6. SUMMARY AND CONCLUSIONS

Canine transmissible venereal tumour is a tumor of the dog and other canids that mainly affects the external genitalia and is transmitted from animal to animal through sexual contacts, but may also be passed on as the dog bites, sniffs or licks the tumour-affected areas. Vast majority of probable aetiological factors, behavioral pattern, treatment regimens and outcome leaves clinician in confusion to choose a rational approach in handling the malady. The present work is an attempt to evolve rationality in establishing various aspects of CTVT for clinical application with the objectives as to study efficacy of different therapeutic approaches viz. sub-mucosal resection followed by chemotherapy with either Vincristine sulphate, Methotrexate and Doxorubicin or chemotherapy alone with Vincristine sulphate, Methotrexate and Doxorubicin; to study the histopathological changes and the hemato-biochemical changes before, during and after the treatment and to study the chromosomal aberrations in the lymphocytes in CTVT by karyotyping so also in granulomatous tissue to ascertain the aetiology of acquired diseased condition.

A survey of the urban area of Nagpur district was conducted and data collected from three veterinary hospitals viz. Advanced Referral Clinic and College Hospital, Government Veterinary Polyclinic, Nagpur and Veterinary Dispensary, Mahal, Nagpur for the period from April 2003 to March 2006 to study various aspects of CTVT. Thirty-six Clinical cases of CTVT in both sexes, presented at Veterinary Polyclinic, North Ambazari Road, Nagpur reported during January 2005 to December 2006 were used as experimental material. These animals were divided in six groups with five females and one male in group I, II and III, three females and three males in group IV and VI and six females in group V. The dogs of group I, III and V were treated with the five weekly cycles of chemotherapeutic drugs Vincristine sulphate (0.025 mg/kg body weight intravenously), Methotrexate (0.3mg/kg body weight intravenously) and Doxorubicin hydrochloride (1 mg/kg body weight intravenously), respectively, whereas in the dogs of group II, IV and VI submucosal resection was carried out under dissociative anaesthesia using Ketamine hydrochloride, followed by the chemotherapy with above three respective drugs. Various parameters viz. prevalence, effect of therapeutic regimen on clinical, haematological and biochemical parameters, regression of growth and histopathological changes
were studied at scheduled intervals of 0th, 7th, 14th, 21st, 28th and 35th day of drug administration. The side effects, tumour immunity, and chromosomal aberrations in lymphocytes and the CTVT tissue were also studied. The dogs were observed for a period of six months for recurrence.

Prevalence of Canine Transmissible Venereal Tumour

The survey analysis of data from urban area of Nagpur for three years revealed that 39,582 dogs were treated in the three hospitals, which included 5301 cases of surgical entity. The cases of various types of tumours accounted for 507 cases out of which, 212 (0.54%) cases were of CTVT with PC: SP ratio 1: 5.4.

The cases of canines presented from Jan 2005 to December 2006 at Veterinary Polyclinic, Nagpur were 3585 out of which 821 canines had a surgical entity and only 36 cases (1.004%) of different breed, age and sex were suffering with CTVT with the PC: SP ratio 1: 10.04. The maximum cases of CTVT i.e. 15 were recorded in winter season, followed by rainy season 13 cases and 8 cases in summer season. The maximum incidence of CTVT was documented in non-descript dogs (23) followed by Pomeranian (6) and less number of cases was documented in Doberman pinscher (3), German shepherd (2), Labrador and Boxer breeds (1 each).

Amongst 36 cases of affected canines, males were 9 and females were 27. However, more numbers of the females affected with Venereal Granuloma enrolled in non-descript breeds (16) followed by Pomeranian (5), Doberman and German shepherd (2 each) and Boxer and Labrador (1 each).

The present study revealed more incidence in non-descript breed at 4.36 years while in females, the mean age was 4.53 years. The higher age of incidence was documented in Pomeranian males i.e. 11 years and in females i.e. 6 years. Thus, the CTVT was observed to be a disease of young to middle aged dogs.

Aetiology of CTVT / Karyotype study of CTVT

Tissue and Lymphocyte culture and chromosome analysis

The study was aimed with identification of neoplasia associated chromosomal aberration in canine transmissible venereal tumour.

In the present study 20 metaphase cells each from blood of 6 dogs suffering with
canine transmissible venereal tumor and also the CTVT tissues of same dogs were identified and studied.

The study revealed that good metaphase spreads were obtained by direct culture as well as long term culture method. The modal chromosome number in metaphases of peripheral blood lymphocytes in all the 6 cases of dogs suffering with CTVT was found to be 76 acrocentric autosomes and either a pair of large metacentric X chromosomes in cases of female and a large metacentric X chromosome and a small metacentric Y chromosome in males (2n = 78).

There was no variation in the number of chromosomes in lymphocytes in CTVT cases as compared to lymphocytes of normal dogs. Similarly, there was no change in the number of chromosomes between males and females in the lymphocytes of CTVT and normal dogs.

However, the karyotype of granuloma tissue from both male and female dogs revealed hypoploidy i.e. 58.8 ± 0.28 (range 58-60). The gross morphology of the chromosomes was different than the normal morphology of canine cells, out of these chromosomes (44.00 ± 1.13, range 39-48) were acrocentric 8.17± 0.83 (range 4-10) metacentric and 6.67± 0.61 (range 6-9) submetacentric. Thus, the karyotype picture of the tissue was different than the normal canine karyotype.

All the tumours studied showed high aneuploidy. The genome diversity at the chromosomal level was observed. There was reduction in the number of chromosomes from 78 in normal to 58-60 in CTVT tumour samples.

Thus, considering the findings of the investigation and the research work done by the other scientists that the karyotype of CTVT tissue is different than the normal dog tissue almost similar karyotype observed in all the tumour cells both in male and female dogs; the tumour cells can be grown in-vitro and cell lines can be maintained for several passages, the tumour can be transplanted naturally or experimentally from male to female dog and female to male dog in immunosuppressed dogs; the transplanted tumour has similar histological and cytogenetic appearance; the tumour can regress spontaneously; the tumour shows immune response and no pathogens could be identified till date as the causative agent for this malicious malady, it was concluded that the CTVT tissue is different than the host tissue and CTVT itself is responsible for its spread and causative agent. The findings of present investigation supports the views of other scientists (Parker et al, 2004 and Murgia et al, 2006) who
undertook microsatellite genotyping by using PCR amplification in canines and other specific cytogenetic and molecular genetic studies concluded that tumour and the host cells were genetically separate.

Thus, from the present study, it could be stated that the CTVT tissue is a parasitic tissue and it itself is responsible for its transplantation from one susceptible host/donor dog to the recipient dog.

Tumour Immunity

The Lymphocyte Migration Inhibition Test was performed against the soluble cell membrane antigens for assessing the cell mediated immune response. Alloantigens were prepared and used against the separated leucocytes of the CTVT infected dogs. It was observed that there was 100% migration inhibition in all the dogs suffering from the CTVT.

Radiography

Thoraco-abdominal radiographs taken in cases of infiltrative type of growth (7 cases) or in cases where external metastases were visible (3 cases) did not reveal any metastatic lesions.

Submucosal resection

The surgery in groups II, IV and VI revealed that the submucosal resection was convenient in males where the growth is restricted to the shaft or prepucial area whereas in females episiotomy was required for better exposure of growth for its removal.

Effect of various therapeutic regimen on the clinical, haematological and biochemical parameters

Effect on body weight

The body weight was found to be decreased in all the groups, but the differences were statistically non-significant. Chemotherapy with Methotrexate and Doxorubicin hydrochloride resulted in more weight loss as compared to chemotherapy with Vincristine sulphate.
Effect of various therapeutic regimens on the haematological parameters

Effect on total leucocyte Count

Animals of group I and II revealed non-significant, decreasing, undulating trend.

Animals of group III and IV revealed a regular linear decreasing trend up to day 35. The observations were found statistically significant although the values remained within normal physiological limits. The animals of group V and VI had significant linear decreasing trend throughout the observation period but within normal physiological limits.

Effect on Differential Leucocytic Count

The animals of all groups revealed significant decreasing trend in neutrophil count and revealed neutropenia.

Animals of groups I, II, III, IV and V revealed significant increasing trend that was significant from day 21st onwards whereas the animals of group VI revealed significant increasing trend in lymphocyte count from day 14 onwards and all groups showed significant lymphocytosis.

Significant eosinopenia with decreasing trend in values of eosinophil count was observed in animals of all the groups.

The monocyte count in all the groups was observed to be significantly increased but the values remained within normal physiological limits in all the groups.

The animals of all groups showed irregular trend in basophil count that was statistically non-significant throughout the observation period.

Haemoglobin

The animals of group I and II revealed decreasing trend throughout the course of observations. However, values remained within normal physiological range and the differences were non-significant.

The Methotrexate chemotherapy adversely affected the haemoglobin percentage in animals of group III and IV and revealed a regular decreasing trend that was statistically significant. The observations indicated anaemia due to the effect of the chemotherapeutic drug.
The animals of group V and VI revealed decreasing trend but the values remained within normal physiological range.

**Packed cell volume**

The animals of group I and II adopted decreasing trend, however the values remained within normal physiological range.

The animals of groups III, IV, V, and VI, revealed regular decreasing trend throughout the course of observation. The decreasing trend of the PCV was found statistically significant in all these groups. Thus, Methotrexate and Doxorubicin hydrochloride adversely affected the haemoglobin percent and indicated anaemia.

**Total Erythrocyte Count**

The total erythrocyte count was adversely affected by the chemotherapy and animals of all the groups indicated decreasing trend that was statistically significant although the erythrocyte count remained within normal physiological limits.

**Platelet Count**

The chemotherapy with Vincristine sulphate in animals of groups II and I surprisingly revealed significant thrombocytosis although the values remained within normal physiological limits.

The Methotrexate and Doxorubicin hydrochloride adversely affected the platelet count and the animals of groups III, IV, V and VI decreasing trend uptio day 35\textsuperscript{th} and indicated thrombocytopaenia, however, the values remained within normal physiological limits.

**Effect of various therapeutic regimens on the biochemical parameters**

**Blood Glucose**

The Vincristine sulphate chemotherapy did not affect the blood glucose levels and animals of group I and II showed undulating trend that was statistically non-significant. However, the Methotrexate and Doxorubicin hydrochloride chemotherapy affected the blood glucose levels, the animals of group III, IV, V and VI showed significantly increased values but within normal physiological limits.
Blood Urea Nitrogen

The animals of groups I and II treated with Vincristine sulphate did not show any significant changes in BUN levels. However, the animals of group III, IV, V and VI wherein Methotrexate and Doxorubicin hydrochloride were administered showed increasing trend throughout the observation period and revealed significant increase in BUN levels, although the values remained within normal physiological limits.

Serum Creatinine

The Vincristine sulphate therapy did not alter the serum creatinine levels significantly in groups I and II. However, Methotrexate and Doxorubicin hydrochloride chemotherapy revealed significant increase in the levels of serum creatinine in groups III, IV, V and VI.

Serum Calcium

The Vincristine sulphate therapy did not alter the serum calcium levels significantly in groups I and II. However, Methotrexate and Doxorubicin hydrochloride chemotherapy adversely affected serum calcium levels and revealed significant increase in the levels of serum calcium in groups III, IV, V and VI.

Serum Total Proteins

The animals of all groups showed irregular trend that was statistically non-significant. Thus Vincristine sulphate, Methotrexate and Doxorubicin hydrochloride therapy did not affect the serum total proteins levels.

Aspartate Amino Transferase

The chemotherapy adversely affected the AST levels in animals of all groups and revealed increasing trend throughout the observation period that was statistically significant although the inclined values of AST remained within normal physiological range in all the groups.

Alanine Amino Transferase

The animals of group I, II and V did not show significant changes in the levels of serum ALT values.
However, the animals of group III, IV and V revealed increasing
trend that was statistically significant although the values remained within normal
physiological limits.

Histopathological studies
In the present investigation, the tumour regression as a result of
chemotherapy was seen from the histopathological studies of biopsy samples
taken at scheduled interval before, during and after the completion of the
chemotherapy.

Size of growth and its regression following chemotherapy
The regression of the tumour growth as a result of chemotherapy
was studied in females only. The size of the tumified vulva was measured (n=5
females in each group) on day 0 i.e. before the initiation of chemotherapy. The
volume of this day was considered as 100% growth and the regression was
noted at scheduled intervals in groups I, III, and V. The regression study in three
groups treated with chemotherapeutic drugs Vincristine sulphate, Methotrexate
and Doxorubicin respectively, it is stated that the drug Vincristine sulphate
causd the maximum regression (96.98%) followed by Methotrexate (93 %) and
least regression of granulomatous growth was observed with Doxorubicin
chemotherapy (83.92%) at the end of observation period. Thus the Vincristine
sulphate appeared to be more effective in regression of the venereal granuloma
as compared to Methotrexate and Doxorubicin.

Side effects of chemotherapy:
The common side effects observed as a result of chemotherapy
included fatigue, inappetence and vomiting, alopecia, anemia and infection. The
drug Vincristine sulphate (groups I and II) had exhibited minimum side effects
whereas the drugs Methotrexate (groups III and IV) and Doxorubicin
hydrochloride (groups V and VI) showed severe toxic side effects.

Recurrence
The canines of all the groups were observed for a period of six
months for recurrence of the growth, if any. In none of the cases recurrence was
noted.
Extragenital form of canine transmissible venereal tumour

Extragenital TVT was observed in 2 females and a male. The granulomatous growth in one female was seen on the ventro-lateral abdominal region just above the posterior abdominal mammary gland and several subcutaneous nodules all over the body, whereas in another female, the growth was observed on the lateral abdomen. The diagnosis was confirmed by histological examination of the extragenital growth, which revealed TVT. Surgical excision of the extragenital growth and adjunct chemotherapy resulted in complete cure.

Comparative analysis of effects of antineoplastic agents

Vincristine Sulphate

A vinca alkaloid was found more effective in treatment of CTVT when administered in 5 cycles at scheduled interval, revealed regression of granulomatosus growth up to 96.98 percent in both sexes, however, Vincristine therapy alone or submucosal resection followed by Vincristine therapy revealed no recurrence or metastasis. The results were satisfactory. The toxic effects of Vincristine were documented as loss of appetite, loss of hair coat, and occasional vomiting.

Methotrexate

An antimetabolite administered in 12 CTVT affected dogs revealed significant reduction in body weight, regression of CTVT and also affected haemogram significantly so also the biochemical parameters. In the present study, toxic effects of Methotrexate were vomiting, loss of appetite, diarrhoea, behavioral change in animal, loss of hair coat and liver toxicity.

Doxorubicin hydrochloride

A powerful myelosuppressive antitumour, antibiotic, chemotherapeutic agent, revealed less regression of tumour, more loss of body weight and significantly affected haematological and biochemical parameters in the present study. The toxic symptoms exhibited by the animals were diarrhoea, frequent vomiting, loss of hair coat, and anorexia.

Therefore, considering all the aspects of different chemotherapeutic regimen, it was concluded that Vincristine sulphate therapy in
venereal granuloma could be used/advocated successfully without any adverse effects on different haematological and biochemical parameters. The comparative analysis of effect of three different antineoplastic drugs revealed that Vincristine sulphate had minimum side effects, minimum effect on haematological and biochemical parameters, and maximum regression without recurrence or metastasis which had proved its superiority and is safe to administer, economic and easily available.

The submucosal resection reduce the number of malignant cells, thus the killing effect of chemotherapy has a better chance of 100% kill effect. It is therefore recommended to supplement antineoplastic therapy soon after the surgical intervention with Vincristine sulphate.

CONCLUSIONS

1) The CTVT is an acquired condition wherein; the affection was more in females (75%) as compared to males (25%)
2) The incidence of CTVT was more in non-descript dogs. The incidence of CTVT was more in young to adult middle-aged dogs.
3) There was no variation in the number of chromosomes in lymphocytes of CTVT affected dogs as compared to lymphocytes of normal dogs.
4) The karyotype of granulomatous tissue from both male and female dogs revealed hypoploidy i.e. 58.8 ± 0.28 (range 58-60) with 44.00 ± 1.13 (range 39-48) acrocentric; 8.17± 0.83 (range 4-10) metacentric and 6.67± 0.61 (range 6-9) submetacentric chromosomes and chromosomal aberrations were noted. Thus, the CTVT tissue is cytogenetically different than the host tissue and CTVT itself is responsible for its spread and acted as a causative agent.
5) The CTVT showed immunogenic properties.
6) The antineoplastic therapy has caused non-significant reduction in the body weight in all the groups; non-significant leucopenia in group I and II and significant leucopenia in group III, IV, V and group VI, significant neutropenia, significant lymphocytosis, significant eosinopenia; significant monocytosis and significantly reduced haemoglobin percentage in group III, IV, V and VI and erythrocytopenia in all groups.
7) Vincristine sulphate therapy revealed thrombocytosis in group I and II and Methotrexate and Doxorubicin hydrochloride therapy caused significant thrombocytopenia.

8) In group I and II there was non-significant hypoglycemia while in group III, IV, V and VI, significant hyperglycemia. There was significantly increased BUN and serum creatinine and serum calcium in group III, IV, V and VI; non-significant changes in serum total protein in all the groups; significant increase in AST in all the groups. However, ALT was increased significantly in group III and IV.

9) Comparatively effective regression was observed as much as 96.98 percent in group treated with Vincristine sulphate.

10) The tumour regression as a result of chemotherapy was seen from the histopathological studies of biopsy samples taken at scheduled interval before, during and after the completion of the chemotherapy.

11) Vincristine sulphate was found more effective, less toxic with less adverse effects on clinical, haematological and biochemical parameters as compared to Methotrexate and Doxorubicin therapy. Doxorubicin therapy was superior to Methotrexate therapy considering clinical, haematological, biochemical and regression parameters.

12) Submucosal resection followed by chemotherapy with Vincristine sulphate is the most feasible, convenient and effective in treatment for CTVT.