared to be an increasing trend in Lymphocyte percent with advancement of

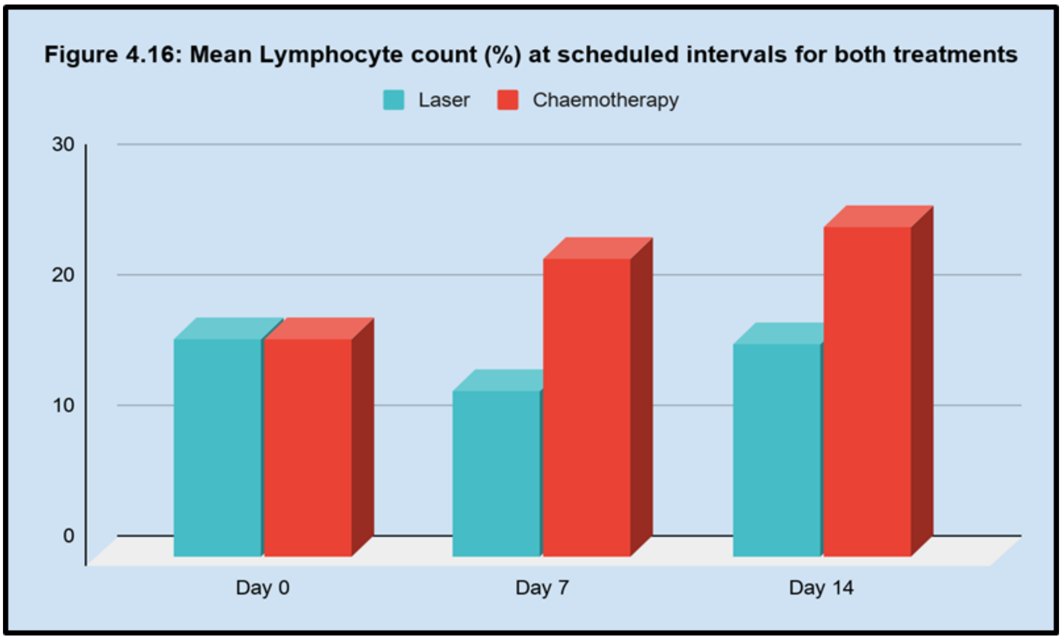


Figure 4.16: Mean Lymphocyte count (%) at scheduled intervals for both treatments
Laser
Chaemotherapy
30
20
10
0
Day 0
Day 7
Day 14

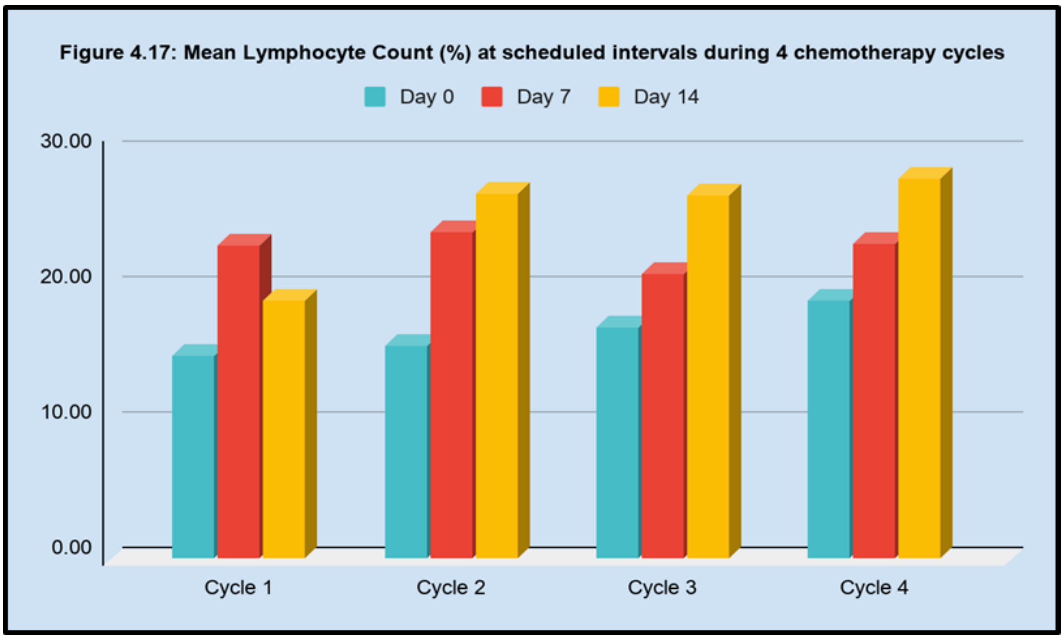


Figure 4.17: Mean Lymphocyte Count (%) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
30.00
20.00
10.00
0.00
Cycle 1
Cycle 2
Cycle 3
Cycle 4

chemotherapy, this could however be justified based on the concurrent Neutropenia that developed as a result of chemotherapy and was not true lymphocytosis.

**4.2.5.3 Monocyte (Percent)**

Mean of percent monocytes was calculated for day 0, 7 and 14 of laser excision of tumour and chemotherapy. It is depicted in Table 4.8 and Figure 4.18.

**Table 4.8: Mean ± SE of Monocyte (Percent) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 5.03 ± 0.49 | 3.83 ± 0.27 | 4.65 ± 0.41 |
| Adjuvant Chemotherapy | 03 | 12 | 5.23 ± 0.16 | 5.25 ± 0.15 | 5.33 ± 0.12 |

The mean value of monocyte (percent) on day 0 before surgery was 5.03 ± 0.49%. A slight decrease in monocyte count on 7$^{th}$ day was observed and the value was 3.83 ± 0.27%. The mean monocyte count on 14$^{th}$ day was 4.65 ± 0.41%. A non-significant undulating trend, within normal physiological range was noted in the mean monocyte percentage.

The trend could be correlated to the concurrent change in the neutrophil percent associated with inflammatory changes following surgery.

The mean monocyte count was calculated for the scheduled days during the different cycles of chemotherapy. The mean monocyte count on day 0 was 5.23 ± 0.16%. The mean monocyte count following chemotherapy, on day 7 and 14 were 5.25 ± 0.15% and 5.33 ± 0.12%, respectively. The changes were non-significant at different scheduled intervals and all values were within the normal physiological range.

The mean monocyte count for each of the scheduled days during the different cycles of chemotherapy is depicted in Figure 4.19.  During the study, the irregular trend noted could be attributed to the changes in Neutrophil value.

**4.2.5.4 Eosinophil (Percent)**

Arithmetic mean of the eosinophil count estimated on day 0, 7 and 14 following surgery and chemotherapy was calculated. The values are presented in depicted in table 4.9 and figure 4.20

**Table 4.9: Mean ± SE of Eosinophil (Percent) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 4.41 ± 0.29 | 3.47 ± 0.35 | 4.09 ± 0.22 |
| Adjuvant Chemotherapy | 03 | 12 | 4.93 ± 0.18 | 5.02 ± 0.16 | 5.25 ± 0.12 |

The mean eosinophil count on day 0 before laser excision of tumour was 4.41 ± 0.29% which decreased slightly to 3.47 ± 0.35% on day 7. However, the count on day 14 increased marginally and the value was 4.09 ± 0.22%. An undulating trend was noted in the eosinophil count. These changes were non-significant and within normal physiological limits. The changes could be attributed to the concurrent changes in percent neutrophil that occurred as a result of inflammatory changes following surgery which ultimately reflected on the eosinophil count.

The mean eosinophil count of all dogs, calculated for 4 cycles of chemotherapy on day 0 was 4.93 ± 0.18%. The mean counts on day 7 and 14 were 5.02 ± 0.16% and 5.25 ± 0.12%, respectively.

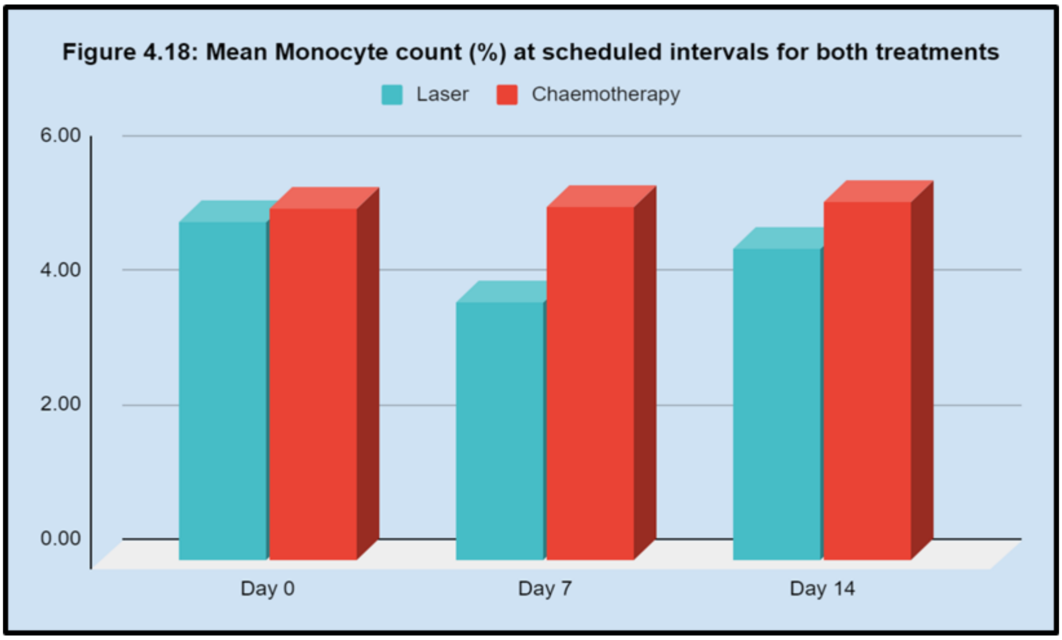



Figure 4.18: Mean Monocyte count (%) at scheduled intervals for both treatments

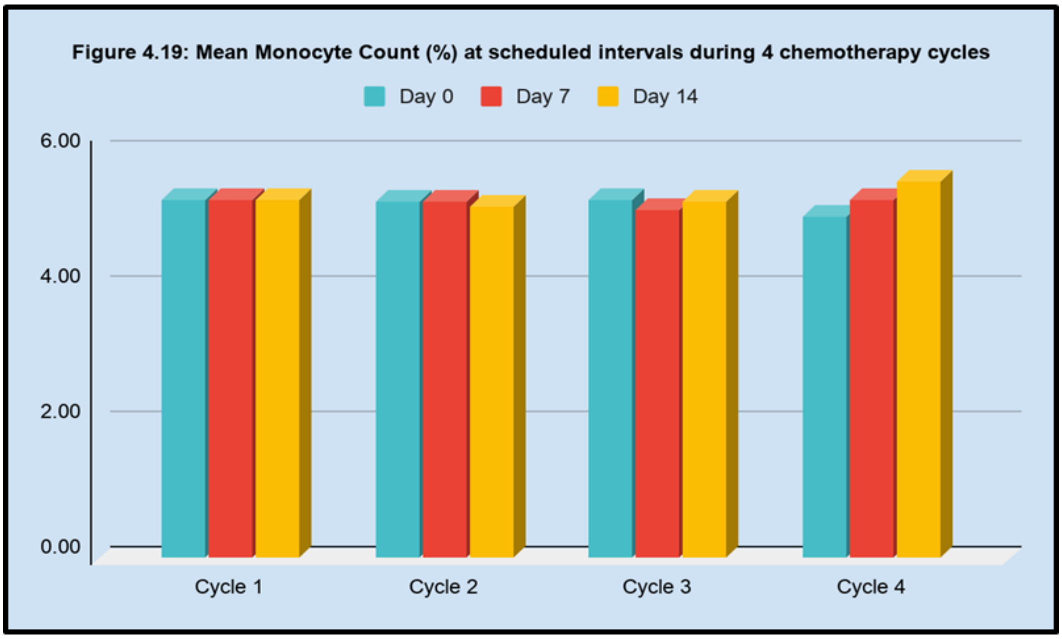



Figure 4.19: Mean Monocyte Count (%) at scheduled intervals during 4 chemotherapy cycles

Figure 4.20: Mean Eosinophil count (%) at scheduled intervals for both treatments

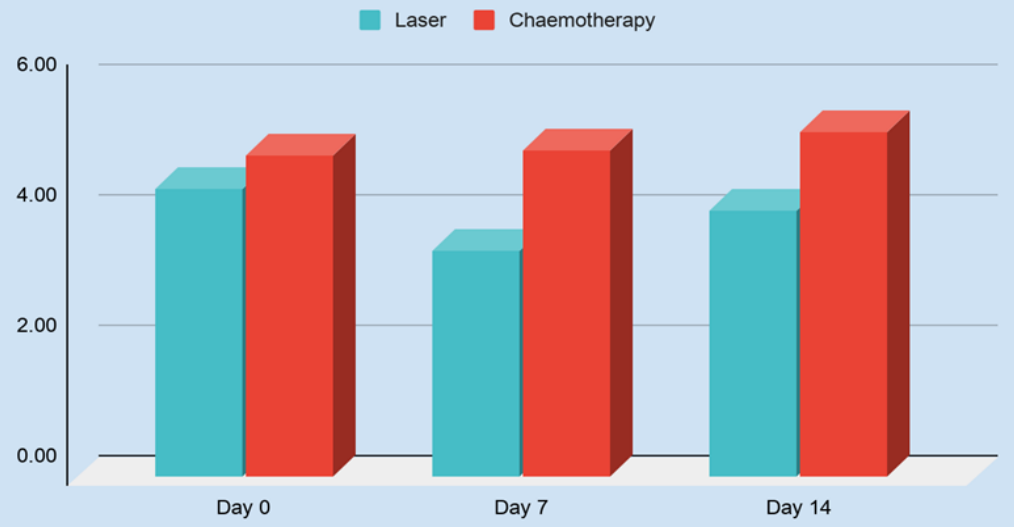
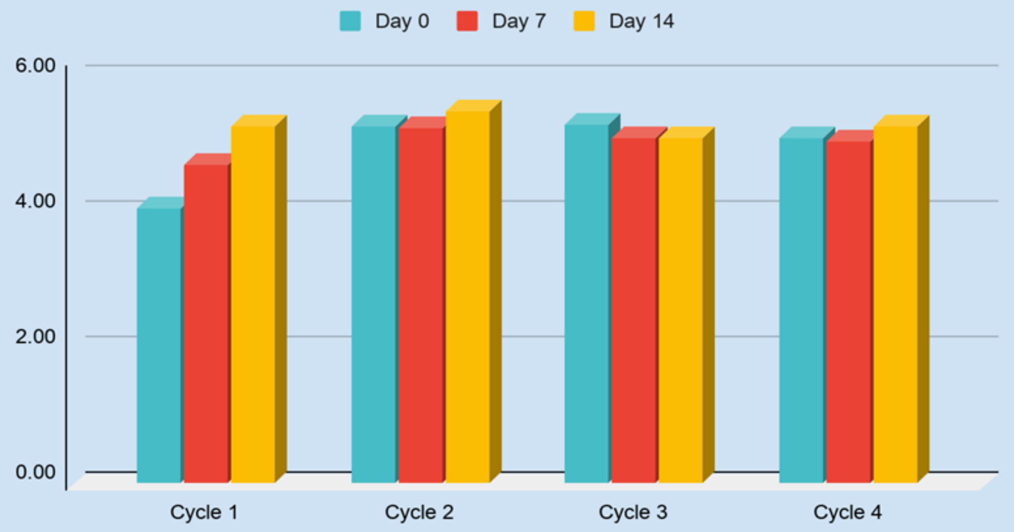



Figure 4.21: Mean Eosinophil Count (%) at scheduled intervals during 4 chemotherapy cycles



The mean eosinophil count on each of the scheduled days for each chemotherapy cycle is depicted in figure 4.21. An undulating trend was noted, however, it was within the normal physiological range and no specific reason could be attributed to these observations, other than fluctuations as a result of the concurrent Neutropenia.

### 4.2.6 Platelet count (lakh/cumm)

Platelet count was estimated on day 0, 7 and 14 for all animals following both tumour excision and chemotherapy. Arithmetic mean of all dogs for the different days was calculated and depicted in Table 4.10 and Figure 4.22.

**Table 4.10: Mean ± SE of Platelet Count (lakh/cumm) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 3.30 ± 0.33 | 3.05 ± 0.25 | 3.08 ± 0.23 |
| Adjuvant Chemotherapy | 03 | 12 | 2.58 ± 0.12$^{a}$ | 2.07 ± 0.09$^{b}$ | 1.53 ± 0.06$^{c}$ |

Different superscripts row-wise wise indicates significance

The mean platelet count on day 0 was 3.30 ± 0.33 lakh/cumm. The mean count decreased on 7$^{th}$ day and was 3.05 ± 0.25 lakh/cumm. The count thereafter slightly increased by day 14 and was 3.08 ± 0.23 lakh/cumm. The changes were statistically non- significant and within the reference range.

The mild decrease in the platelet count could be attributed to haemodilution following intravenous fluid infusion or perioperative platelet consumption. Similar findings were stated by Skeith *et al.* (2020).

The mean platelet count on day 0 in dogs subjected to chemotherapy was 2.58 ± 0.12 lakh/cumm. The mean platelet count on day 7 was 2.07 ± 0.09 lakh/cumm. The platelet count further decreased to 1.53± 0.06 lakh/cumm by day 14. The critical difference calculated at 5% level of significance was 0.26. Thus the changes were statistically significant.

This decreasing trend and significant change observed in the mean platelet count following chemotherapy is explained by the thrombocytopenia caused by carboplatin therapy. The mean platelet count calculated for the different cycles of chemotherapy on the scheduled days is represented in Figure 4.23. A significant decrease was observed in the mean platelet count following chemotherapy, the thrombocyte count following the last cycle of chemotherapy being significantly low, when compared with the count on day 0 of $1^{st}$ cycle.

Decrease in the platelet count is one of the adverse effects of Carboplatin drug, which causes myelosuppression and thereby decreased platelet production. Similar effects of Carboplatin therapy have been discussed by Edelman and Rupard (2006), Ehrhart *et al.* (2013), Papich (2016) and Ramalingam *et al.* (2018).

### 4.3 Serum Biochemistry

#### 4.3.1 Blood Urea Nitrogen (mg/dL)

Serum Biochemical values were estimated on day 0, 7 and 14 before and after, both laser tumour excision and chemotherapy. Mean values of Blood Urea Nitrogen were recorded in all animals at scheduled intervals and the values are depicted in Table 4.11 and Figure 4.24.

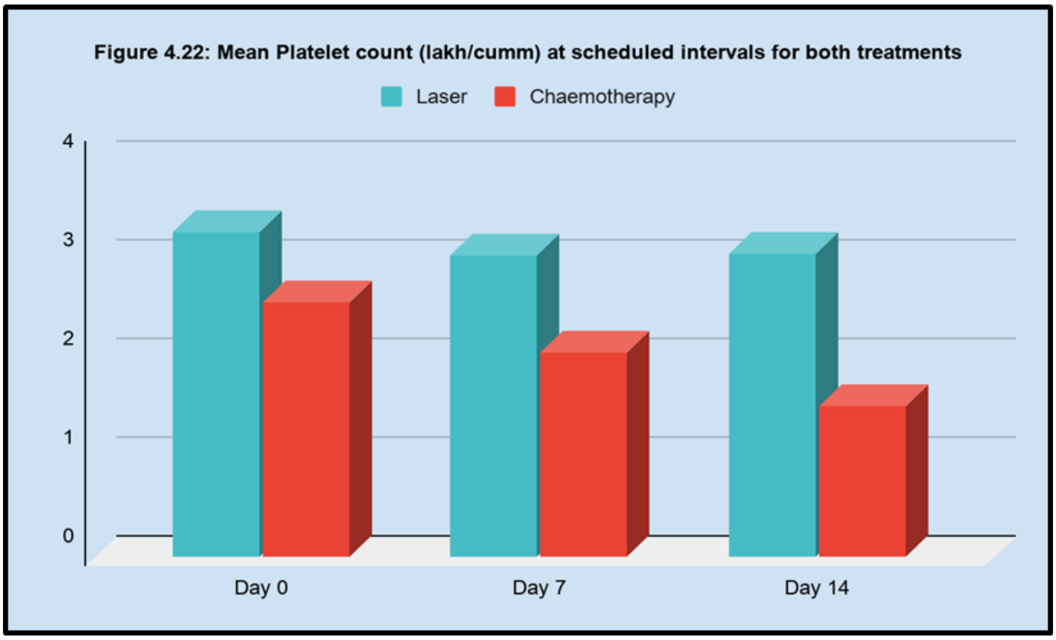


Figure 4.22: Mean Platelet count (lakh/cumm) at scheduled intervals for both treatments
Laser
Chaemotherapy
4
3
2
1
0
Day 0
Day 7
Day 14

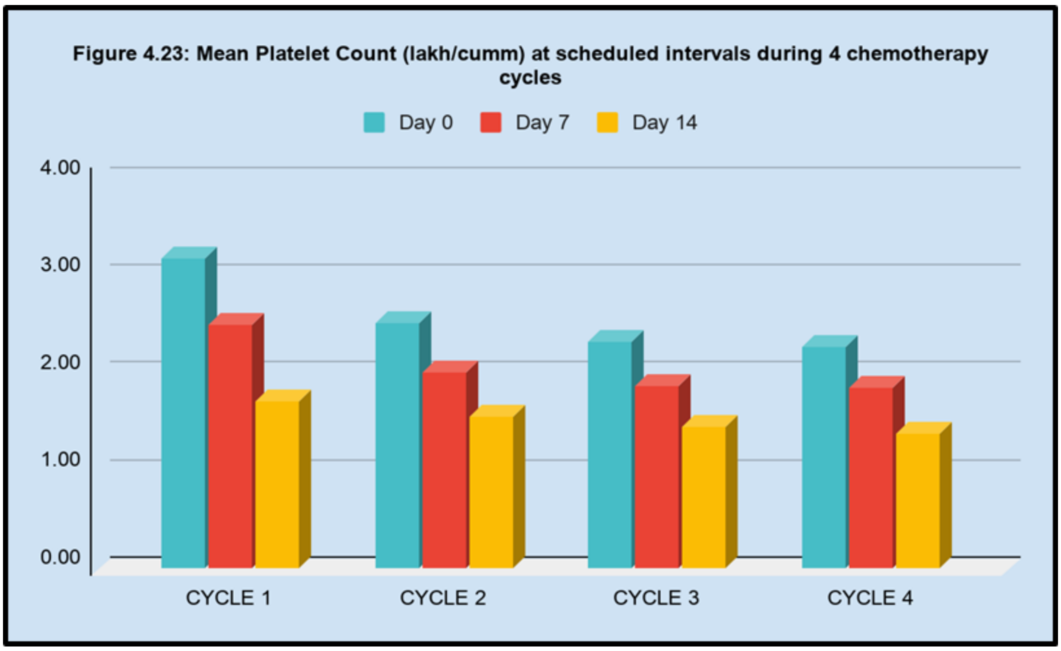


Figure 4.23: Mean Platelet Count (lakh/cumm) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
4.00
3.00
2.00
1.00
0.00
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4

**Table 4.11: Mean ± SE of Blood Urea Nitrogen (mg/dL) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 17.43 ± 1.52 | 20.72 ± 2.55 | 19.27 ± 1.45 |
| Adjuvant Chemotherapy | 03 | 12 | 26.33 ± 1.67$^{c}$ | 56.05 ± 2.81$^{a}$ | 34.43 ± 2.20$^{b}$ |

Different superscripts row-wise wise indicates significance

The mean BUN on day 0 was 17.43 ± 1.52 mg/dL. The mean BUN values on day 7 and 14 were 20.72 ± 2.55 mg/dL and 19.27 ± 1.45 mg/dL, respectively. All the values were within the normal physiological range.

An undulating trend was noticed in the mean values during the study. The changes were statistically non-significant and no specific reason could be attributed to these observations.

The mean value of BUN on day 0 i.e., before chemotherapy was 26.33 ± 1.67 mg/dL. The significant increase in the mean BUN recorded on day 7 and the value was 56.05 ± 2.81 mg/dL. The mean value on 14$^{th}$ day was decreased to 34.43 ± 2.20 mg/dL. The critical difference at 5% level of significance was calculated as 6.54. Thus, there was a statistically significant difference ($P < 0.05$) in the values of day 0, 7 and 14 which was outside the normal reference values.

The mean BUN value calculated for each of the cycles on the various scheduled days is represented in Figure 4.25. An undulating trend is observed, where there is a rise in BUN value on day 7 which decreases by day 14, in every cycle. A general increasing trend, indicating rise in BUN value is also observed with advancement of chemotherapy.

The nephrotoxic effects of Carboplatin have also been discussed by Edelman and Rupard (2006), Papich (2016) and Ramalingam *et al.* (2018).

These changes could be associated with the nephrotoxic effects of Carboplatin, thereby impairing renal function and clearance. Carboplatin has been stated to be nephrotoxic but it is significantly lesser than Cisplatin.

### 4.3.2 Serum Creatinine (mg/dL)

Serum Creatinine values were estimated in all dogs on 0, 7$^{th}$ and 14$^{th}$ day. Arithmetic mean was calculated of the values from all animals. The mean values are presented in Table 4.12 and Figure 4.26.

**Table 4.12: Mean ± SE of Serum Creatinine value (mg/dL) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 1.02 ± 0.05 | 1.10 ± 0.04 | 1.07 ± 0.04 |
| Adjuvant Chemotherapy | 03 | 12 | 1.11 ± 0.05$^{c}$ | 1.63 ± 0.06$^{a}$ | 1.31 ± 0.04$^{b}$ |

Different superscripts row-wise wise indicates significance

Before laser excision of tumour, the mean serum creatinine was 1.02 ± 0.05 mg/dL. On day 7 and 14, the mean values were 1.10 ± 0.04 mg/dL and 1.07 ± 0.04 mg/dL, respectively. The changes observed were non-significant and displayed an undulating trend within physiological limits. No specific reason could be attributed to these changes.

In dogs subjected to the chemotherapy, the mean serum creatinine value on day 0 was 1.11 ± 0.05 mg/dL. The values following chemotherapy were increased and the values on day 7 and 14 were 1.63 ± 0.06 mg/dL and 1.31 ± 0.04 mg/dL, respectively. The critical difference was 0.14, when calculated at 5% level of

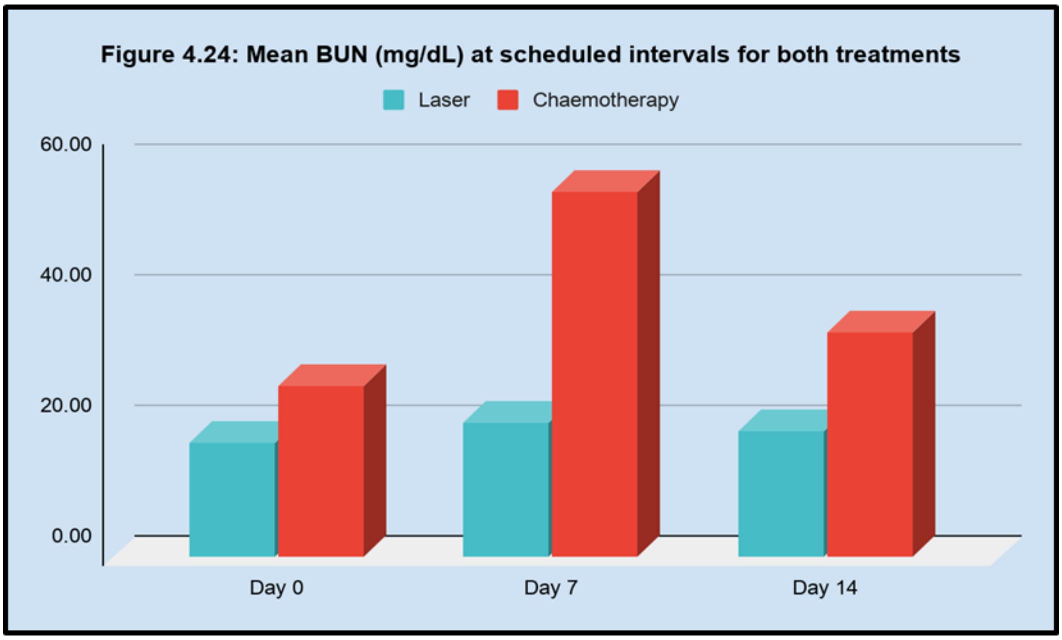


Figure 4.24: Mean BUN (mg/dL) at scheduled intervals for both treatments
Laser
Chaemotherapy
60.00
40.00
20.00
0.00
Day 0
Day 7
Day 14

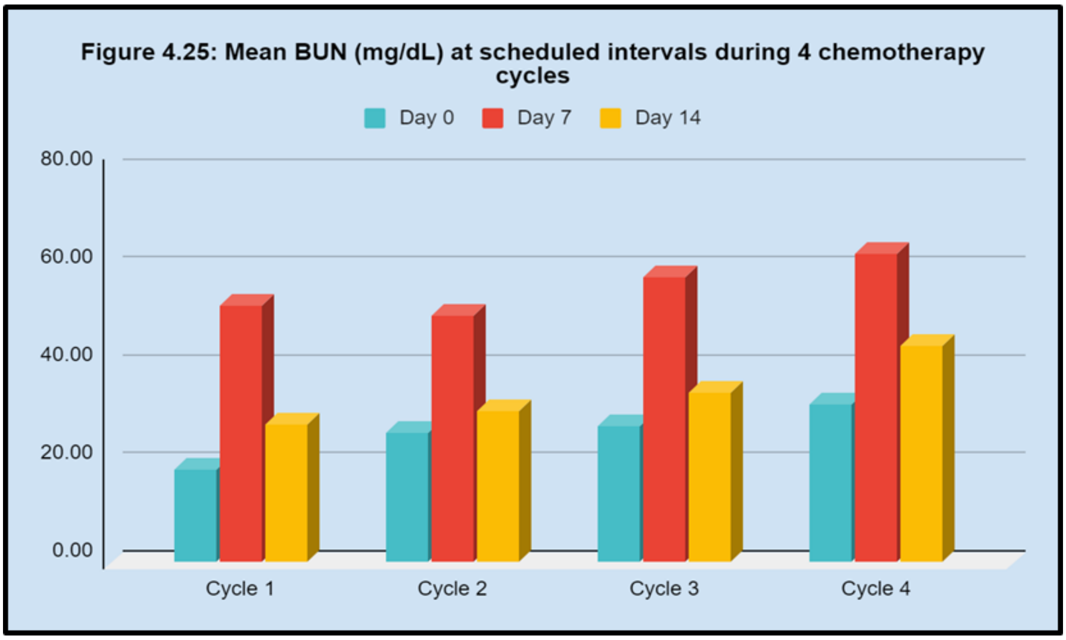


Figure 4.25: Mean BUN (mg/dL) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
80.00
60.00
40.00
20.00
0.00
Cycle 1
Cycle 2
Cycle 3
Cycle 4

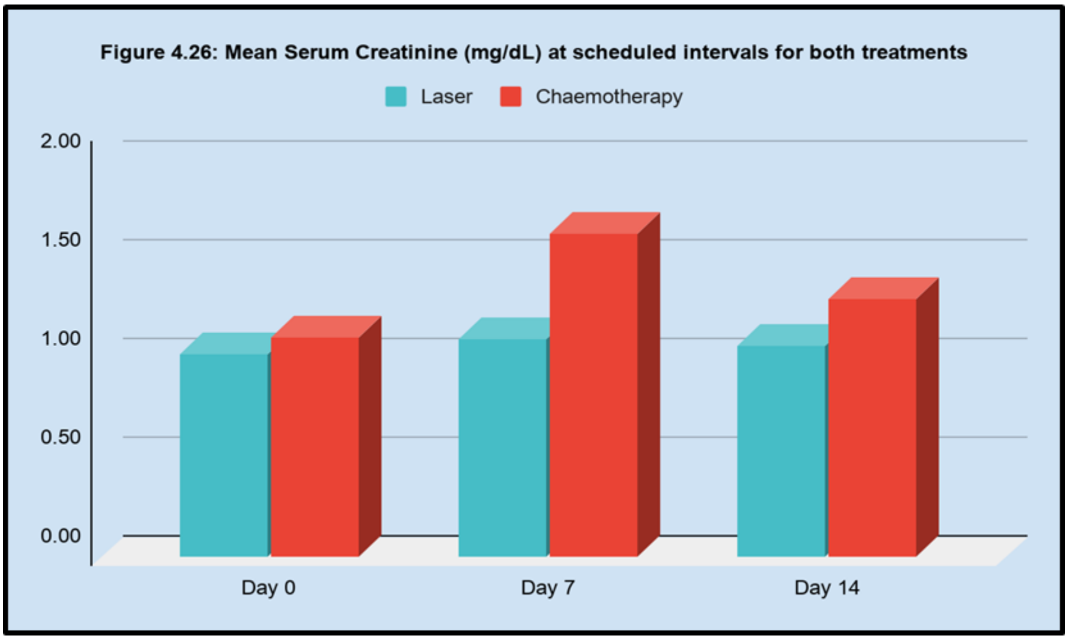



Figure 4.26: Mean Serum Creatinine (mg/dL) at scheduled intervals for both treatments

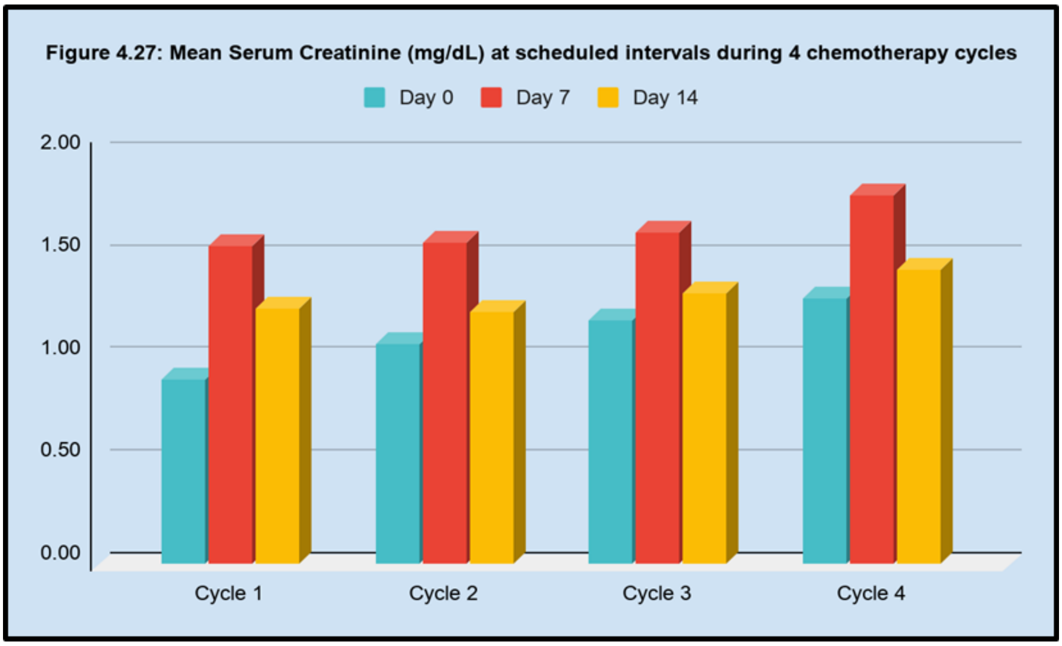



Figure 4.27: Mean Serum Creatinine (mg/dL) at scheduled intervals during 4 chemotherapy cycles

significance. Thus, the changes noted were statistically significant (P<0.05).

Figure 4.27 graphically represents the undulating trend that was observed between the days during each cycle of chemotherapy, where there was an increase by day 7 and a decrease by day 14 after carboplatin administration. The mean Serum creatinine on both these days was not within the normal physiological limit and could be attributed to the chemotherapy induced nephrotoxicity. Overall, there was an elevation in serum creatinine in all dogs, after the 4th cycle, as compared to before the start of chemotherapy.

These changes could be attributed to the mild nephrotoxic effects of Carboplatin, as stated by Edelman and Rupard (2006), Papich (2016) and Ramalingam *et al.* (2018).

### 4.3.3 Serum Alkaline Phosphatase (IU/L)

Serum Alkaline Phosphatase was estimated in all dogs on day 0, 7 and 14. Arithmetic mean was calculated and presented in table 4.13 and figure 4.28.

**Table 4.13: Mean ± SE of Serum Alkaline Phosphatase (IU/L) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 197.50 ± 20.63 | 221.99 ± 20.65 | 189.54 ± 18.42 |
| Adjuvant Chemotherapy | 03 | 12 | 195.31 ± 8.60[c] | 279.90 ± 8.19[a] | 239.95 ± 4.49[b] |

Different superscripts row-wise wise indicates significance

The mean serum alkaline phosphatase value on day 0 i.e. before tumour excision was 197.50 ± 20.63 IU/L. Following laser excision, on day 7, the value increased slightly and was 221.99 ± 20.65 IU/L. The value on 14th day was 189.54 ± 18.42 IU/L. The values showed an undulating trend and the changes were non-significant. However, the values were not within normal physiological range.

In the present study, the changes could be attributed to bone infiltration by oral tumour, chronic stress leading to endogenous corticosteroid release and use of corticosteroids for the patient at some point during or before the study (Ginel *et al.*, 2002).

Prior to chemotherapy on day 0, the mean serum alkaline phosphatase value was 195.31 ± 8.60 IU/L. The value thereafter increased significantly after chemotherapy and values on day 7 was 279.90 ± 8.19 IU/L which by day 14 decreased to 239.95 ± 4.49 IU/L. The critical difference calculated at 5% level of significance was 21.10. Thus, the changes noted in mean SAP values recorded at scheduled intervals was statistically significant ($P<0.05$). Figure 4.29 depicts graphically the undulating trend that was observed. The values increased on day 7 followed by a decrease on day 14 following all cycles of chemotherapy. An increase was observed in SAP level following completion of all cycles of chemotherapy, when compared to the value before 1$^{st}$ cycle, in all dogs.

The changes in the present study could be related to Carboplatin related hepatotoxicity and gastro-intestinal toxicosis as well as the stress related with chemotherapy leading to rise in the serum ALP. Major producers of the enzyme are the liver, bone and to an extent the intestine. Injury to any of these tissues will cause a rise in SAP (Eclinpath, 2020).

### 4.3.4 Serum Glutamic Oxaloacetic Transaminase (U/L)

Serum Glutamic Oxaloacetic Transaminase was estimated in all dogs on day 0, 7 and 14 of laser excision and all cycles of chemotherapy. Table 4.14 and Figure 4.30 represent the mean SGOT values.

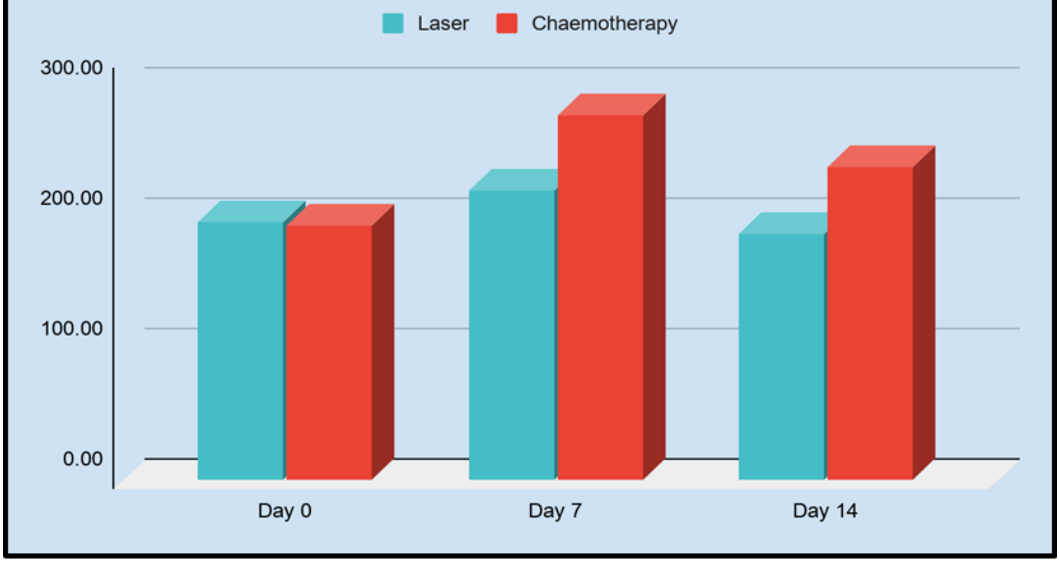



**Figure 4.28: Mean SAP (IU/L) at scheduled intervals for both treatments**

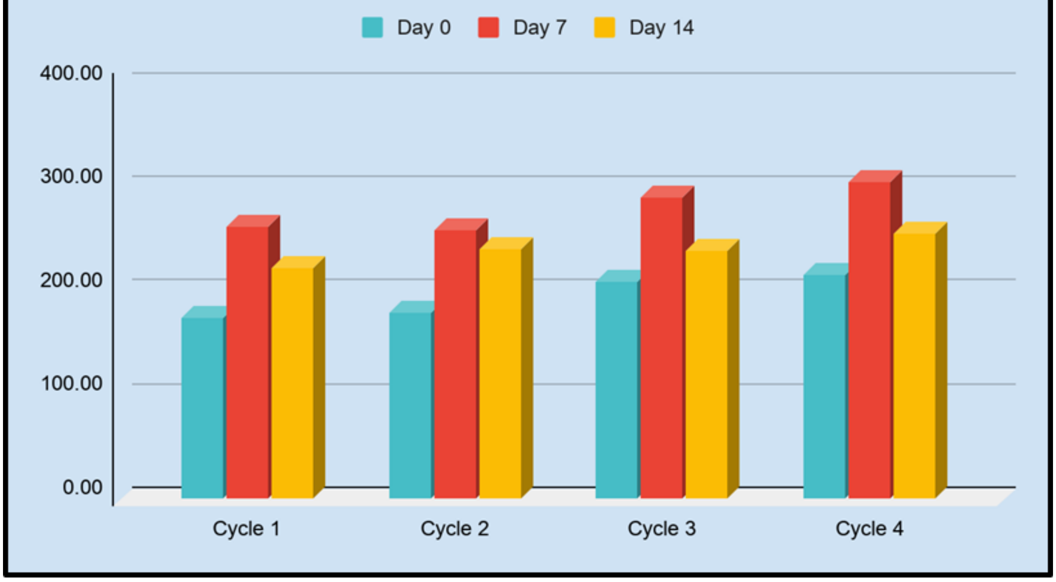



**Figure 4.29: Mean SAP (IU/L) at scheduled intervals during 4 chemotherapy cycles**

**Table 4.14: Mean ± SE of Serum Glutamic Oxaloacetic Transaminase (U/L) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 30.11 ± 3.26 | 36.99 ± 4.16 | 36.39 ± 3.46 |
| Adjuvant Chemotherapy | 03 | 12 | $59.32 \pm 5.43^{c}$ | $106.24 \pm 5.80^{a}$ | $86.63 \pm 2.29^{b}$ |

Different superscripts row-wise wise indicates significance

The mean SGOT on day 0 i.e. before laser excision was 30.11 ± 3.26 U/L. The mean values on day 7 and 14 following the surgery were 36.99 ± 4.16 U/L and 36.39 ± 3.46 U/L, respectively. The value on 7$^{th}$ day was slightly increased, which remains almost constant till day 14. The changes were statistically non-significant and all values were within the normal physiological limit.

Mean ± SE SGOT values were calculated for the scheduled intervals for all cycles of chemotherapy. Prior to chemotherapy on day 0, the mean SGOT value was 59.32 ± 5.43 U/L. The SGOT value on 7$^{th}$ day increased to 106.24 ± 5.80 U/L which again decreased on 14$^{th}$ day to 86.63 ± 2.29 U/L. The critical difference calculated at 5% level of significance was 13.74. Figure 4.31 represents the undulating trend noted on the scheduled days for each individual cycle.

Similar transient rise in SGOT value was stated by LiverTox (2012). The rise in SGOT values in the present study could be attributed to the hepatotoxic effects of Carboplatin.

### 4.3.5 Serum Glutamic Pyruvic Transaminase (U/L)

Serum Glutamic Pyruvic Transaminase was estimated in all dogs on day 0, 7 and 14 of surgery and chemotherapy. Arithmetic mean was recorded at scheduled time intervals and is depicted in Table 4.15 and Figure 4.32.

**Table 4.15: Mean ± SE of Serum Glutamic Pyruvic Transaminase (U/L) recorded at different intervals**

| Treatment | n | t | Day 0 | Day 7 | Day 14 |
|---|---|---|---|---|---|
| Laser Excision | 15 | 15 | 33.51 ± 2.18 | 45.35 ± 5.32 | 38.13 ± 3.33 |
| Adjuvant Chemotherapy | 03 | 12 | 67.68 ± 5.55[c] | 121.71 ± 7.44[a] | 89.03 ± 2.13[b] |

Different superscripts row-wise wise indicates significance

The mean SGPT on day 0 before surgery was 33.51 ± 2.18 U/L. Non-significant increase in the mean SGPT level was recorded on day 7 and the value was 45.35 ± 5.32 U/L, which decreased to 38.13 ± 3.33 U/L on day 14. An undulating trend was observed in the mean values at scheduled interval during the study. However, the changes were non- significant and could not be attributed to any specific reason.

The mean SGPT on day 0 prior to chemotherapy was 67.68 ± 5.55 U/L. The mean value increased on 7$^{th}$ day and was 121.71 ± 7.44 U/L. Thereafter, the value showed declining trend up to 14$^{th}$ day and the value was 89.03 ± 2.13 U/L. The critical difference calculated for 5% level of significance was 15.82. Thus the changes observed were statistically significant ($P<0.05$).

Figure 4.33 graphically represents the undulating trend that was observed on the scheduled days for each chemotherapy cycle, where increase in mean SGPT was noted on day 7, followed by a decrease by day 14. An overall increase in SGPT following all cycles of chemotherapy is also noted in all dogs.

Chemotherapeutic drugs, including Carboplatin have a hepatotoxic effect. In the present study, the transient increase in the SGPT level could be attributed to hepatotoxic effect of Carboplatin.

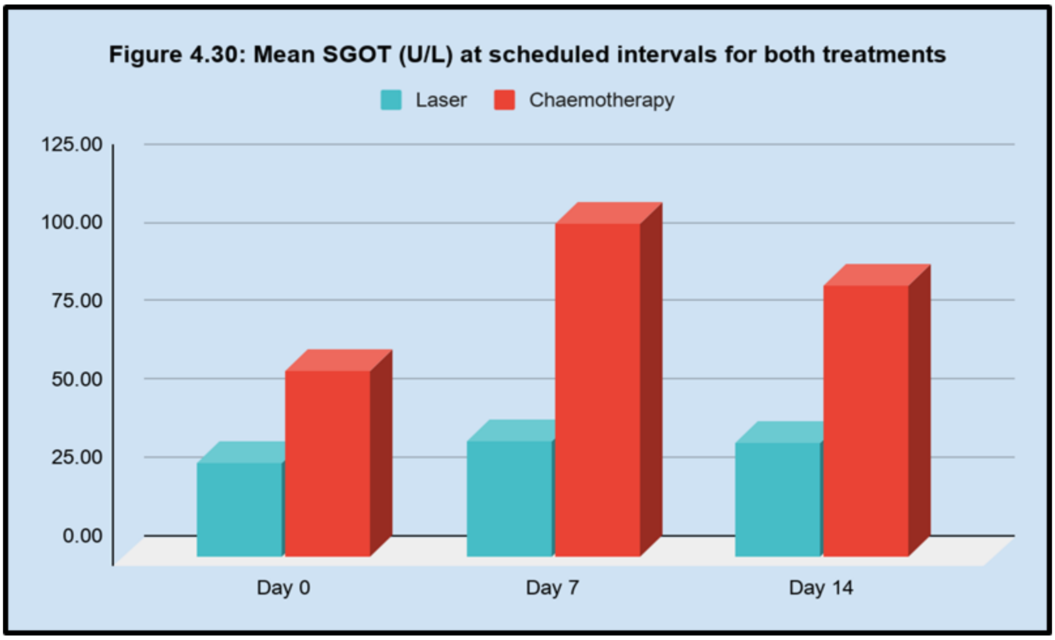


Figure 4.30: Mean SGOT (U/L) at scheduled intervals for both treatments
Laser
Chaemotherapy
125.00
100.00
75.00
50.00
25.00
0.00
Day 0
Day 7
Day 14

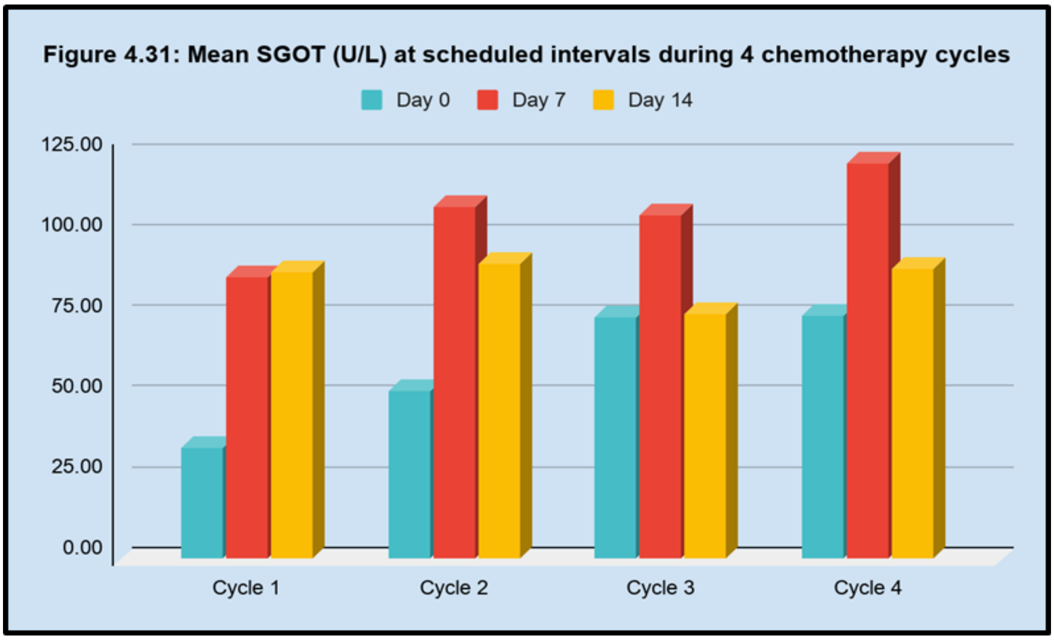


Figure 4.31: Mean SGOT (U/L) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
125.00
100.00
75.00
50.00
25.00
0.00
Cycle 1
Cycle 2
Cycle 3
Cycle 4

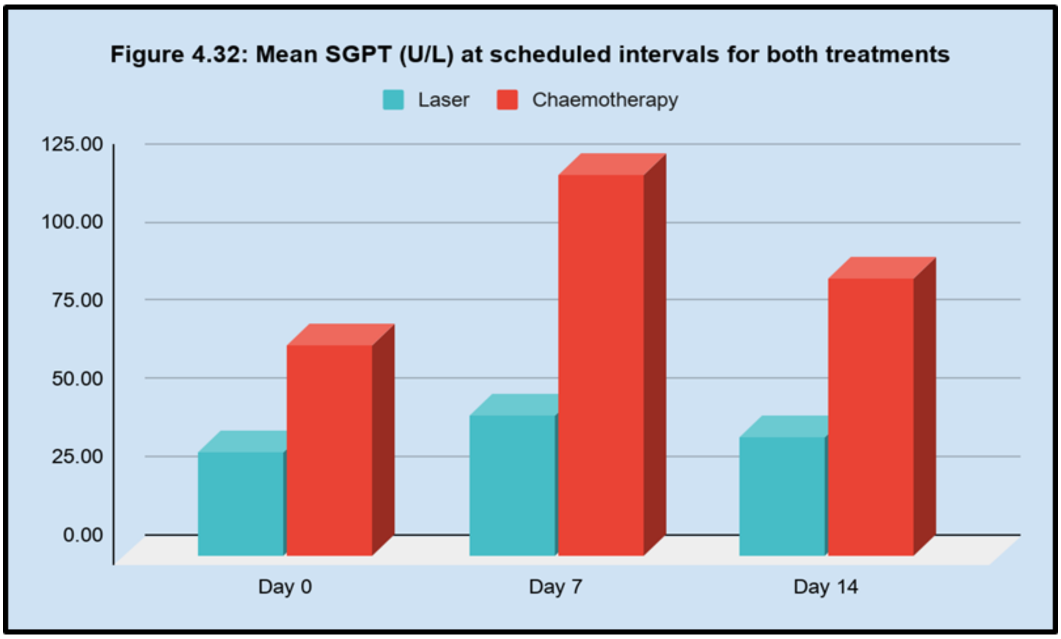


Figure 4.32: Mean SGPT (U/L) at scheduled intervals for both treatments
Laser
Chaemotherapy
125.00
100.00
75.00
50.00
25.00
0.00
Day 0
Day 7
Day 14

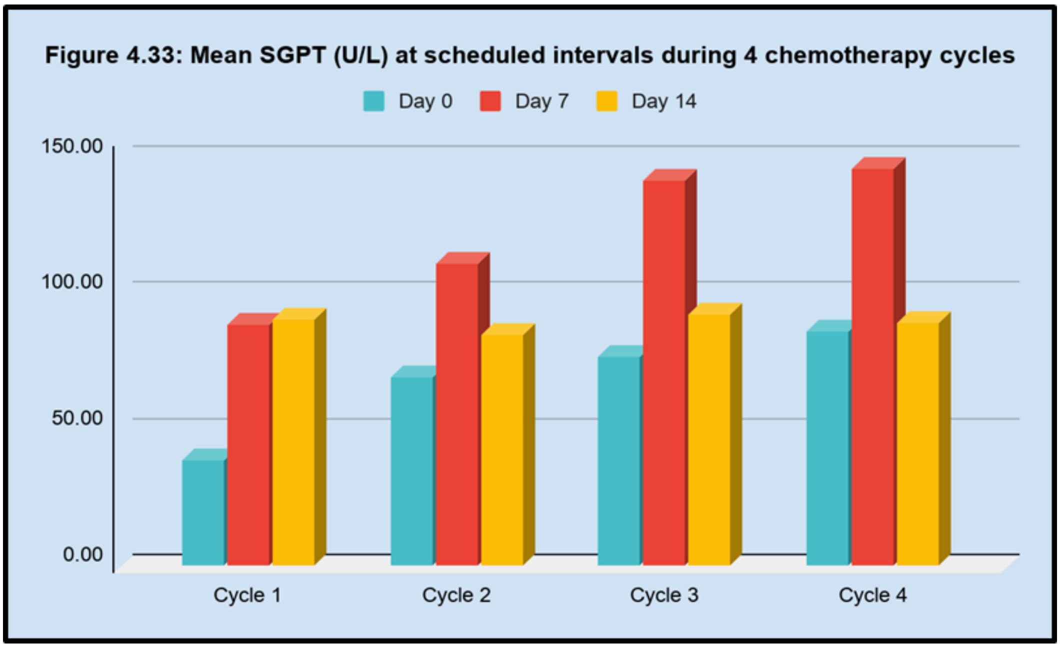


Figure 4.33: Mean SGPT (U/L) at scheduled intervals during 4 chemotherapy cycles
Day 0
Day 7
Day 14
150.00
100.00
50.00
0.00
Cycle 1
Cycle 2
Cycle 3
Cycle 4

### 4.4 Surgical Parameters

Various surgical parameters such as duration of surgery, intraoperative haemorrhage and ease of surgery were noted so as to evaluate the efficacy of diode laser for surgical excision of oral neoplasms (Plates 4.17- 4.26).

Mean ± SE duration required for completion of surgical laser excision of oral neoplasm in the 15 dogs was 19.73 ± 2.64 minutes. The least amount of time required was 7 minutes and maximum amount of time required was 39 minutes. The variation in time was mainly due to the size, location, number and extent of infiltration of neoplasm into surrounding tissue and bone. The tumours situated on the palate and invading the surrounding bone required more time of laser excision since an attempt was made to remove all the neoplastic tissue and obtain clean surgical margins as far as possible. In 3 cases, where there was bone infiltration by tumour, it was not possible to obtain clean surgical margins.

Oral tissue is highly vascular and any attempt at surgical manipulation of any tissue in the oral cavity resulted in voluminous haemorrhages. This was particularly true for oral neoplasms which were typically highly vascularized. The use of diode laser in the excision of these oral neoplasms greatly decreased intraoperative haemorrhage and volume of blood loss was minimal.

The use of laser allowed access to remote parts of the mouth and deeper areas such as the soft palate with ease. The minimal haemorrhage also led to improved field of visualization and thus improving ease of surgery. Due to coagulative properties of laser, no sutures were required in most cases; thus further reducing surgical time (Romanos and Nentwig, 1999).

Due to these properties, use of laser was indicated by many authors such as Guerry *et al.* (1986), Gaspar (1994), Maiorana and Salina (2006), Fornaini *et al.* (2007), Dunié-Mérigot *et al.* (2010), Neukam and Stelzle (2010), Azma and Safavi (2013), De Lorenzi *et al.* (2015), Derikvand *et al.* (2016) and Igna *et al.* (2016) for oral surgery.

**4.5 Healing of Surgical Wound and Complications**

Following laser excision the surgical wounds were examined routinely at regular intervals. There was a whitish layer of coagulated tissue over the wound surface by the 7$^{th}$ day and complete healing was observed by the 14$^{th}$ day. Healing occurred without much scar tissue formation, hence without major deformation of surgical site. **(**Plates 4.27- 4.30**)** Post surgery, all dogs were able to resume eating by 2$^{nd}$ or 3$^{rd}$ postoperative day. Minimal inflammation was observed during wound healing. *Elizabethan Collar* used after the surgery helped in avoiding self-mutilation.

In one case, on 3$^{rd}$ postoperative day wound dehiscence (Plate 4.31) occurred where sutures were placed to close the flap of gingival mucosa created during tumour removal. The wound was not re-sutured and healed via secondary intention, in 3 weeks.

**4.7 Histopathology findings**

Based on histomorphological findings, the tumour type was identified in all the cases. Identification of tumor type helps in deciding the treatment protocol. Histopathological identification is also necessary as was to determine whether the tumor is benign or malignant. Typically in canines the most common benign oral tumors are Epulis and Acanthomatous ameloblastoma; whereas, the most common malignant tumors are Malignant melanoma, Squamous cell carcinoma, Fibrosarcoma and Osteosarcoma (Duncan and Lascelles, 2011).

## Surgical Excision of Palatine and Gingival Tumour Using Laser (Plates 4.17 - 4.26)

**Plate 4.17:** Epulis in a non-descript dog

**Plate 4.18:** Mouth gag placed and dog intubated prior to surgery

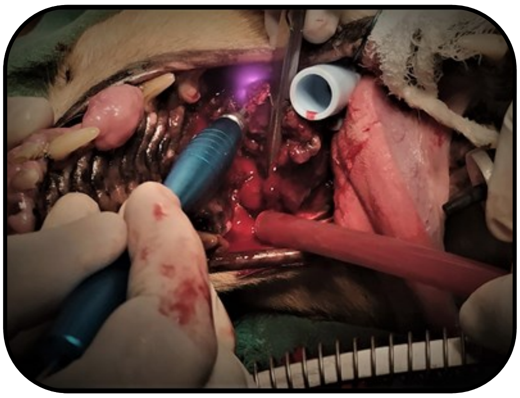


**Plate 4.19:** Laser beam directed to surgical site via optical fibre, handled using handpiece

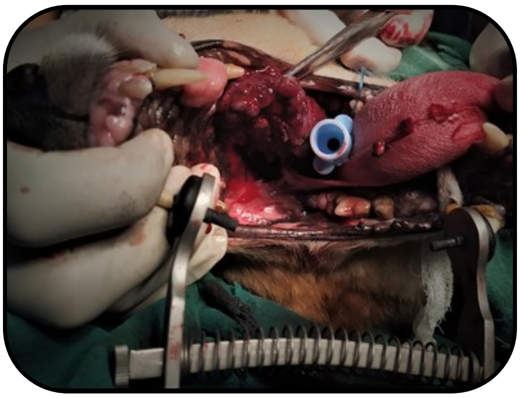


**Plate 4.20:** Partially excised tumour from soft palate

**Plate 4.21:** Separation of tumour from soft palate

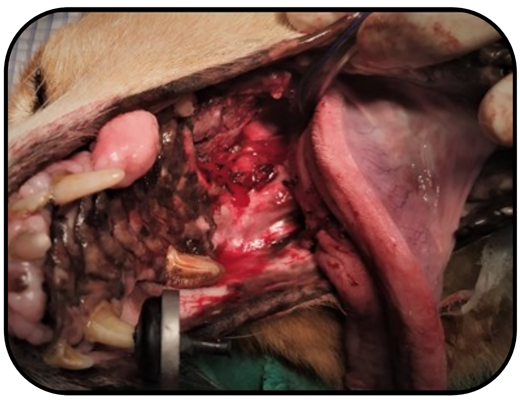


**Plate 4.22:** Post-operative site showing complete excision of palatine tumour

**Plate 4.23:** Gingival tumour exposed for surgery

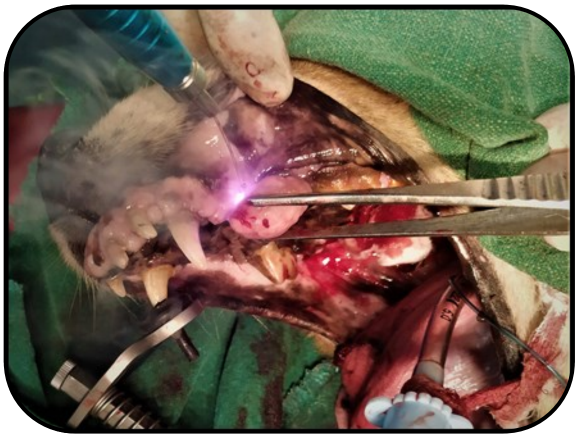


**Plate 4.24:** Laser excision of tumour from gingival border

**Plate 4.25:** Post-operative view of oral cavity after excision of both tumours

**Plate 4.26:** Complete healing of surgical wound - day 15, post-surgery

## Healing of Surgical Wound (Plates 4.27 - 4.31)

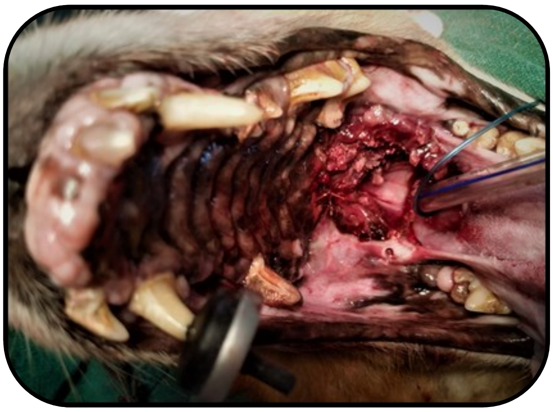


**Plate 4.27:** Post-operative view of oral cavity after excision of both tumours

**Plate 4.28:** Surgical site on day 3, post surgery

**Plate 4.29:** Surgical site on day 7

**Plate 4.30:** Complete healing of surgical wound with minimal cicatrix - day 15

**Plate 4.31:** Suture Dehiscence on 3rd post-operative day

**Table 4.16: Distribution of tumour based on histomorphology**

| Tumour type | Number | Percent |
|---|---|---|
| Epulis | 4 | 25.00 |
| Malignant Melanoma | 3 | 18.75 |
| Acanthomatous ameloblastoma | 3 | 18.75 |
| Fibrosarcoma | 3 | 18.75 |
| Squamous cell carcinoma | 2 | 12.50 |
| Osteoma | 1 | 6.25 |

In the present study, the most common form of oral tumor identified was Fibromatous epulis (25%), followed by Malignant Melanoma (18.75%), Acanthomatous ameloblastoma (18.75%), Fibrosarcoma (18.75%), Squamous Cell Carcinoma (12.50%) and Osteoma (6.25%). Distribution of tumour based on histomorphology during the study is presented in Table 4.16 and Figure 4.34.

Similar findings were recorded by Vos and van der Gaag (1987), Kosovsky *et al.* (1991), Wallace *et al.* (1992), Roza *et al.* (2009) and Duncan and Lascelles (2011). Mikiewicz *et al.* (2019) stated that epulis was most common benign tumour and that malignant melanoma, squamous cell carcinoma and osteosarcoma were the most common malignant tumours.

Simons (2015) published contradictory findings. He found in his study that the most common oral tumour was Acanthomatous Ameloblastoma, followed Fibrosarcoma, Squamous Cell Carcinoma and then Malignant Melanoma. Goldschmidt *et al.* (2017) also found in a study that the most common odontogenic tumour was Acanthomatous Ameloblastoma, which is in disagreement with this study.

Based on this study, the distribution of benign and malignant oral tumours is 50%

each (Figure 4.35). Similar findings are stated by Bronden *et al.* (2009) who stated that 51.1% of oral tumours were malignant. Cray *et al. (2020)* stated that most oral tumours were malignant (53.6%) in nature.

**Fibromatous Epulis**

Neoplastic fibrocytes were arranged in interwoven fascicles (H&E x100). The inset in Plate 4.32 shows neoplastic fibrocytes with uniform oval normochromatic nuclei with abundant collagen and indistinct cytoplasm that are blending into the extracellular collagenous stroma (H&E x400). Infiltration of neutrophils, lymphocyte and macrophages into collagenous stroma was observed in case of ulceration of the tumour.

**Acanthomatous Ameloblastoma**

The histopathological section revealed sheets of ameloblastic epithelial cells forming cord-like structures. Sub epithelial connective tissue comprises stellate shaped cells with oval to elongated nuclei. Squamous metaplasia and variable keratinization of stellate reticulum-like cells. Section showed irregular epithelial stratification at the periphery of ameloblastomatous islands (Plate 4.33). Hemorrhages were also revealed.

**Osteoma**

The histopathological section revealed cancellous trabecular bone. Neoplastic cells were round in shape. Neoplastic cells were arranged in compact masses. There was no mitotic activity or atypia of the neoplastic cells noted (Plate 4.34).

**Malignant Melanoma**

The section revealed neoplastic melanocytes in mid and lower subepithelial parenchyma (Plate 4.35). Neoplastic cells are arranged in broad sheets and were round in shape with small nuclei. Pigment granules obscured the nucleus in few

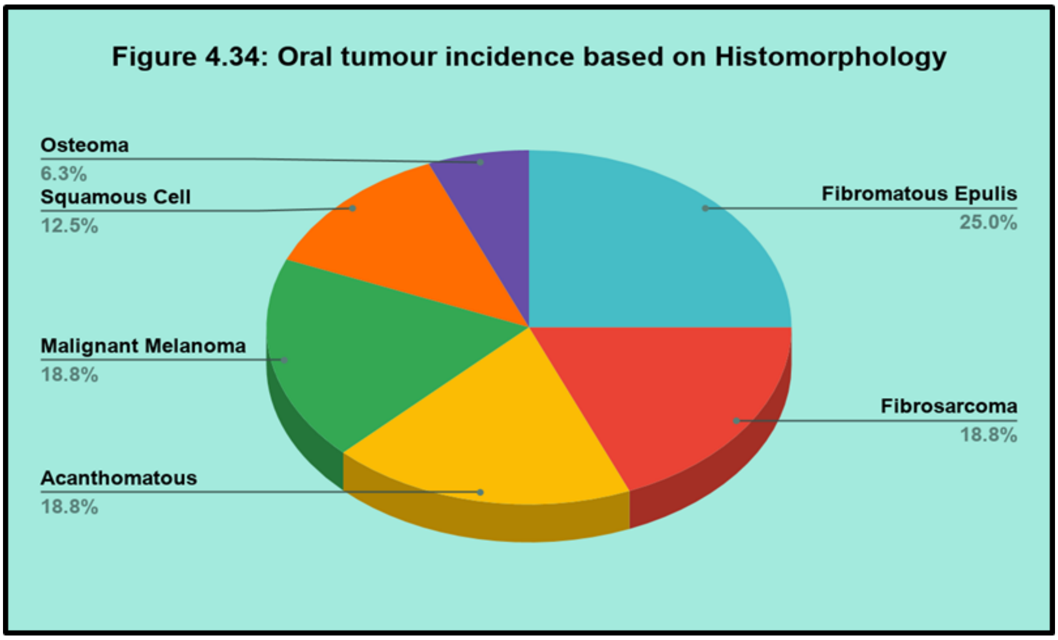


Figure 4.34: Oral tumour incidence based on Histomorphology
Osteoma
6.3%
Squamous Cell
12.5%
Malignant Melanoma
18.8%
Acanthomatous
18.8%
Fibromatous Epulis
25.0%
Fibrosarcoma
18.8%

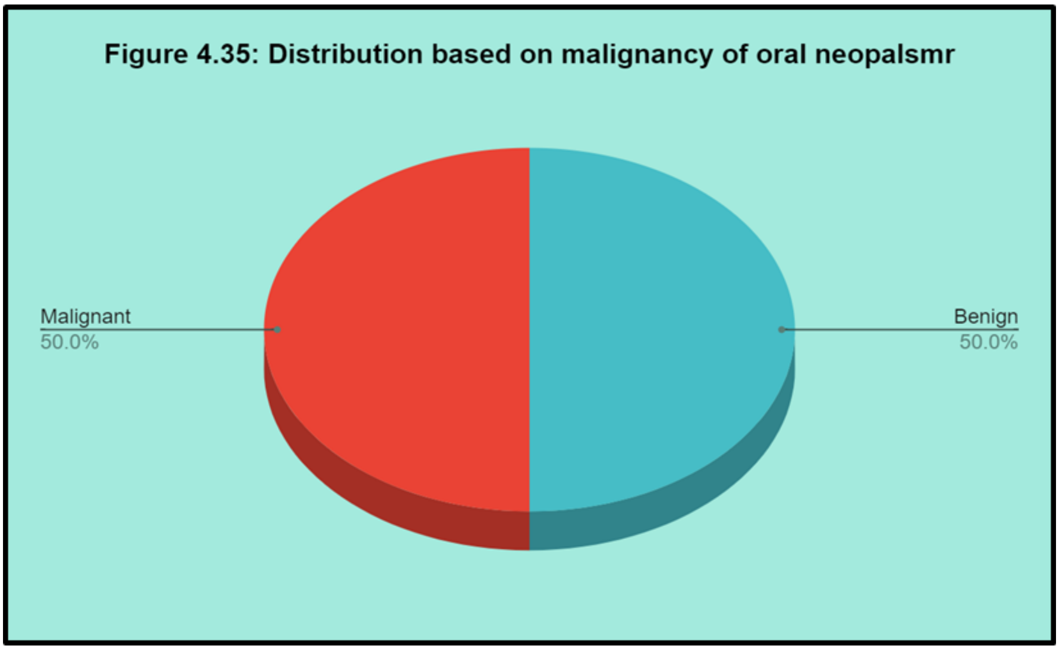


Figure 4.35: Distribution based on malignancy of oral neopalsmr
Malignant
50.0%
Benign
50.0%

## Histomorphology of Oral Tumours (Plates 4.32-4.39)

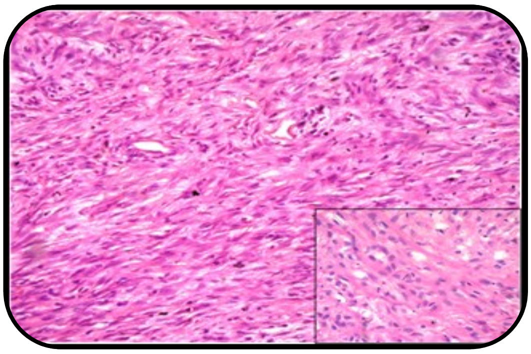


**Plate 4.32:** Fibromatous epulis (H&E 10X), Inset: H&E x40

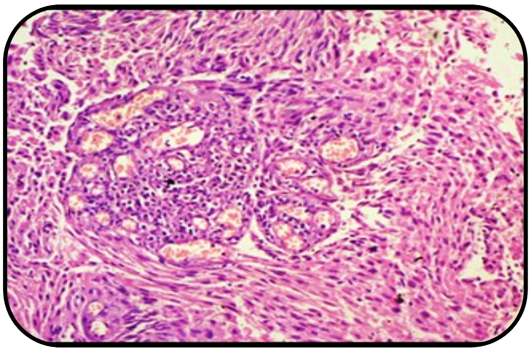


**Plate 4.33:** Acanthomatous Ameloblasto ma (H&E 20X)

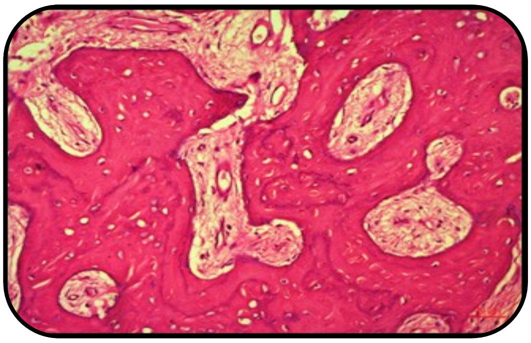


**Plate 4.34:** Osteoma (H&E 40X)

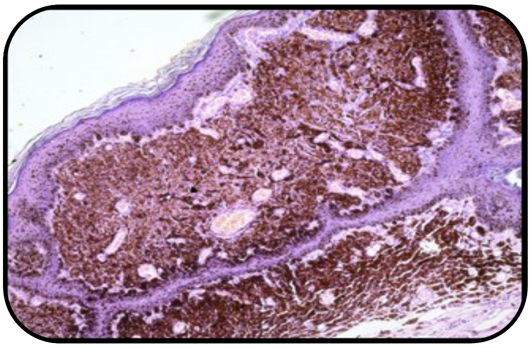


**Plate 4.35:** Malignant Melanoma (H&E 10X)

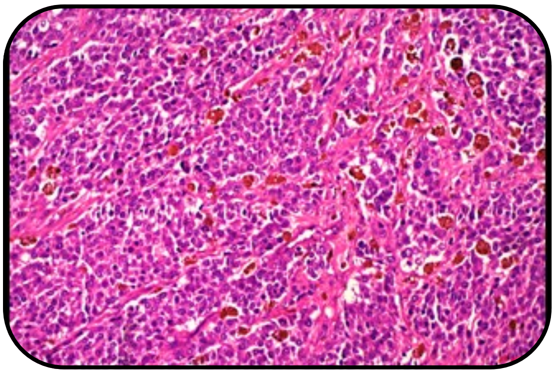


**Plate 4.36:** Malignant Melanoma (H&E 20X)

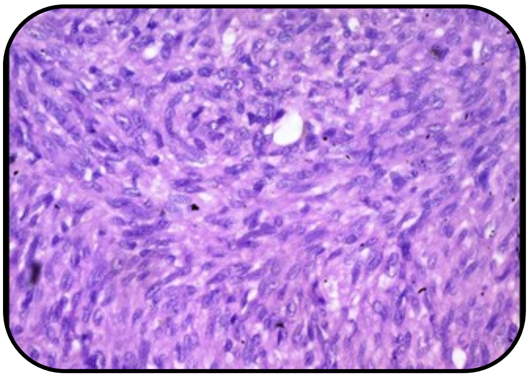


**Plate 4.37:** Fibrosarcoma (H&E 40X)

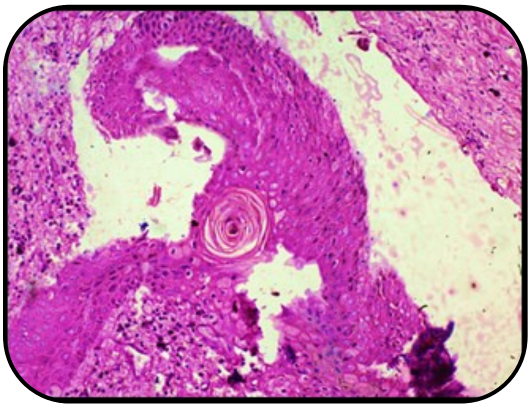


**Plate 4.38:** Squamous cell carcinoma (H&E 20X)

neoplastic cells whereas few cells lack melanin pigment. Focal areas of hemorrhages at the periphery of the section could be seen**.** Mitotic count was low with 2 mitoses per 20 HPFs. Focal area of hemorrhages at the periphery of section.

In another section (Plate 4.36) round neoplastic cells with small nuclei were arranged as broad sheets. Some neoplastic cells contained brown granular melanin pigment and few fibrous connective tissue. Mitotic count was moderate with 3 mitoses per 20 HPFs. Focal area of neutrophilic infiltration were also revealed

**Fibrosarcoma**

The histopathology section revealed interlacing bundles of fibrous connective tissue. Neoplastic fibroblasts showing moderate pleomorphism, spindle and round in shape with hyperchromatic nuclei were observed. Focal infiltration of lymphocytes could be noted. Neoplastic spindle shaped fibroblasts are arranged in a “herringbone” pattern. (Plate 4.37)

**Squamous Cell Carcinoma**

Section revealed sheets of neoplastic squamous epithelial cells. Neoplastic cells were elongated to spindle in shape. Nest of neoplastic squamous epithelial cells termed as keratin pearl is visible in Plate 4.38. Abundant connective tissue and numerous blood vessels could also be visualized.

**Radiography**

Radiography was carried out for ruling out the metastasis in all the animals. During the study, radiograph in one dog revealed evidence of metastasis (Plate 4.39) with reduced density of rib while small nodules were visible all over the lung. The reduced density may be due to advance age of the dog.

**4.6 Recurrence**

In the present study, all the cases were followed up to 60$^{th}$ days for recurrence of the tumour. The local recurrence was judged on the basis of visual observation and palpation under sedation. Although 08 tumours were malignant and 08 tumours were benign on histopathological examination, the local recurrence was observed in 08 cases. The recurrence was observed in all the dogs (3) with malignant melanoma. (Plate 4.40 and 4.41) One dog with squamous cell carcinoma and one dog with Fibrosarcoma showed recurrence. Despite being classified as a benign tumour, in all the dogs (3) with acanthomatous ameloblastoma, recurrence was observed. The recurrence in these benign tumours was mainly due to bone invasion of the tumour, where partial maxillectomy or partial mandibulectomy, along with radiotherapy was suggested to be curative. During the present study, the dog owners were reluctant for partial maxillectomy or partial mandibulectomy and radiotherapy was not feasible

### 4.7 Effects of Chemotherapy and response to treatment

Chemotherapy was administered to 3 dogs with malignant oral tumours. After surgical excision the tumour sample was sent for histopathological examination to determine tumour type. On confirmation of malignant tumour type, suitable candidates were selected for adjuvant chemotherapy. Out of 8 cases of malignant tumours only 3 dogs were suitable for adjuvant chemotherapy, due to age considerations and owner compliance. Chemotherapy was started 15 days after laser excision of tumour. Two dogs with malignant melanoma and one dog with squamous cell carcinoma were administered Carboplatin at 300 $mg/m^2$. The dogs with oral melanoma at time of surgery were at stage 2 and 3. The dog with squamous cell carcinoma showed tumour stage 3 based on TNM classification by Grier and Mayer (2007) and Bergman (2007).

After $1^{st}$ cycle of chemotherapy the adverse events were recorded. One dog with oral melanoma showed gastrointestinal disturbance and vomiting after $2^{nd}$ cycle. This was managed by supportive therapy and resolved with time. All the dogs showed leukopenia, neutropenia, decreased haematocrit, thrombocytopenia on $7^{th}$ day. Biochemical alterations were also noted on day 7 with mild increase in serum

## Radiographic Examination of Thorax

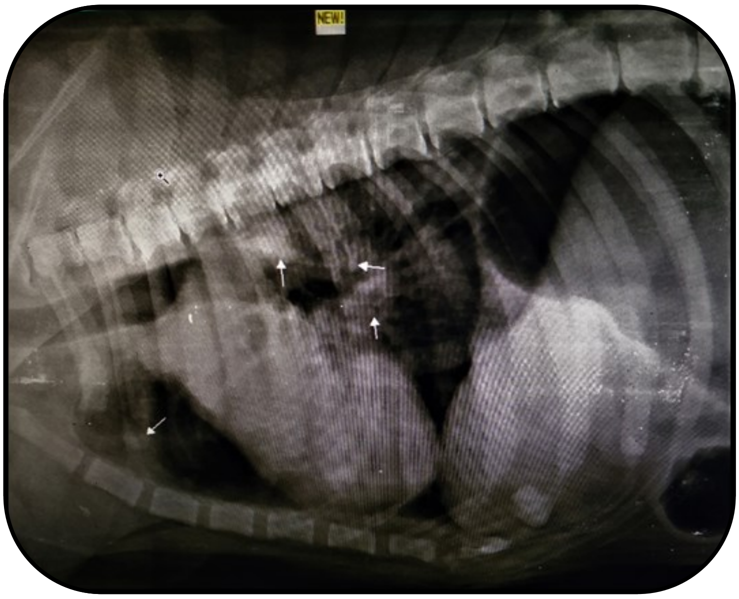


**Plate 4.39:** Metastastic nodule in lung (arrows) (Lateral view)

## Tumour Recurrence

**Plate 4.40:** Tumour recurrence in dog with malignant melanoma

**Plate 4.41:** Tumour recurrence in dog with Squamous Cell Carcinoma

creatinine, SGOT, SGPT and SAP up to grade 1 and BUN upto grade 3, which decreased by day 14. Similar findings were stated by Rassnick *et al.* (2001), Brockley *et al.* (2013), Dank *et al*. (2014) and Woodruff *et al*. (2019).

In the dog with signs of gastrointestinal toxicosis (grade 3), a dosage adjustment to 250 $mg/m^2$ was made for the subsequent cycles. All dogs underwent 4 cycles of chemotherapy, following which one dog with squamous cell carcinoma showed stable disease (33.33%) with no increase in size of ipsilateral submandibular lymph node, no evidence of metastasis to lungs on radiograph and no local recurrence. However, chemotherapy was stopped following the fourth cycle due to adverse events related to gastrointestinal toxicosis as well due to lack of response of tumour to treatment. The two dogs with oral melanoma showed no response to therapy and there was evidence of disease progression (66.67%), based on metastasis to lymph nodes. One dog showed local tumour recurrence as well. One dog with oral melanoma was euthanized before end of the study period due to disease progression. The other two dogs were continued on Ocoxin liquid following chemotherapy. Based on follow up, the dogs showed no signs of discomfort and activity was near normal throughout the period of study.

Rassnick *et al.* (2001) stated that the response rate to Carboplatin therapy was 28%, including 1 dog with complete remission and 24% dogs with a partial response to treatment; whereas, 36% dogs each showed stable disease and disease progression.

**4.8 Efficacy of Laser excision**

During the study, the surgical excision of the oral tumour using LASER required less operating time. The variation in operating time was mainly based on the size, location, number and extent of infiltration of neoplasm into surrounding tissue and bone. The tumours situated on the palate and invading the surrounding bone required more time in an attempt to remove all the neoplastic tissue and obtain clean surgical margins as far as possible. The use of diode lasers greatly decreased intraoperative haemorrhages due to its coagulative properties, vastly improving the field of visibility. Decreased inflammation observed during healing of wound, reduced discomfort to the animal. There was also minimal scar tissue formation following healing. No other systemic or local complications were as a result of laser surgery, during the study.

Thus, Laser surgery proved to be a safe alternative for oral tumour resection. Thus surgical excision using LASER, should be followed as the first line of therapy prior to the use of any other treatment modality.

# SUMMARY AND CONCLUSIONS

The current study on oral tumours in dogs was conducted at the Teaching Veterinary Clinical Complex, Nagpur Veterinary College between December 2019 and December 2020. A total of 15 dogs were included in this study, of which all the dogs underwent surgical excision of the tumour using diode laser. Based on histopathological findings and eligibility of dogs, 3 out of 15 dogs underwent chemotherapy using Carboplatin.

In all the dogs, surgery was performed under dissociative anaesthesia using a combination involving Xylazine, Ketamine and Diazepam.

The prevalence of Oral neoplasms (n=18) based on surgical cases (n=1732) presented to TVCC was 1.03%. Oral neoplasms constituted 13.04% of all cases of neoplasms presented to the TVCC, NVC, Nagpur. During the study, the majority of the dogs presented with oral tumours were old; i.e., 73.33% of the dogs were more than 8 years of age and male (66.67%). Non-descript dogs (46.67%) showed the highest incidence of oral tumour during the study followed by Spitz (13.33%). The incidence appeared higher in non-descript and male dogs. This was however attributed to the fact that majority of the dogs presented to TVCC were males and of a non-descript breed.

Within the oral cavity the most common location of the tumours was the gingiva (53.33%), followed by the palate (26.67%) and buccal mucosa (20%) and on visual examination, about equal number of tumours were ulcerated and non-ulcerated.

All the dogs underwent surgical excision of the oral tumour using 980 nm GaAlAs diode laser, operated in Continuous Wave mode, at a power setting of 15 to 20 Watt. The tumours were excised with clean, wide surgical margins, wherever possible. In case of oral tumours invading the adjacent maxilla or mandible, achieving clean surgical margins was not possible.

The tumour thus excised was processed for histopathological examination. Post operatively the dogs were maintained on antibiotic and analgesic therapy and advised to be fed only soft foods, till resolution of surgical wound. In almost all dogs the wounds healed without major complication, only in one case, suture dehiscence was noted.

Based on histomorphology, tumour types were identified. Three suitable dogs with malignant tumours, one dog with squamous cell carcinoma and two dogs with malignant melanomas, underwent chemotherapy. Carboplatin was used as a single drug, dosed at 300 $mg/m^2$, administered for 4 cycles, with an interval of 21 days between each cycle.

Following each treatment haematological and biochemical parameters were assessed on 0, $7^{th}$ and $14^{th}$ day. In case of chemotherapy, both parameters were assessed at similar intervals following all 4 cycles.

Mean ± SE haemoglobin following laser excision, showed a non-significant undulating trend, with slight decrease on day 7, followed by mild increase by day 14. The values during the observation period were within normal physiological limits. The Mean ± SE haemoglobin of all cycles decreased by day 7, which then increased by day 14. This undulating change was also observed during each individual cycle. The changes were statistically significant.

Following laser excision mean TEC showed mild decrease by day 7 and increased by day 14. The undulating trend noted was statistically non-significant and within physiological reference range. The mean TEC value decreased significantly by $7^{th}$ day following all cycles of chemotherapy which then increased by $14^{th}$ day. This undulating change was observed during each individual cycle, at the scheduled intervals as well.

Similar undulating trend was observed in mean PCV value, following laser excision. There was a decrease in mean PCV from day 0 to day 7 and an increase by day 14. The changes in the mean packed cell volume were non-significant. Mean ± SE PCV for all cycles on day 7 decreased, which then showed an increasing trend till day 14. This undulating trend was recorded across all cycles. The changes observed in the mean PCV were statistically significant, and a deviation from the normal reference range was also noted.

Mean TLC values showed an undulating trend. Following laser excision the mean value increased by day 7 which then decreased by day 14 The differences were found statistically significant, however the values were within normal physiological limits. The mean leukocyte count, following chemotherapy showed a decrease by day 7 and a further decreased by day 14. These changes were found to be statistically significant at 5% level of significance. A regular declining trend was observed in all cycles at scheduled intervals.

Mean percent of neutrophils following laser excision of tumour was decreased by $7^{th}$ post-operative day, which resolved by day There was a statistically significant difference between the mean of different days, attributed to the inflammatory processes as a result of the surgical wound. The mean Neutrophil count following chemotherapy decreased to on day 7, and further by day 14. The changes recorded were statistically significant ($P<0.05$). A similar decreasing trend was noted during all cycles except the first which showed an undulating change with decrease in mean on $7^{th}$ day, which then increased by $14^{th}$ day.

After laser excision of tumour, the mean percent of lymphocyte by $7^{th}$ day was slightly decreased, which then increased by $14^{th}$ day. There was a statistically significant change in the values recorded. Mean lymphocyte percent appeared to have an inclining trend till day 7 and day 14. This was an apparent increase and not true lymphocytosis, as a result of the Neutropenia.

The mean monocyte (percent) shows non-significant undulating trend, within normal physiological range was noted in the mean monocyte percentage. The mean monocyte count was calculated for the scheduled days during the different cycles of chemotherapy showed a mild undulating trend. The changes were statistically non-significant and within the normal physiological range. The mean monocyte count following chemotherapy, showed statistically non-significant undulating changes.

. The undulating changes recorded in mean eosinophil count following laser excision were statistically non-significant and within physiological reference range. The mean eosinophil count calculated for all 4 cycles of chemotherapy, showed a non-significant undulating change at the scheduled intervals.

The mean platelet count following, laser excision decreased day 7, which then recorded a very mild increase by day 14. The changes were statistically non-significant and within physiological reference range. The mean platelet count showed a decreasing trend for all cycles of chemotherapy on all days. The changes observed were statistically significant.

The mean BUN calculated for the scheduled intervals during laser excision showed a non-significant undulating change. Mean BUN value calculated during all cycles of chemotherapy showed a undulating trend with significant change in values calculated for the scheduled days.

The mean serum creatinine following laser excision showed a mild undulating change which were statistically non-significant and were within physiological limits. The mean serum creatinine value calculated for all cycles of chemotherapy at the scheduled intervals. A significant undulating change was noted with the mean serum creatinine level elevated on day 7, which declined by day 14. A similar undulating trend was noted on the scheduled days during each cycle.

The mean serum alkaline phosphatase value showed non-significant changes following laser excision, however the values were not within normal physiological range. The elevated SAP values was attributed to chronic stress because of the disease as well infiltration of bone by the tumour. The mean SAP calculated for all cycles of chemotherapy on scheduled days showed changes that were statistically significant. There was an increase by day 7 followed by a decrease on day 14.

Following laser excision, the mean SGOT showed non-significant changes and all values were within normal reference range. Mean ± SE SGOT showed significant increase by day 7 which then decreased by day 14 following each cycle of chemotherapy. The changes were statistically significant.

Mean SGPT, following laser excision of tumour showed a non-significant undulating trend was noted. The calculated mean SGPT, during each cycle of chemotherapy showed significant increase on day 7 followed by decrease on day 14.

The surgical parameters assessed were duration of surgery, intraoperative haemorrhage and ease of surgery. The average duration of surgery, for all 15 dogs was 19.73 ± 2.64 minutes. Invasive tumours required more surgical time. Intraoperative haemorrhage assessed visually was minimal, due to the coagulative properties of laser. Use of laser greatly aided surgery and lead to increased ease of surgery, since it allowed access to remote parts of the mouth. Also due to minimal bleeding, the field of visualization improved greatly. Minimal inflammation was observed at surgical site following laser excision of tumour. Complete healing occurred in all but one dog within 2 weeks following surgery. All dogs were able to resume feeding latest by 3$^{rd}$ post-operative day in all cases.

Histopathological examination revealed the various types of oral tumours. The most common was Fibromatous epulis (25%), followed by Malignant Melanoma, Acanthomatous Ameloblastoma and Fibrosarcoma, each 18.75%. Other tumour types identified were Squamous Cell Carcinoma (12.5%) and a relatively rare tumour: Osteoma (6.25%). On radiographic examination of thorax, metastasis to the lungs was identified in one dog.

Recurrence was noted in a total of 9 dogs, within the observation period of 60 days. All dogs with malignant melanoma, acanthomatous ameloblastoma and one dog with squamous cell carcinoma showed local tumour recurrence.

In 3 dogs that underwent chemotherapy, 1 dog with squamous cell carcinoma showed stable disease (33.33%), while both dogs with malignant melanoma showed progressive disease (66.67%). Adverse events related to Carboplatin therapy that were recorded were, gastrointestinal toxicosis (Grade 1-Grade 3), haematological and biochemical disturbance (Grade 1 to Grade 3).

The use of diode laser for the surgical excision of oral tumours, proved beneficial in this study since it eased and hastened the surgery, reduced intraoperative bleeding and aided healing leading to reduction in the discomfort to the animal.

**CONCLUSIONS:**

From this study, the conclusions that could be drawn were:

1. The prevalence of Oral neoplAsms (n=18) based on surgical cases (n=1732) presented to TVCC was 1.03%. Oral neoplasms constituted 13.04% of all cases of neoplasms presented to the TVCC, NVC, Nagpur.
2. The distribution of benign and malignant oral neoplasms was equal i.e., 50% each. The types of oral tumours recorded during the study were Fibromatous Epulis, Acanthomatous Ameloblastoma and Osteoma amongst benign tumours, while Malignant Melanoma, Fibrosarcoma and Squamous Cell Carcinoma were the malignant neoplasms identified.
3. The use of 980 nm diode laser for surgical excision of oral neoplasms proved to be beneficial since it improved ease of surgery, reduced surgical time and minimizes intra-operative haemorrhages.
4. Healing was also improved as a result of use of laser since there was less inflammation during wound healing following surgery as well as reduced scar tissue formation.
5. The use of Carboplatin as an adjuvant therapy, along with antioxidant Ocoxin, proved to be mildly beneficial in 2 cases where the life of patient was prolonged, while the animal was relatively comfortable throughout the study period.

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# VITA

The author, Miss Ambika Arvind was born on 1st October 1995, in Mumbai, Maharashtra. She has been an avid lover of all animals since her childhood. She completed her primary education from Academic International Primary School, Dar-es-Salaam, Tanzania. She then moved to Mumbai, Maharashtra in 2007 and completed her Secondary Education from Gopal Sharma International School, Mumbai. She passed her X standard ICSE board examinations in 2011. She completed her Higher Secondary education from S.I.E.S. College for Arts, Science and Commerce and passed her HSC examination in 2013.

She took admission in Mumbai Veterinary College in August 2013, in order to pursue graduation in B. V. Sc. & A.H. Course. Through her graduation, the author has attended continuing education programs on Wild Animal Health Care and Management (2016), Small Animal Clinical Case Studies (2017), Small Animal Radiology, Haematology and Clinical Pathology (2017) and Emergency Applications in Critical Care Management (2018). In the fourth year of her graduation she was appointed Joint Secretary of the BVC Student Council for 2016-2017. During her tenure she co-organized an animal adoption camp and dog blood donation camp in 2017. While pursuing her graduation, she was also an active member of the National Service Scheme and participated in several animal treatment camps and Clean up Camps, organized by the college. She completed her graduation in July 2018.

In October 2018, the author took admission in Nagpur Veterinary College, so as to pursue post-graduation, in the discipline of Veterinary Surgery and Radiology. During post-graduation the author delivered oral and poster presentations at various conferences, such as: "Third Clinical Case Conference (January 2019) on Diagnostic and Therapeutic Challenges in Farm and Companion Animals held at Akola and 43rd National Symposium of Indian Society for Veterinary Surgery (November 2019) held at Hisar (Haryana). She also participated and presented a study in the Online National Clinical Case Conference, hosted by PGIVAS, Akola in 2020.

The author is a lifetime member of the Indian Society for Veterinary Surgery and a member of the Bombay Veterinary College Alumni Association.

## THESIS ABSTRACT

| | | | |
|---|---|---|---|
| a) | Title of thesis | : | **"SURGICO-THERAPEUTIC MANAGEMENT OF ORAL TUMOUR IN DOG WITH REFERENCE TO LASER"** |
| b) | Full Name of Student | : | **Ambika Arvind** |
| c) | Name & Address of Advisor/ Guide | : | **Dr. S. B. AKHARE**<br>Professor<br>Department of Veterinary Surgery and Radiology<br>Nagpur Veterinary College, Nagpur |
| d) | Degree to be awarded | : | **MASTER OF VETERINARY SCIENCE** |
| e) | Year of award of degree | : | **2021** |
| f) | Major subject | : | **VETERINARY SURGERY AND RADIOLOGY** |
| g) | Total number of pages in the thesis | : | **80** |
| h) | Number of words in the thesis abstract | : | **495** |
| i) | Signature of student | : | |
| j) | Signature, Name & Address of forwarding authority | : | |

**Associate Dean**
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## ABSTRACT

The study "Surgico-therapeutic management of oral tumours in dog with reference to Laser" was carried out at Teaching Veterinary Clinical Complex, Nagpur Veterinary College, between December 2019 and January 2021.

The study included total 15 dogs with oral tumours. Common clinical signs in such dogs were haemoptysis, dysphagia, inappetance, excessive salivation and halitosis.

All 15 dogs underwent surgical excision of the oral tumors using 980nm diode laser. Three dogs with malignant tumours (one squamous cell carcinoma and 2 dogs with malignant melanoma) underwent chemotherapy using Carboplatin.

Prevalence of oral tumours recorded at the TVCC was 1.03% of all surgical cases and 13.04% of all tumour cases in dogs presented to the TVCC. Highest incidence was noted in non-descript (46.67%), old (73.33%), male (66.67%) dogs. The most common location of tumour was gingiva (53.33%), followed by the palate, buccal and labial mucosa.

Tumours were excised under dissociative anaesthesia using Xylazine-Ketamine-Diazepam combination, using diode laser operated at power setting of 15-20 Watt, with clean margins, as far as possible.

Following laser excision, no haemato-biochemical alterations were noted, except for mild neutrophilia and leukocytosis in few cases.

The mean time required for surgical excision was 19.73 ± 2.64 minutes. All the surgeries were quick, with minimal blood loss.

Healing of surgical wounds occurred with less inflammation and cicatrix formation. All dogs were able to resume feeding by $3^{rd}$ post-operative day.

Carboplatin was administered @ of 300 $mg/m^2$, at an interval of 21 days, for 4 cycles; in 3 dogs with confirmed malignancies, based on histopathological examination. Adverse events recorded were gastro-intestinal toxicosis (grade 1- grade 3) and haemato-biochemical disturbance (grade 1-grade 3). In one dog, gastrointestinal toxicosis warranted a dose change to 250 $mg/m^2$ for $3^{rd}$ and $4^{th}$ cycles.

Chemotherapy was discontinued following 4 cycles due to unmanageable toxicosis in a dog and disease progression in another two dogs with malignant melanoma. One dog was euthanized before the end of study period due to disease progression. Other two dogs were maintained on Ocoxin liquid and appeared comfortable for the rest of the study period.

Based on histopathology reports the types of tumours identified in this study were Fibromatous Epulis (25%), Acanthomatous Ameloblastoma (18.75%) and Osteoma(6.25%) among benign tumours and Malignant Melanoma (18.75%), Fibrosarcoma (18.75%) and Squamous Cell Carcinoma (18.75%) among malignant tumours.

Local tumour recurrence was observed in 8 cases, where the dogs suffered from malignant melanoma (3), acanthomatous ameloblastoma (3), one dog with squamous cell carcinoma and fibrosarcoma, each.

The availability of Laser for excision of oral tumours was extremely beneficial due to its advantages. It greatly reduced surgical time, minimized intra-operative haemorrhage vastly and improved field of visibility. Laser also improved healing by minimal inflammation during wound healing as well less scar tissue formation, thereby improving comfort of animals.

Thus, Laser surgery proved to be a safe alternative for oral tumour resection. The surgical excision using laser, should be followed as the first line of therapy prior to the use of any other treatment modality.

# प्रबंध सारांश

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| अ. | प्रबंधाचे शिर्षक | : | **श्वानांच्या मुखातील अर्बुदांचे लेझर कीरणांच्या संदर्भाने शल्यचिकीत्सालयीन व्यवस्थापन** |
| ब. | विद्यार्थ्याचे पुर्ण नांव | : | अंबिका अरविंद |
| क. | मार्गदर्सकाचे नांव व पत्ता | : | डॉ. संदीप भा. आखरे<br>प्राध्यापक<br>पशूशल्यचिकित्सा व क्ष-किरणशास्त्र विभाग, नागपूर पशूवैद्यकीय महाविद्यालय, नागपूर |
| ड. | प्रदान करण्योत येणारी पदवी | : | स्नातकोत्तर पदवी (एम. व्ही. एससी.) |
| इ. | पदवी प्रदान करण्याचे वर्ष | : | २०२१ |
| फ | मुख्य विषय | : | पशूशल्यचिकित्सा व क्ष-किरणशास्त्र |
| ग. | प्रबंधातील एकूण पृष्ट | : | ८० |
| ह. | सारांशातील एकूण शब्द | : | ४४८ |
| ई. | विद्यार्थ्याची सही | : | |
| ज. | अग्रेषित करणाऱ्या अधिकाऱ्यांची सही, नांव आणी पत्ता | : | |

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## सारांश

**श्वानांच्या मुखातील अर्बुदांचे लेझर कीरणांच्या संदर्भाने शल्यचिकीत्सालयीन व्यवस्थापन हा अभ्यास पशुवैद्यकीय शैक्षणीक चिकीत्सा संकुल, नागपूर पशुवैद्यकीय**

**महाविद्यालय, नागपूर येथे डीसेंबर २०१९ ते जानेवारी २०२१ या कालावधीत घेण्यात आला.**

**सदरचा अभ्यास मुखामध्ये अर्बुद असलेल्या एकुण १५ श्वानांवर घेण्यात आला. या श्वानांमध्ये लाळेतुन रक्त येणे, अन्न खातांना त्रास होणे, भुक मंदावणे, अती प्रमाणात लाळ गाळणे आणि तोंडाचा घाण वास (उग्र दर्प) येणे ईत्यादी लक्षणे कमी अधीक प्रमाणात आढळुन आली.**

**अभ्यासादरम्यान सर्व १५ श्वानांचा मुखातील अर्बुदांचा डायोड लेझर द्वारे शस्त्रक्रीया करण्यात आली. यापैकीच ज्या तीन श्वानांमध्ये पसरणारा अर्बुद होता त्यांच्यामध्ये कार्बोप्लेटीन ह्या औषधीने किमोथेरपी करण्यात आली.**

**पशुवैदयकीय शैक्षणीक चिकीत्सा संकुल येथे शस्त्रक्रीयेकरिता येणाऱ्या श्वानांमध्ये मुखाच्या अर्बुदाचे १.०३ टक्के प्रमाण तर अर्बुद नोंदी पैकी मुखाच्या अर्बुदाचे १३.०४ टक्के प्रमाण आढळुन आले. अभ्यासादरम्यान गावठी श्वानांमध्ये (४६.६७%), वयस्कर श्वानांमध्ये (७३.३३%) तर आणि नर श्वानांमध्ये (६६.६७%) मुखाच्या अर्बुदाचे जास्त प्रमाण आढळुन आले. त्याच प्रमाणे अर्बुदाचे स्थान सर्वात जास्त हीरडीवर व त्यानंतर टाळु आणि तोंडातील स्लेष्मल त्वचेवर आढळुन आले.**

**सर्व शस्त्रक्रीयेंकरिता झ्कॉयलॅझ्कीन, कीटॅमीन आणि डायझेपाम चा वापर भुल देण्याकरीता करण्यात येवुन शस्त्रक्रीया डोयोड लेझर (१५-२० वॅट) च्या सहाय्याने करण्यात आल्या. सर्व शस्त्रक्रीयेंदरम्यान संपूर्ण प्रमाणात अर्बुदाच्या उती काढण्याचा प्रयत्न करण्यात आला.**

**लेझर द्वारे शस्त्रक्रीयेंनंतर करण्यात आलेल्या रक्त आणि रक्तजल जैवरासायनीक चाचण्यांमध्ये न्युट्रोफीलीया आणि ल्युकोसायटोसीस वगळता कोणताही आमुलाग्र बदल आढळला नाही.**

**लेझर द्वारे केलेल्या शस्त्रक्रीयेंकरिता साधारणत: १९.७३±२.६४ मिनींटाचा अवधी लागला. सर्व शस्त्रक्रीयेंकरिता कमी वेळ आणि कमी रक्तस्त्राव आढळुन आला.**

**शस्त्रक्रीयेच्या जखमेच्या ठिकाणी कमी सुज आढळुन येवुन जखमेच्या ठिकाणी व्रण तयार झाल्याचे दिसुन आले. सर्व श्वानांनी साधारणत: शस्त्रक्रीयेनंतर तीसऱ्या दिवसापासुन अन्न प्राशन केल्याचे दिसुन आले.**

**अभ्यासादरम्यान तीन श्वानांमध्ये ३०० मी.ग्रॅ/मी□ या प्रमाणात कार्बोप्लेटीन दर २१ दिवसांनी ४ वेळा देण्यात आले. कार्बोप्लेटीन दिल्यानंतर जठर आणि आतड्याची दर्जा १ आणि २ ची विषाक्तता आणि रक्त व रक्तजल जैवरासायनीक मापदंडावर सुद्धा प्रतिकुल परिणाम आढळुन आला. एका श्वानामध्ये तर जठर आणि आतडीच्या विषाक्ततेमुळे कार्बोप्लेटीनची तीसरी व चौथी मात्रा २५० मि.गॅ/मी□ इतकी कमी करण्यात आली. जठर आणि आतडीच्या जास्त विषाक्ततेमुळे तसेच कार्बोप्लेटीनचा जास्त उपयोग नसल्याचे आढळल्याने सर्व श्वानांमध्ये कीमोथेरपी ४ वेळा दिल्यानंतर बंद करण्यात आली. अभ्यासादरम्यान उपचारास प्रतिसाद नसल्याने व प्रकृती जास्त खालावण्यामुळे एका श्वानास वेदनारहित मरण देण्यात आले. इतर दोन श्वानांना ओकॉक्सीन ही औषधी रोज देण्यात आली आणि ही दोनही श्वान अभ्यासादरम्यान स्वस्थ आणि आरामदायक आढळुन आले.**

**उती विकृतीशास्त्रीय परिक्षणावर आधारित अर्बुदे ही-फायब्रोमॅटस इप्युलीस (२५%), ॲकँथामॅटस अमीलोब्लास्टोमा (१८.७५%) आणि ऑस्टीओमा (६.२५%) ही न पसरणारी अर्बुदे तर मेलॅब्लास्टोमा (१७.७५%), फायब्रोसार्कोमा (१८.७५%) आणि स्कॉमस सेल कार्सीनोमा (१८.७५%) ही पसरणारी अर्बुदे आढळुन आलीत.**

**अभ्यासादरम्यान एकुण आठ श्वानांमध्ये अर्बुदाची पुनश्च: वाढ आढळुन आली. लेझर उपकरणाची अभ्यासादरम्यान उपलब्धता अत्यंत उपयोगी असल्याचे आढळुन आले. लेझरद्वारे शस्त्रक्रीयेस लागणारा वेळ, शस्त्रक्रीयेदरम्यान कमी रक्तस्त्राव, आणि शस्त्रक्रीयेकरीता आवश्यक दृश्यता वाढल्याचे आढळुन आले.**

सदर कारणांमुळे लेझर द्वारे शस्त्रक्रीया ही मुखातील अर्बुदाचे उच्चाटन करण्यास अत्यंत उपयुक्त असल्याचे आढळुन आले आणि त्यामुळेच लेझर द्वारे मुखातील अर्बुदाची शस्त्रक्रीया करण्यास इतर उपचार पद्धती आधी प्रथम प्राधान्याने करण्यात यावी.