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Cancer in Pet Animals: Incidence, Diagnosis and its Management

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Cancer still remains an enigmatic life threatening disease of human as well as animals, especially pet animals, despite the significant progress made in its diagnosis and treatment in recent years. This dreaded disease is a combination of different illnesses that hallmarks self sufficiency in growth signal, insensitivity to antigrowth signal, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and metastasis. Cancer related problems gained much importance in pet animals owing to the affection, love and increased awareness among the people towards animal sufferings and pain. Cancer is considered as the second most frequent cause of death in humans and the first one in canines and felines (Jemal *et al.* 2008). Due to continuous rise in the number of cancer patients every year, it becomes essential to develop effective diagnostic measures which may help in early detection and to devise suitable management of this malady to save lives of human beings and animals. As dog and cat are the most preferred companion animals to human, they shares intimately man's environment and thus subjected to almost the same kind of neoplasia observed in mankind. Of all species, dogs develop neoplasms twice as frequently as humans (Moulton *et al.*, 1970), with incidence of skin and mammary tumours being the highest (Rungsipipat *et al.*, 2003).

The diagnosis and management of neoplasms therefore, represent the major challenges faced by a veterinary oncologist. Tumour markers are the unique attributes of this dynamic process that may reflect the neoplastic process by a high/low level of expression relative to that of normal cells, offering a putative use in diagnosis of cancer. They can be measured quantitatively or qualitatively by chemical, immunological or molecular methods. Non-subjective biological parameters such as tumour ploidy, cell proliferation and hormone receptor status can provide more precise diagnostic and prognostic information (Bacus *et al.*, 1989). Screening and surveillance of cancer risk can be tailored by using current diagnostic methods of imaging which includes magnetic resonance imaging, computed tomography, positron emission tomography, magnetic resonance spectroscopy, ultrasound, mammography and digital mammography. There is no doubt that emerging genomic and proteomic technologies will facilitate more precise and accurate investigations to know about the cause of cancer. Techniques such as immunohistochemistry, *in situ* hybridization (FISH, CSH), PCR (RT-PCR, real time-

PCR), flow cytometry and microarray are paving a new era for cancer diagnosis at early stage. Until the last decade, veterinary options for the treatment of neoplasms in dogs were very limited and arriving at prognosis was bleak. New combinations of drugs, improved surgical procedures for removing neoplasms and novel neoplasm-targeting drugs are successfully used nowadays to prolong and improve the quality of life in many dogs diagnosed with neoplasms.

CAUSE

Exact cause for cancer development is still obscure, mainly it attributed are the genetic factors and also diverse nature of agents such chemical carcinogens, periodic injury (physical, heat, etc.), ionizing radiations, hormones, infectious agents, immunological dysfunction etc. Chemical carcinogens causing DNA mutations are known as mutagens. Tobacco smoking in humans is associated with many forms of cancer (Sasco *et al.*, 2004) and causes 90% of lung cancer (Beisalski *et al.*, 1998). Decades of research has demonstrated the link between tobacco use and cancer in the lung, larynx, head, neck, stomach, bladder, kidney, oesophagus and pancreas. Ionizing radiations also produce mutations such as radon gas and prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies (English *et al.*, 1997). Non-ionizing radio frequency radiation from mobile phones has also been proposed as a cause of cancer, but there is currently little established evidences of such a link. Recently, electronic media reported that radiation from mobile towers is suspected for brain tumours (Star TV – Tehlka). Mammary tumour is thought to be an inherited disease. In canine, there are some breeds (English Setter, Chihuahua, miniature Poodle and Afghan Hound) which have more incidence of mammary tumours (Goldschmidt *et al.*, 2001; Misdorp *et al.*, 2002) but breedwise incidence of mammary tumours differ in different regions due to differences in availability of breeds in these regions. Schneider *et al.* (1969) reported that female dogs spayed before their first oestrus have only 0.5% incidence of mammary tumour as compared with higher incidencs of 8 and 26%, if the females were spayed (ovarihyterectomised) after first and second oestrus, respectively suggesting the hormonal influence on mammary tumour.

INCIDENCE

The age dependent overall cancer incidence per 100000 individual per year is approximately 381 in dogs and 264 in cats as against 105 in humans (Vail and MacEwen, 2000; Jemal *et al.*, 2008). When analyzed site wise, the mammary tumour is the most frequent of all the neoplasia (52%) occurring in bitches and ranks third (17%) in cats after lymphohaemopoietic and skin tumours (MacEwen and

Withrow, 1996; Jemal *et al.*, 2003). Different studies have been carried to find out the incidence of neoplasms in pet animals and due to the difference in the geographical location and existence of different breeds the incidence rates varied among them. Tulsa Registry reported an incidence of 1,126 cases of neoplasms per 100,000 dogs per annum (MacVean *et al.*, 1978) while Dobson *et al.* (2002) collected a database of 130,684 insured dogs, out of which a total of 2,546 were tumour related and found that skin and soft tissues were the most common sites for tumour development, with a standardized incidence rate of 1,437 per 100,000 dogs per year, followed by alimentary (210), mammary (205), urogenital (139), lymphoid (134), endocrine (113) and oropharyngeal (112).

Mukaratirwa *et al.* (2005) studied the prevalence of different cutaneous neoplasms from 540 dogs at University of Zimbabwe and diagnosed thirty different histological types of tumour. According to them, the prevalence of epithelial, mesenchymal, lymphohistiocytic and melanocytic tumours was 39.4, 44.4, 7.4 and 8.7, respectively, and the ten most common tumours comprising 73.7% of all cutaneous neoplasms were mast cell tumours, squamous cell carcinomas, perianal gland adenomas, lymphomas, benign melanomas, haemangiosarcomas, sebaceous gland adenomas, fibrosarcomas, lipomas and malignant melanomas. Findings of Animal Tumour Registry Genoa, Italy (Merlo *et al.*, 2008) revealed that mammary cancer was the most frequently diagnosed cancer in female dogs, accounting for 70% of all the cancer cases. Incidence of all cancers was 99.3 per 100,000 dogs in male and 272.1 in female dogs. The highest incidence rates were detected for mammary cancer (191.8) and for non-Hodgkin's lymphoma (22.9) in bitches, and for non-Hodgkin's lymphoma (19.9) and skin cancer (19.1) in male dogs. All cancers incidence rate increased with age ranging between 23.7 and 763.2 in bitches and between 16.5 and 237.6 in male dogs aged d"3 years and >9–11 years. From these findings they concluded that incidence of all cancers was 3 times higher in female than in male dogs, which was due to the high rate of mammary cancer observed in bitches.

INDIAN SCENARIO

The scenario of cancer incidences in India is not much clear due to lack of systemic study at national level and absence of Animal Cancer Registry. In India, the incidence of tumours is found to be highest in canines followed by equines and bovines. Until 1982, dogs were not included in the India's national livestock census when their population stood at 18.54 million. It increased to 29 million in 2003 [16.7 million (57.59%) licenced dogs and 12.3 (42.41%) million others].

In studies pertaining to epidemiology and survey of canine tumours in India, Choudhary and Rao (1982) reported canine neoplasms encountered in Andhra

Pradesh and found the skin tumours to be most common type of all cancers in dogs. Phangcho *et al.* (1990) studied canine neoplasms in Assam with highest incidence of transmissible venereal tumours (42.5%). Singh *et al.* (1991) conducted a survey of tumours in domestic animals in Hisar and reported low occurrence of tumours in dogs (19.5%) when compared to cattle (53.56%).

Mukhopadhayay and Som (1990), in their study on 136 canine tumours from Calcutta found highest incidence of skin tumours (46.6%), followed by genital organs (23.5%), mammary gland (20.6%) and other organs (13.2%). Shekhar *et al.* (2001), in an epidemiological study on canine mammary tumours, reported a high incidence of malignant mammary tumours (59 out of 72). Rohitash (2003) recorded high occurrence of skin and subcutis tumours (35.92%), followed by mammary gland tumours in dogs (30.92%), tumours of genital system (21%), tumours of vascular and lymphoid tissues (7.4%) and tumours of muscle and cartilage (5.26%) in various districts of Rajasthan. Adak (2005) reported 33.14 % incidence of mammary tumors among 347 tumour bearing dogs recorded at the BSPCA Hospital, Mumbai during January to December 2004. Out of 115 canine mammary tumors, 65% occurred between 8 -12 years of age while 25 % of tumours occurred between 13 to 18 years of age and only 10 % of tumors occurred in 0 to 8 years of the age group. Further Pomeranian (40%) was most susceptible breed for mammary tumours followed by Doberman (25 %), Mongrels (15 %), Alsatian (10%), Dachshund (5 %) and Cocker Spaniel (5 %). Krithiga *et al.* (2005) observed 8 mammary tumours (simple tubular adenocarcinoma, complex adenocarcinoma and cystic papillary adenoma, mixed mammary tumours, squamous cell carcinoma and papillary adenocarcinoma) out of 65 cases of tumours. Nair (2005) reported higher incidence of mammary tumours (41.6%) than skin tumours (31.25%) in dogs.

Reddy (2007) in his study on canine tumours found 105 cases as tumourous out of 113 suspected cases. Among them 60% were mammary tumours and 40% skin tumours. On histopathological examination they found 36 as benign and 69 as malignant. The histologically classified benign skin tumours were canine cutaneous histiocytoma, cavernous hemangioma, mast cell tumour, perianal gland adenoma, fibroma and fibromyxoma and malignant skin tumours includes basal cell carcinoma, squamous cell carcinoma, fibrosarcoma, myxosarcoma, perianal gland adenocarcinoma, liposarcoma, epidermoid carcinoma and sebaceous basal cell carcinoma. Among mammary tumours, benign includes cystic fibroadenoma, benign mixed mammary tumour, papillary adenoma, myoepithelioma and malignant counterparts malignant mixed tumour, papillary adenocarcinoma, adenocarcinoma, solid carcinomas and malignant myoepithelioma.

Pawan (2008) studied spontaneously occurring canine mammary tumours, classified them histopathologically and investigated the correlation of mitotic index,

AgNOR count and c-erbB2 oncoprotein expression. In this study, out of 74 grossly suspected cases, 65 were diagnosed as mammary tumours, of which 11 (16.92%) were benign and 54 (83.08%) were malignant tumours. Benign tumours included benign mixed mammary tumour, fibroadenoma, duct papilloma and simple adenoma. The malignant mammary tumours comprised of papillary adenocarcinoma, malignant mixed mammary tumour, solid carcinoma, squamous cell carcinoma, fibrosarcoma, infiltrative adenocarcinoma, mucinous carcinoma and one case each of osteochondrosarcoma, carcinosarcoma, myxosarcoma, intraductal carcinoma and spindle cell carcinoma (malignant myoepithelioma). Rangnath (2009) also studied 103 cases (out of 109 suspected cases) of canine mammary tumours and found that most of tumours (95.25%) were malignant in nature.

DIAGNOSIS

The diagnosis of cancer involves the analysis of tumorous tissue and cells for the histopathological and cytological examination obtained through several procedures, including surgical biopsy, core or aspirational needle biopsy, pleural or ascitic tap, scraping of tissue surfaces and collection of exfoliative cells from urine and sputum. Despite the major advances in diagnostics and management, the survival of pet animals with cancer has not been substantially improved. The success of treatment to cure cancer lies in early detection of the disease. However, early detection is often one of the most challenging aspects of this disease. This is primarily because not all cancers present as tumor masses on the surface of the body where they may be easily noticed and examined for changes. In many instances, malignant tumors arising in the organs of the body will eventually cause symptoms directly related to the location of the tumor.

CLINICAL SYMPTOMS

Clinical symptoms of cancer vary according to the type and nature of the cancer and its location in different organs. These include gastrointestinal obstruction which may be accompanied by bleeding which is presented as diarrhea and vomiting (commonly associated with tumors invading the stomach, small intestine, large intestine, or colon), hematuria (in tumors of the kidney or bladder), Cushing's disease, hypoglycemia, etc. (in hormone-producing tumors such as some pancreatic, thymic and hepatic tumors) and neurologic symptoms such as loss of coordination or seizures (in tumors of the brain or spinal cord). Cancers producing nonspecific symptoms are extremely difficult to be diagnosed for their location, referred to as paraneoplastic disorders. These include weight loss, low-grade fever, seizures, lethargy, loss of appetite, diarrhea, skin rash, hair loss, and general arthritic-like symptoms. These types of cancers require specialized diagnostic tech-

niques such as laboratory screening tests, X-rays, CT scan, MRI etc. which can provide a means for earlier diagnosis and perhaps better long-term prognosis.

IMAGING

Imaging is an important tool to get information about malignant nature of the tumour. With the help of imaging techniques such as ultrasound, radiography (X-ray), computed tomography (CT) and magnetic resonance imaging (MRI), one can frequently suspect the patient possessing any internal tumour based on imaging information, which is later confirmed on histology. Until now, exploratory surgery or limited radiologic evaluations are most commonly used techniques for cancer diagnosis and staging in the pet animals. These techniques often provide the important structural and anatomic information about tumour and also allow differentiation of benign from malignant lesions and assist in accurate staging of disease. With the advent of molecular imaging techniques i.e. magnetic resonance spectroscopy (MRS) and positron emission tomography (PET), it is currently possible to know about the functional, biochemical and physiologic nature of cancerous mass and which lead to designing of appropriate therapeutic and managemental measures.

HISTOPATHOLOGICAL TECHNIQUE

Histolopathology is still a gold standard for diagnosis of tumours but it alone does not provide sufficient details of the cellular changes which could predict the clinical behaviour of the tumour. Even then histopathological examination of the tumour cells by any expert oncologist can give an accurate diagnosis about the type of tumour and possible malignancy status. Presence of hyperchromatic nuclei, more nucleus to cytoplasm ratio, disorientation of the cells, etc. are the cellular changes observed in cancer cell. Presence of mitotic figures in the cells is also related with neoplastic changes in the tissues. During studies on the canine mammary and skin tumours it was found that number of mitotic fiqures in the malignant tumours was significantly higher than the benign tumours (Pawan *et al.*, 2010; Reddy *et al.*, 2007). Special staining procedures can differentiate the different types of tumours and thus help in diagnosis such as touludine blue stain differentiate mast cell tumour from other tumours as it stains the metachromatic granules present in mast cells (Reddy *et al.*, 2009).

AgNOR count

AgNOR is a molecular marker used to study the rapidity of cell proliferation in various types of tumours (Crocker and Nar, 1987). The number of interphase AgNORs in continuously proliferating cells had been strictly related to the rapid-

ity of cell proliferation (Trere, 2000). AgNOR method had been applied in tumour pathology for both diagnostic and prognostic purposes. These counts can be used as a measure to differentiate canine transmissible venereal tumour from canine cutaneous histiocytomas (Pawaiya *et al.*, 2006). Canine mammary and skin tumours studies showed that the tumours having malignant nature had more AgNOR count than the benign ones (Pawan *et al.*, 2010; Reddy *et al.*, 2007).

TUMOUR MARKERS

The attempts to find malignant nature of the tumours first started about 2000 years ago with the identification of tumour biomarkers. Tumor markers are biologic or biochemical substances produced by tumours and secreted into blood, urine, other body fluids or present on body tissues in higher than normal amounts. Tumor markers can be detected by various methods including antigen-antibody based techniques (enzyme linked immunosorbant assay, radio-immunoassay, precipitin tests, flow-cytometry, immunohistochemistry, immunoscintigraphy) and molecular genetic methods. Measurement of tumor markers levels, when used along with other diagnostic tests, can be useful in the detection and diagnosis of some types of cancers.

SEROLOGICAL METHODS

Mainly two serological methods are used in estimation of serum tumour markers- ELISA (Enzyme Linked Immunosorbent Assay) and RIA (Radio Immuno Assay). The ELISA is typically used to detect and quantify antigen within biological fluids, in which the Dual- Antibody Sandwich ELISA is being used for measuring the concentration of 80% of tumor markers in blood or serum. RIA is one of the most sensitive technique for detecting antigen or antibody. Gamma emitting isotope such as Iodine and beta emitting isotope such as tritium are also routinely used as labels. The presence of CEA, AFP, PSA and other markers in the serum of the cancer patients can be detected with the help of ELFA (Enzyme linked fluorescent assay). The test measures the amount of CEA that may appear in the blood of some people who have certain kinds of cancers, especially large intestine (colon and rectal) and breast cancer. It may also be present in people with cancer of the pancreas, ovary, or lung (Threiveni *et al.*, 2007).

IMMUNOHISTOCHEMISTRY (IHC)

IHC is of great value in the diagnosis of undifferentiated tumours where light microscopy is unable to discern diagnostic features such as poorly differentiated carcinoma, anaplastic large cell lymphoma, amelanotic melanoma or, less com-

monly sarcoma. It has a major assistance in defining metastatic tumors of unknown primary site. For example, expression of leukocyte common antigen (LCA) is evidence of lymphoid origin, cytokeratins strongly suggests an epithelial origin while expression of S 100 protein and HMB 45 is characteristic of malignant melanoma. IHC has been utilized extensively to determine the expression of oncoproteins (c-erbB2, c- myc), hormonal receptors (estrogen, progesterone) and proliferative markers (PCNA, Ki67) in the animal such as ovine pulmonary adenocarcinoma, canine mammary tumours, canine skin tumours, buffalo cutaneous histocytoma and chemically induced rat tumours. It was found positive for different oncoprotein expression and tumour proliferative markers by various researchers using IHC technique (Reddy *et al.*, 2007; Pawaiya *et al.*, 2008; Pawan *et al.*, 2009; Pawaiya and Ram Kumar, 2009). Particularly in pet animals tumours such as canine mammary tumours, the expression of the c-erbB2 has been found to be more in tumours of epithelial origin (Rungsipipat *et al.*, 1999; Pawan *et al.*, 2009) and can be corelated with the prognosis of the case (Rungsipipat *et al.*, 1999). All the advantages of IHC can be nullified if it is used without an expertise. Strict adherence to laboratory practices is essential. A panel of antibodies is generally recommended to characterize a diagnostic problem. Results need to be interpreted in appropriate context; possibility of false positive and false negative remains.

FLOW CYTOMETRY

Flow cytometry is a modern clinical laboratory test used to assist physicians in patient diagnosis, disease classification and patient prognosis. Fluorescence-activated cell sorting (FACS), a specific type of flow cytometry utilizes fluorescent markers placed on the cells for the purpose of recognizing and sorting the cells. FACS possess the ability to perform multiparameter analyses on a single cell and offers promise for the early detection of apoptotic cells in tumours during treatment. Sampling of tumours for apoptotic cells may offer a very early marker of tumour response, predictive of cancer's sensitivity to a given treatment (Bertho *et al.*, 2000). Over the decade, flow cytometry has evolved as an indispensable tool in the diagnosis of hematologic malignancies. Many new antibodies, improved gating strategies, and routine use of multiparameter techniques have dramatically improved the diagnostic utility of flow cytometry. Typically, light scatter is combined with staining for tumor-specific antigen combinations (Stelzer, 1996). The strategy of detecting malignant cells using expression of aberrant phenotypes which is rarely found in normal cells requires thorough knowledge of the frequency of normal patterns of expression. For example TdT is only expressed in T - cells that reside in the thymus and a limited number of bone marrow cells. The majority of cases of ALL and lymphoblastic lymphoma express TdT. Therefore, if TdT cells are found

in the peripheral blood or cerebrospinal fluid, one can identify them as malignant cells. The majority of B-lineage ALL cells expresses TdT, CD19, and CD10, with a smaller number expressing CD34. Any combination of these markers (all of which are found on normal cells in the bone marrow) with the addition of certain aberrant markers such as CD13, CD33, or CD15 may uniquely identify the ALL cells from normal bone marrow or peripheral blood cells. The use of flow cytometry in the veterinary clinical laboratories including at IVRI has increased considerably during the past decade. The most common applications of flow cytometry in small animal oncology are measurement of DNA content in tumours and immunophenotyping of haematopoietic malignancies (Culmsee and Notle, 2002).

POLYMERASE CHAIN REACTION (PCR)

Molecular oncology deals with the study of the alterations in genetic and biochemical processes at the molecular level. The changes can be studied on chromosomes, DNA or RNA. Not all mutations in cancer genes are apparent at cytogenetic level, so it has become increasingly important to identify genes themselves, and relevant changes within their structure. PCR allows early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the quite developed tool in cancer research and is already being used routinely. Mackay *et al.* (2002) opined that Real-time quantitative PCR is a very powerful and accurate technique to examine expression patterns of different oncogene and suppressor genes in different cancerous conditions. Gene expression analysis has become increasingly important in cancer research where different gene expression profiles lead to establish the definite prognosis and therapeutics.

MICROARRAY

Microarray technology has emerged as a powerful tool to increase the potential of standard methods through genomic biology studies. Carcinogenesis is a multistep process that is the outcome of the accumulation of several genetic and epigenetic events. Detection of mutations in cancer is of major importance for both basic understanding of the cancer development process and clinical practice. DNA array is a powerful and effective tool for detecting specific mutations, small insertions and deletions in non repetitive sequences. Other examples include determination of mutation in *K-ras* and *p53* genes by microarray. Among genetic changes occurring during carcinogenesis, chromosomal rearrangements with gene copy number fluctuations (including gains and losses of nucleic material) occur frequently.

Relevance of cancer markers identified by genomic or proteomic analysis in the diagnostic, prognostic, and therapeutic of cancer can be evaluated with tissue microarrays or tissue chips (Kononen *et al.*, 1998). This consist of a set of small cylindrical sections (600 µm in diameter, 5 µm thick) acquired from formalin-fixed tissues and arrayed on a glass slide. Typical tissue microarrays contain 500 to 1,000 sections. They are used in large-scale screening of tissue specimens for *in situ* detection of DNA, RNA, and protein targets or to survey gene amplification. Pathologic evaluation of molecular alteration is of most importance in cancer research and treatment. Tissue microarray technology contributes greatly in advances of cancer research and enhanced translation of experimental discoveries of potential markers into clinical practice (Horvath and Hanshell, 2001). Immunohistochemical staining of arrayed tissue allows measurement of protein levels in cancer specimens. Given its marked advantages, the tissue microarray has become a mainstay in a two-phase strategy with microarray based gene expression profiling. Indeed, tissue arrays may become a validation tools used in a second analysis to focus on individual targets differentially expressed in cancer by global methods. Camp *et al.* (2000) in his study on 38 breast carcinomas been screened for three antigens (estrogen receptor, progesterone receptor, and Her2/*neu*) found similar results in the whole section when they tested at least two samples. Furthermore, they validated use of archival fixed and embedded tissue to construct tissue arrays in tissue-based molecular research.

THERAPEUTIC MANAGEMENT

Recognizing that cancer is a multi-factorial disease, prevention and treatment requires a pro-active approach using a wide variety of both traditional and non-traditional modalities. Common cancer therapies include surgery, chemotherapy and radiation but nowadays the age of more targeted therapeutics has come. Novel cancer targeting drugs like anti angiogenesis agents, monoclonal antibodies, telomerase therapy for canines (Nasir, 2007), gene therapy, tyrosine kinase inhibitors etc. are beginning to be explored in the treatment of canine tumours. The search for better prognostic markers and predictive factors is now focused on the molecular mechanisms which underlie tumour behaviour such as altered cell cycle progression, proliferation, apoptosis and angiogenesis. The ultimate goal is to identify reliable markers that can accurately diagnose and predict tumour's clinical behaviour, prognosis and response to therapy (Mukaratirwa, 2005).

SURGERY

Surgery is the oldest method to get rid off from cancer. Approximately 70% of patients presenting with cancer already have distant micrometastases at the time

of diagnosis so in those cases surgery alone is not effective to cure cancer. Surgical treatment of cancer recommends aggressive, radical approaches for removing tumors as well as regional lymph nodes and underlying tissues, however, clinical studies have not demonstrated any therapeutic advantage to this approach. In certain conditions surgical intervention act as preventive measure for certain tumours such as in unilateral or bilateral cryptorchidism which increases risk of testicular cancer in male dogs and benign mammary tumors which increase risk for mammary cancer in female dogs, castration (neutering) and ovariohysterectomy (spaying), respectively, can prevent pet animals form contracting these tumours.

RADIATION

Because of the clinical evidence of the efficacy of radiation treatment of dogs with particular tumors, more and more veterinary facilities are beginning to offer this form of treatment for cancer and some benign tumors. Radiation therapy is beneficial particularly when tumor invasion is widespread or if the dog's general health places him at high risk for surgical complications. The higher the dose of radiation that a tumor is exposed to, the greater the chance for destroying all of the tumor cells. Unfortunately, however, high dose radiation also compromises the normal tissue that surrounds the tumour. A way to administer a high total dose of radiation is to divide it up and administer it in small, equal amounts over a period of time a procedure termed fractionation, which reduces excessive complications in normal tissues. Brain tumours respond well to radiation with either complete cures (as in the case of small pituitary tumors), or longer survival times (intracranial tumors and spinal lymphomas). Limitations includes higher possibility of survival of cancer cells at the center of the large tumour mass which may lead reappearance of the tumour. So it is recommended that radiation therapy must be given in combination with surgery or chemotherapeutic drugs to remove remaining tumour mass. Care should be taken that radiation therapy must be postponed until the surgical incision is completely healed.

In veterinary oncology, radiation is most often used following surgery to destroy remaining cancer cells that may have been left behind. If too much tumor remains, then advantages of using the combination are significantly reduced. Radiation prior to surgery may reduce risk for the spread of tumor cells during surgical excision of the tumor.

HYPERTHERMIA

In some human cancer patients, high fever has been found to be associated with subsequent disease remission which led to idea that hyperthermia might pro-

vide a new treatment approach to this disease. Research into the use of hyperthermia for the clinical treatment of cancer has indicated that it is lethal to cells, causes tumour regression, increases the efficacy of radiation therapy and enhances the action of many anti-cancer drugs. Local heating of tumors can be given by microwave radiation, infrared radiation, radiofrequency or ultrasound. Though cancer cells are destroyed by hyperthermia treatment alone, many factors including the nature and size of the tumour will influence the success of hyperthermia to eradicate the entire disease. Populations of cancer cells that may escape the lethal effects of hyperthermia are often resistant to subsequent heat exposure. Therefore, as with other methods of treatment, hyperthermia is often used in combination with radiation or chemotherapy to increase overall treatment efficacy. In canine cancers, treatment with hyperthermia is more commonly administered in combination with radiation.

PHOTODYNAMIC THERAPY (PDT)

In veterinary oncology, PDT has been used limitedly and usually in dogs with localized, superficial, and minimally invasive tumors. PDT treatment for cancer patients is accomplished in two steps. First, dog is administered a photosensitizer drug which is preferentially uptaken and retained by the cancer cells but excreted from the normal cells of the body. Second, tumor is then exposed to light of a certain wavelength that will activate the photosensitizer. Therapeutic limitations to PDT include the inability of light to penetrate deeply into tumor tissue. Therefore, treatment with PDT has been primarily aimed at superficial mucosal cancers, those affecting the skin, lining of the bladder, and the lining of the oral cavity.

CHEMOTHERAPEUTIC DRUGS

Many studies indicating the efficacy of chemotherapy to control and sometimes cure cancer in the dog has led many veterinary oncologists to include chemotherapy, either as the primary treatment or in combination with other forms of therapy, to treat cancer in the dog (Table 1). Major limitations to chemotherapy are toxicities associated with the non-specific action of many of these drugs against normal cells particularly cells of the bone marrow, gastrointestinal lining, and hair follicles. Common side effects resulting from toxicities include immunosuppression, anemia, nausea and vomiting, delayed wound healing, reproductive failure and hair loss.

Table - 1

Sl. No.	Class of chemotherapeutic agent	Agent	Mechanism of action	Tumours treated
1.	**Alkylating agents**	Cyclophosphamide Ifosfamide Chlorambucil Melphalan Busulfan	Cross linking and strand breakage of DNA	Lymphoma, Mast cell tumors, Mammary tumours, Hemangiosarcomas, Soft tissue sarcomas, Leukemias, Multiple myeloma
2.	**Plant alkaloid**	Vincristine Vinblastine	Cell cycle arrest by interacting with tubulin	Lymphoma, Venereal tumours, Mast cell tumours, Sarcomas
3.	**Antimetabolites**	Methotrexate Cytosine Arabinoside Fluoropyrimidines Hydroxyurea	Cell cycle arrest by interfering in folic acid synthesis and DNA synthesis	Lymphoma, Osteosarcoma, Central nervous system lymphoma, Leukemias, Skin tumours, Mammary carcinoma, Gastrointestinal tract tumours
4.	**Antitumour antibiotics**	Doxorubicin Epirubicin Methoxymorpholino -doxorubicon Mitoxanthrone Bleomycin Actinomycin D	DNA damage	Hemolymphatic malignancies, Carcinomas, Sarcomas, Lymphoma, Oral squamous cell carcinoma
5.	**Platinum compounds**	Cisplatin Carboplatin Lobaplatin	DNA synthesis and replication (crosslink damage)	Osteosarcoma, Skin and nasal carcinomas
6.	**Nitrosoureas**	Lomustine Carmustine	DNA (crosslink damage)	Brain and central nervous system tumours, Lymphomas, Mast cell tumours
7.	**Topoisomerase I Inhibitors**	Camptothecins	Topoisomerase I (DNA replication	Lymphoma
8.	**Hormonal agents**	Prednisone	DNA (cleavage)	Lymphomas, Mast cell tumours
9.	**Biologic Response Modifiers**	Piroxicam, Liposome-encapsulated-muramyl tripeptide phosphatidylethanol	Immune system	Squamous cell carcinoma, Mammary adenocarcinoma, Transmissable venereal tumours, Splenic hemangiosarcoma,

GENE THERAPY

Principle of gene therapy is to insert a foreign DNA into the tumorous cells which when after incorporation with host cell DNA get expressed and ultimately synthesize different proteins or other molecules which usually facilitate that cells destruction. Gene therapies utilize a number of methods, including viral and non-viral vectors, to deliver genetic material into cells. The mechanism of action of these genetic inserts includes production of protein product which changes an inactive drug into a toxic drug only in the cancer cell carrying the foreign gene or by expressing certain molecules on their surfaces that will attract components of the immune system to attack and destroy the cancer cells or by replacing the mutated gene in the cancer cell that has caused unregulated cell growth in the cancer cells or by making normal cells of patient body more resistant to chemotherapy drugs so that the patient may be administered higher doses of chemotherapy with reduced toxic side-effects.

For example, to target genetic lesions in the tumour cell, antisense molecules have been widely used in human trials. Antisense molecules are synthetic oligodeoxynucleotides (ODN) which are designed such that they can hybridize specifically to the coding (sense) mRNA inside the cell (Helene and Toulme, 1990). Targeting mRNA with ODNs is attractive as they form Watson–Crick base pairs with the targeted mRNA. The double stranded RNA cannot be translated and is easily destroyed. *In vivo*, the ODNs can be injected systemically into the patient's body. However, one of the problems is that the ODNs are easily destroyed by the nucleases in the blood. In order to make ODNs stable one of the most common modification of ODNs is replacing the nonbridging oxygen atoms in each of the inter-nucleotide phosphate linkages with sulphur atom (Stein *et al.*, 1988). This makes the ODN stable against nucleases, easily soluble in water and simple and inexpensive to synthesize..There are other derivatives of ODNs which are notable for both, extremely high nuclease resistance and tight binding to single stranded RNA (Toulme, 2001). The anti-sense nucleic acid can also be expressed from a plasmid transfected into the cell. Synthetic DNA and RNA can also be engineered to contain inherent cleaving activity like ribonucleases H which are ubiquitous enzymes cleaving the RNA part of RNA/DNA hybrids (Haseloff, 1988).

STEM CELL THERAPY

Stem cells have been used in the replenishment of blood and immune systems damaged by the cancer cells or during treatment of cancer by chemotherapy or radiotherapy. Apart from their use in the immuno-reconstitution, the stem cells have been reported to contribute in the tissue regeneration and as delivery vehi-

cles in the cancer treatments. The recent concept of 'cancer stem cells' has directed scientific communities towards a different wide new area of research field and possible potential future treatment modalities for the cancer.

Although the idea of the therapies focused on the cancer stem cells may look exciting, targeting the cancer stem cells may not be easy. The cancer stem cells are relatively quiescent compared to other cancer cells and do not appear to have the hyper-proliferation signals activated such as tyrosine kinase. These make the cancer stem cells resistant to the toxicity of the anti-cancer drugs, which traditionally target the rapidly dividing cells. One approach to target the cancer stem cells may be the identification of the markers that are specific for the cancer stem cells compared to normal stem cells such as haematopoietic stem cells express Thy-1 and c-kit whereas leukaemic stem cells express IL-3 (interleukin-3) receptor á-chain (Blair *et al.*, 1997; Blair and Sutherland, 2000).

Much of the research is now focused on targeting the essential genes or pathways crucial for the cancer development through the cancer stem cells, with any possible therapies targeted against TICs. One such example is the use of Gleevec® in chronic myeloid leukaemia that targets the ATP-binding domain of the Abl kinase. Most patients in this study experienced the complete cytogenetic responses (Druker *et al.*, 1996; O'Brien *et al.*, 2003). although the therapy may not be curative due to reported presence of the fusion transcript (Branford *et al.*, 1999). A comparison of the pathways that regulate the stem cell homing with those responsible for metastasis may prove useful to minimise the toxic effects of the drugs. Treatment of mice with a Hedgehog (Hh) pathway inhibitor such as cyclopamine (Berman *et al.*, 2002) inhibits the growth of medulloblastomas in mouse models, without any apparent toxicity. Thus, the Hh pathway may be inactive in most normal adult tissues, thus minimising the toxicity effects of these inhibitors (Beachy *et al.*, 2004). Thus, the concept of the cancer stem cells has opened new areas of research in carcinogenesis and future treatment options.

NANOTECHNOLOGY

Nanotechnology refers to the interactions of cellular and molecular components and engineered materials, typically clusters of atoms, molecules, and molecular fragments at the most elemental level of biology. Such nanoscale objects typically, though not exclusively, with dimensions smaller than 100 nanometers can be useful by themselves or as part of larger devices containing multiple nanoscale objects. Cancer nanotechnology is emerging as a new field of interdisciplinary research, lead to major advances in cancer detection, diagnosis, and treatment (Ferrari, 2005; Srinivas *et al.*, 2002). Medical applications have also appeared,

such as the use of superparamagnetic iron oxide nanoparticles as a contrast agent for lymph node prostate cancer detection (Harisinghani *et al.*, 2003) and the use of polymeric nanoparticles for targeted gene delivery to tumor vasculatures (Hood *et al.*, 2002). Therapeutic and diagnostic agents can be encapsulated, covalently attached, or adsorbed onto nanoparticles. For example, paclitaxel is one of the most widely used anticancer drugs in the clinic. It is a microtubule-stabilizing agent that promotes tubulin polymerization, disrupting cell division and leading to cell death (Diaz *et al.*, 2000; Nicolou *et al.*, 1993). In a new formulation approach used recently approved by the FDA to treat metastatic breast cancer, paclitaxel was conjugated to albumin nanoparticles (Garber, 2004). For enhanced tumor-specific targeting, the differences between cancerous cells and normal cells may be exploited. As efforts in proteomics and genomics uncover other molecules unique to cancer cells, targeted nanoparticles could become the method of choice for delivering anticancer drugs directly to tumor cells and their supporting endothelial cells. In fact, at least one research group is using the empty RNA virus capsules from cowpea mosaic virus and flockhouse virus as potential nanodevices. The premise is that 60 copies of coat protein that assemble into a functional virus capsule offer a wide range of chemical functionality that could be put to use to attach homing molecules such as monoclonal antibodies or cancer cell-specific receptor antagonists, and reporter molecules such as magnetic resonance imaging (MRI) contrast agents, to the capsule surface, and to load therapeutic agents inside the capsule.

HERBAL MEDICINAL THERAPIES

Plants have been used for treating various diseases of human beings and animals since time immemorial. They maintain the health and vitality of individuals, and also cure diseases, including cancer without causing toxicity. According to the estimates of the WHO, more than 80% of people in developing countries depend on traditional medicine for their primary health needs. A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy (Madhuri and Pandey, 2008; Sivalokanathan *et al.*, 2005). Plants are used against various types of tumours/cancers such as sarcoma, lymphoma, carcinoma and leukaemia. Medicinal plants possess immunomodulatory and antioxidant properties, leading to anticancer activities.

Plants contain several phytochemicals, which possess strong antioxidant activities. Phytochemicals such as vitamins (A, C, E, K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have been found to elicit antioxidant activities (Kathiresan *et al.*, 2006; Heber, 2004; Kaur and Kapoor, 2002). Ellagic acid and a whole range of flavonoids, carotenoids and terpenoids present in *Fragaria vesca* (strawberries) and *Rubus idaeus*

(raspberries) have been reported to be responsible for antioxidant activity. These chemicals block various hormone actions and metabolic pathways that are associated with the development of cancer (Caragay, 1992; Steinmetz and Potter, 1991). Quercetin is the major flavonol in the Western diet. Rich sources of quercetin are red and yellow onions, kale, broccoli, red grapes, cherries, French beans, apples and cereals. Quercetin possesses both anticarcinogenic activity and the ability to inhibit LDL oxidation (Craig, 2006; Smith and Yang, 1994). Examples of the plants having therapeutic effects against some cancers includes *Agrimonia pilosa* in sarcoma, *Ailanthus altissima* in intestinal cancer, sarcoma and leukaemia, *Akebia quinata* in sarcoma, *Chelidonium jajus* var. *asiaticum* in stomach cancer; *Chimaphila umbellate* in breast tumour; *Fritillaria thunbergii*16 in tumours of the throat, chest, neck and breast; *Nidus vespae* in gastric and liver cancers etc. Commercialization of the products of these plants needed to design an effective combination of therapies for cancer.

CONCLUSIONS

Cancer is an important disease identity in the pets and accounts for a significant loss of their lives. Due to increased awareness of pet care among pet owners, it becomes necessary to understand this enigmatic complexity of cancers and to carry functional studies to prevent and cure the disease. Presently, cancer therapy has entered in to an exciting new era, with traditional therapies such as chemotherapy, radiotherapy and surgery on one side while the gene therapy, stem cells and nanotecnology on the other hand. Screening and surveillance should ideally be tailored to an individual's cancer risk and current methods of imaging in cancer, including magnetic resonance imaging, computed tomography, positron emission tomography, magnetic resonance spectroscopy, ultrasound, mammography and digital mammography are not frequently been used by the veterinary oncologists. Microarray technology is a new and efficient approach to extract data of biomedical relevance for a wide range of applications. In cancer research, it will provide high-throughput and valuable insights into differences in an individual's tumor as compared with constitutional DNA, mRNA expression, and protein expression and activity. The resulting product should be a wealth of tumor-associated and tumor specific biomarkers, which may help in cancer etiology, diagnosis, and therapy. Due the high cost of all these new technology, their use in veterinary field is restricted but to improve the early diagnosis and prognosis of cancer patients it is mandatory to use them. There is no doubt that emerging genomic and proteomic technologies will further facilitate investigations related with cancer etiology, pathogenesis, diagnosis and their therapeutics.

REFERENCES

1. Adak A (2005). M.V.Sc. Thesis, Bombay Veterinary College, MAFSU Nagpur.
2. Beachy PA et al. (2004). *Nature*, **432**:324-331.
3. Berman DM et al. (2002). *Science*, **297**:1559-1561.
4. Biesalski HK *et al.* (1998). *Cancer J. Clin.*, 48 (3): 167–76.
5. Blair A and Sutherland HJ (2000). *Exp. Hematol.*, **28**:660-671.
6. Blair A et al. (1997). *Blood*, **89**:3104-3112.
7. Branford S et al. (1999). *Br. J. Haematol.*, **107**:587-599.
8. Camp RL *et al.* (2000). *Lab. Invest.*, 80:1943-1949.
9. Caragay AB (1992). *Food Technol.*, 46:65–68.
10. Choudary C and Rao MRKM (1982). *Ind. Vet. J.*, 59(2): 10-102.
11. Craig WJ (2006). Phytochemicals: Guardians of our health, 2006.
12. Crocker J and Nar P (1987). *J. Pathol.*, 151:111-118.
13. Culmsee K and Nolte I (2002). *Methods Cell Sci.*, 24(1-3): 49-54.
14. Diaz JF et al. (2000). *J. Biol. Chem.* 275:26265–26276.
15. Dobson JM *et al.* (2002). *J. Small Ani. Prac.*, 43: 240–246.
16. Druker BJ et al. (1996). *Nat. Med.*, **2**:561-566.
17. English DR *et al.* (1997). *Cancer causes control : CCC*, 8 (3): 271–83.
18. Ferrari M (2005). *Nat. Rev. Cancer*, 5:161–171.
19. Goldschmidt MH *et al.* (2001). In: Pathobiology of aging dogs, ed. Mohr U, Carlton, W.W., Dungworth ,D.L., Benjamin, S.A., Capen, C.C., Hahn, F.F. 1st edn., pp.168-178.
20. Harisinghani MG et al. (2003). *N. Engl. J. Med.*, 348:2491–2499.
21. Haseloff J and Gerlach WL (1988). *Nature*, 334;585–591.
22. Heber D (2004). *J. Postgrad. Med.*, 50:145–149.
23. Helene C and Toulme J (1990). *Biochim. Biophys. Acta*, 1049;99–125.
24. Hood JD et al. (2002). *Science*, 296:2404–2407.
25. Horvath L and Henshall S (2001). *Pathology*, 33:125-129.
26. Jemal A *et al.* (2003). *CA Cancer J. Clin.*, 53:5-26.
27. Jemal A *et al.* (2008). *CA Cancer J. Clin.*, 58:71-96.
28. Kathiresan K et al. (2006). *Nat. Prod. Rad.*, 5:115–119.
29. Kaur C and Kapoor HC (2002). *Int. J. Food Sci. Technol.*, 37:153–161.
30. Kononen J *et al.* (1998). *Nat. Med.*, 4:844-847.
31. Krithiga K *et al.* (2005). *Indian J. Vet. Pathol.*. 29(2): 118-120.
32. MacEwen EG and Withrow SJ (1996). In : Small animal clinical oncology. 2nd edition, W.B. Saunders Company, Philadelphia. pp. 211-226.
33. Mackay IM *et al.* (2002). *Nucleic Acid Res.*, 30(6):1292-1305.
34. MacVean DW *et al.* (1978). *Vet. Pathol.*, 15(6): 700-715.
35. Madhuri S and Pandey G (2008). *Plant Arch.*, 8:13–16.
36. Merlo DF *et al.* (2008). *J. Vet. Intern. Med.*, 22:976-984.

37. Misdorp W (2002). In: Tumours in domestic animals, ed. Meuten DJ, 4th ed., pp. 575-606. Iowa State Press, Ames, IA.
38. Moulton JE *et al.* (1970). *Vet. Pathol.*, 7:289-320.
39. Mukaratirwa S *et al.* (2005). *J. S. Afr. Vet. Assoc.*, 76(2): 59-62.
40. Mukhopadhayay S and Som TL (1992). *Indian J. Vet. Pathol.*, 16(2):122-123.
41. Nair BC (2005). MVSc thesis submitted to IVRI, Izatnagar.
42. Nicolaou KC et al. 1(993). *J. Natl. Cancer Inst.*, 96:90–91.
43. O'Brien SG et al. (2003). *N. Engl. J. Med.*, **348**:994-1004.
44. Pawaiya RVS and Ramkumar (2007). *Indian. J. Vet. Pathol.*, 31(2):99-107.
45. Pawaiya RVS and Ramkumar (2009). *Indian. J. Vet. Pathol.*, 33(1):46-51.
46. Pawaiya RVS *et al.* (2008). *Indian. J. Vet. Pathol.*, 32(2):251-56.
47. Pawan Kumar *et al.* (2010). *Brazilian J. Vet. Path.*, 3(1):41-45.
48. Pawan Kumar *et al.* (2009). *Indian. J. Vet. Pathol.*, 33(2):125-129.
49. Pawan Kumar (2008). MVSc thesis submitted to IVRI, Izatnagar.
50. Phangcho CV *et al.* (1990). *Ind. Vet. J.*, 67(9): 881-882.
51. Rangnath, GJ (2009). MVSc thesis submitted to IVRI, Izatnagar.
52. Reddy GBM *et al.* (2007). *Indian. J. Vet. Pathol.*, 31(2):108-112.
53. Reddy, GBM (2007). MVSc thesis submitted to IVRI, Izatnagar.
54. Rohitash D (2003). Thesis submitted to Rajasthan Agricultural University, Bikaner.
55. Rungsipipat A *et al.* (1999). *J. Vet. Med. Sci.*, 61(1):27–32.
56. Rungsipipat A *et al.* (2003). *Thai J. Vet. Med.*, 33 (1):59-66.
57. Sasco AJ *et al.* (2004). *Lung cancer*, 45 (2): 53–9.
58. Schneider R *et al.* (1969). *J. Nat. Cancer Inst.*, 43:1249-1261.
59. Shekhar CS *et al.* (2001). *Indian Vet. J.*, 78(2):107-109.
60. Singh P *et al.* (1991). *Indian Vet. J.*, 68(8):721-725.
61. Sivalokanathan S *et al.* (2005). *Indian J. Exp. Biol.*, 43:264–267.
62. SmithTJ and Yang CS (1994). In *Food Phytochemicals for Cancer Prevention I. Fruits and Vegetables* (eds Huang, M. J. *et al.*), ACS, Washington DC, 1994, pp. 17–48.
63. Srinivas PR et al. (2002). *Lab. Invest.*, 82:657–662.
64. Stein CA et al. (1988). *Nucleic Acid Res.*, 16;3209–3221.
65. Steinmetz KA and Potter JD (1991). *Cancer Causes Control (Suppl.)*, 2:325–357.
66. Stelzer GT (1996). *Clin. Immunol.*, 16:137.
67. Thriveni K *et al.* (2007). *Indian J. Clin. Biochem.*, 22:57-60.
68. Toulme JJ (2001). *Nature Biotechnol.*, 19 ;17–18.
69. Trere D (2000). *Micron.*, 31(2): 127-131.
70. Vail DM and MacEwen EG (2000). *Cancer Invest.*, 18: 781-792.