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OMPARATIVE EFFICACY OF VINCRISTINE, DOXORUBICINE AND METHOTREXATE USING CYTOLOGICAL EXAMINATION OF CANINE TRANSMISSIBLE VENEREAL TUMOUR BEFORE AND AFTER CHEMOTHERAPY

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ABSTRACT

The present study was conducted on 18 confirmed CTVT cases in dogs which were randomly placed into following 3 groups comprising of 6 animals each to administer therapeutic protocols viz. Inj. Vincristine sulphate @ 0.025 mg/kg B.wt. intravenously with normal saline at weekly intervals (Group I) , inj. Doxorubicin @ 1 mg/kg B.wt. intravenously with normal saline at weekly intervals (Group II) and inj. Methotrexate @ 0.3 mg/kg B. wt. intravenously with normal saline at weekly intervals (Group III). Therapeutic efficacy of canine transmissible venereal tumour was adjudged by extent of regression of tumour mass. Maximum reduction (99.23%) was observed in vincristine sulphate treatment group at all the treatment time intervals. The reduction in the size of tumour mass was also effectively recorded at different time intervals in doxorubicin (65.92%) treatment group. The least regression (42.26%) of tumour mass was observed in methotrexate treatment group.

Key word: Canine, Doxorubicin , Extent , Methotrexate, Regression.

INTRODUCTION

Canine Transmissible Venereal Tumour (CTVT) is a benign reticulo-endothelial (histiocytic) tumour which are single to multiple, pink-red, cauliflower like lesion, firm to friable nodular mass having a rich blood supply on the external genitalia of either male or female dogs which occur at the same frequencies in both sexes (Dhurvey, 2012). It is also known as canine transmissible venereal sarcoma (CTVS), sticker's sarcoma and infectious sarcoma of the both male as well as female dogs that mainly affects the external genitalia (Dar *et al*., 2017). Hemorrhage is associated with tumor fragility. Other associated clinical signs may include genital discharge, abnormal odor, and excessive licking.

Major advantages of chemotherapy are the high cure rate, ease of administration and potential usefulness in metastatic, multi-focal disease. The disadvantages of treatment are their side effects i.e. loss of appetite, vomiting, diarrhea, neutropenia and alopecia in some animals.

Vincristine sulphate is an alkaloid salt obtained from the common periwinkle (*Vincarosa livia*) also known as leurocristine , Doxorubicin is an anthracycline glycoside antibiotic originally produced by *Streptomyces peucetius var. caesius* (Morrison, 1998) and Methotrexate is an antimetabolite and antineoplastic drug which is a folic acids analogue and has been used to treat variety of malignancies in dogs.

Doxorubicin is an anthracycline glycoside antibiotic originally produced by *Streptomyces peucetius var. caesius* (Morrison, 1998). It exerts cytotoxic effect as a DNA-intercalating agent to inhibit further DNA and RNA biosynthesis. Doxorubicin is widely used either as single agent or in combination with other chemotherapeutic regimens for various types of solid tumours. However, dose limiting toxic side effects such as cardio toxicity, myelosuppression, myositis and alopecia limit its clinical applications, owing to non-specific distribution to healthy normal tissue. Doxorubicin is widely used either as single agent or in combination with other chemotherapeutic regimens for various types of solid tumours (Annasaheb, 2016).

Methotrexate is an antimetabolite and antineoplastic drug which is a folic acids analogue and has been used to treat variety of malignancies in dogs. Cancer cells need to make and repair DNA, so that they can grow and multiply. Methotrexate stops cells making and repairing DNA (Vinaykrao, 2007).

MATERIALS AND METHODS

The present study was carried out on the clinical cases reported to Teaching Veterinary Clinical Complex (TVCC) and referred to the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, MHOW and in nearby clinics suspected for canine transmissible venereal tumour irrespective of their age, breed and sex.

A total of 18 confirmed CTVT cases in dogs were randomly placed into following 3 groups comprising of 6 animals each to administer following protocols.

Group I: In this group 6 animals received chemotherapy by administration of injection Vincristine sulphate @ 0.025 mg/kg B.wt. intravenously with normal saline at weekly intervals.

Group II: In this group 6 animals undergone chemotherapy by administration of injection Doxorubicin @ 1 mg/kg B.wt. intravenously with normal saline at weekly intervals.

Group III: In this group also 6 animals undergone chemotherapy by administration of injection Methotrexate @ 0.3 mg/kg B.wt. intravenously with normal saline at weekly intervals.

Days of observations:

Animals belonging to all the groups which received chemotherapy were monitored by cytological, hematological and bio-chemical observations on 0^{th}, 7^{th} and 14^{th} days.

STATISTICAL ANALYSIS

Data collected from the study was analyzed, using standard statistical method by application of Completely Randomized Design (CRD) as described by Snedecor and Cochran (1994).

RESULT AND DISSCUSSION

All the clinically suspected cases for canine transmissible venereal tumour having a history of bleeding or breeding problems was initially included in this study and after thorough clinical examination such as presence of cauliflower like growth and cytological confirmation 18 cases of

CTVT were randomly allocated in different therapeutic groups. The observations were made, analyzed and interpreted as follows.

CYTOLOGICAL EXAMINATION

In the present study, cytological examination of the vaginal swab and impression smears of tumorous growth was carried out in all the cases and only after confirmations of CTVT, the cases were included in the study.

Cytological smears were made over a clean grease free microslide and stained with Papaincolaou stain before commencement of therapy and after last administration of the drug Microscopic Cytological evaluation (Papaincolaou stain, 100X) showed high cellularity and presence of numerous round cells. In the present study, cytological examination of vaginal swab and impression smear in all the groups revealed numerous neutrophils and round neoplastic cells with vacuolated cytoplasm. A minimum of 200 exfoliated cells were count and classified for different cell types. The mean observations are given in below tables.

Neutrophil count

The mean neutrophil count (Table 01) in vincristine sulphate treated (group-I) at day 0 was 55.16 ± 4.21 which decreased to 29.50 ± 4.01 on day 7^{th} and 3.50 ± 1.17 on day 14^{th} of treatment respectively. Statistical variation in neutrophil count between periods in this group were non significant.

In doxorubicin treated animals (group-II) the mean neutrophil count were 50.33 ± 3.69 on 0 day, 35.50 ± 2.74 on day 7^{th} and 21.33 ± 1.60 on day $14^{th\ day}$.

In methotrexate treated animals (group-III) the mean neutrophil count were 65.66 ± 2.74 on day 0 which decreased to 47.00 ± 2.64 on day 7^{th} and 29.16 ± 2.19 on day 14^{th} of treatment (Table 10 and fig.10) respectively.

Variation in these periodic values of neutrophil irrespective of any treatment group were statistically non significant.

The mean neutrophil count on 0 day was higher in group III (65.66 ± 2.74) in comparison to group I (55.16 ± 4.21) and group II (50.33 ± 3.69) respectively.

The mean neutrophil count on 7^{th} day was higher in group III (47.00 ± 2.64) in comparison to group II (35.50 ± 2.74) and group I (29.50 ± 4.01) respectively.

The mean neutrophil count on 14^{th} day was higher in group III (29.16 ± 2.19) in comparison to group II (21.33 ± 1.60) and group I (3.50 ± 1.17) respectively.

Variations in these periodic values of neutrophil irrespective of any treatment between group were statistically non significant.

In the present study, no significant difference was found. Ayala Diaz (2018) observed moderate amount of neutrophil.

TABLE 01 : Mean (± SE) Neutrophil count at different time intervals in different groups (Papaincolaou stain)

Group Name	0th day	7th day	14th day
Group I(Vincristine sulphate)	55.16 ± 4.21	29.50 ± 4.01	3.50 ± 1.17
Group II (Doxorubicine)	50.33 ± 3.69	35.50 ± 2.74	21.33 ± 1.60
Group III (Methotrexate)	65.66 ± 2.74	47.00 ± 2.64	29.16 ± 2.19

ROUND CELLS

The mean round cells count (Table 02) in vincristine sulphate treated (group-I) at day 0 was 105.00 ± 3.83 which decreased to 44.66 ± 1.71 on day 7th and 0.66 ± 0.42 on day 14th of treatment respectively. Statistical variation between periods in the count was highly significant (P< 0.01).

Similar trend was noticed in (Table 02) group-II animals treated by doxorubicin where the mean round cells count were 119.33 ± 3.74 on 0 day, 88 ± 2.01 on day 7th and 52.16 ± 2.79 on day 14th of treatment. There was statistically highly significant (P< 0.01) decrease in the count between periods.

In animals treated with methotrexate the mean round cells count was 104.66 ± 1.72 on day 0 which decreased to 92.16 ± 3.59 on day 7th and 64.66 ± 2.12 on day 14th of treatment (Table 02) respectively. There was statistically highly significant (P< 0.01) decrease in the count between periods.

The mean round cells count on 0 day was significant (P< 0.05) difference in the number of round cells in doxorubicin (119.33 ± 3.74) in comparison to vincristine sulphate (105.00 ± 3.83) and methotrexate (104.66 ± 1.72) respectively.

The mean round cells count on 7th day was higher in methotrexate (92.16 ± 3.59) in comparison to doxorubicin (88.00 ± 2.01) and vincristine sulphate (44.66 ± 1.71) respectively. There was significant (P< 0.05) difference in the number of round cells in doxorubicin and methotrexate compared to the number of round cells in vincristine sulphate.

The mean round cells count on 14th day was higher in methotrexate (64.66 ± 2.12) in comparison to doxorubicin (52.16 ± 2.79) and vincristine sulphate (0.66 ± 0.42) respectively. There was highly significant (P< 0.01) difference in the number of round cells in methotrexate and doxorubicin compared to number of round cells in vincristine sulphate. Dar *et al*. (2017) observed large round cells with round nucleus.

TABLE 02: Mean (± SE) Round cells count at different time intervals in different groups (Papaincolaou stain)

Group Name	0th day	7th day	14th day
Group I (Vincristine sulphate)	$105.00^{B} \pm 3.83^{a}$	$44.66^{B} \pm 1.71^{b}$	$0.66^{C} \pm 0.42^{c}$
Group II (Doxorubicine)	$119.33^{A} \pm 3.74^{a}$	$88.00^{A} \pm 2.01^{b}$	$52.16^{B} \pm 2.79^{c}$
Group III (Methotrexate)	$104.66^{B} \pm 1.72^{a}$	$92.16^{A} \pm 3.59^{b}$	$64.66^{A} \pm 2.12^{c}$

Superscript bearing different capital letter alphabets differ significantly ($P< 0.05$) between groups.
Superscript bearing different small letter alphabets differ significantly ($P< 0.05$) within group.

VACUOLATED ROUND CELLS

The mean vacuolated round cells count (Table 03) in vincristine sulphate treated (group-I) at day 0 was 40.00 ± 3.09 which decreased to 22.00 ± 2.72 on day 7th and 0.50 ± 0.34 on day 14th of treatment respectively. Statistical variation between periods in the count was highly significant ($P< 0.01$).

Similar trend was noticed in (Table 03) group-II animals treated by doxorubicin where the mean vacuolated round cells count were 28.66 ± 2.17 on 0 day, 19.50 ± 2.02 on day 7th and 8.66 ± 0.76 on day 14th of treatment. There was statistically highly significant ($P< 0.01$) decrease in the count between periods.

In animals treated with methotrexate the mean vacuolated round cells count was 31.16 ± 2.58 on day 0 which decreased to 20.16 ± 2.37 on day 7th and 14.50 ± 0.76 on day 14th of treatment (Table 03) respectively. There was statistically significant ($P< 0.05$) decrease in the count between periods.

The mean vacuolated round cells count on 0 day was significant ($P< 0.05$) in vincristine sulphate (40.00 ± 3.09) in comparison to methotrexate (31.16 ± 2.58) and doxorubicin (28.66 ± 2.17) respectively. There was difference in the number of vacuolated round cells in between groups.

The mean vacuolated round cells count on 7th day was higher in vincristine sulphate (22.00 ± 2.72) in comparison to methotrexate (20.16 ± 2.37) and doxorubicin (19.50 ± 2.02) respectively. There was no significant difference in the number of vacuolated round cells between groups.

The mean vacuolated round cells count on 14th day was highly significant ($P< 0.01$) difference higher in methotrexate (14.50 ± 0.76) in comparison to doxorubicin (8.66 ± 0.76) and vincristine sulphate (0.50 ± 0.34) respectively. There was difference in the number of vacuoles in round cells in between groups.

In the present study, significant difference in the counts of round neoplastic cells with vacuolated cytoplasm was found between different groups. Ayala Diaz (2018) and Vural *et al.* (2018) were observed similar round neoplastic cells with vacuolated eosinophilic cytoplasm, as well as a round hyperchromatic nucleus with one or two evident nucleoli and a large cytoplasm nucleus relation.

TABLE 03: Mean (± SE) Vacuolated round cells count at different time intervals in different groups (Papaincolaou stain)

Group Name	0th day	7th day	14th day
Group I (Vincristine sulphate)	$40.00^{A} \pm 3.09^{a}$	22.00 ± 2.72^{b}	$0.50^{C} \pm 0.34^{c}$
Group II (Doxorubicine)	$28.66^{B} \pm 2.17^{a}$	19.50 ± 2.02^{b}	$8.66^{B} \pm 0.76^{c}$
Group III (Methotrexate)	$31.16^{B} \pm 2.58^{a}$	20.16 ± 2.37^{b}	$14.50^{A} \pm 0.76^{b}$

Superscript bearing different capital letter alphabets differ significantly (P< 0.05) between groups.
Superscript bearing different small letter alphabets differ significantly (P< 0.05) within group.

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