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Haemato-Biochemical Evaluation of Chemotherapy in Canine Transmissible Venereal Tumour

Aja Taj Nazer[1], Bahuleyan Bibin Becha[1]*, Choppillil Jayakumar[1], Melitta Pappu Unnikrishnan[1] and Suma-Thankachy Sreekantan-Thampi Devi[2]

[1]Department of Animal Reproduction, Gynaecology and Obstetrics
[2]Department of Veterinary Pathology
College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, Pookode, Kerala

ABSTRACT

The study was aimed at haematological and biochemical evaluation of chemotherapeutic response in canine transmissible venereal tumour (CTVT). Thirty one CTVT confirmed dogs presented to University Veterinary Hospitals were categorised into three groups. Group I (n=13) were treated with vincristine sulphate, while group II (n=9) and III (n=9) dogs were treated with vincristine along with ivermectin and cyclophosphamide respectively. Haemato-biochemical evaluations were performed on the day of presentation and at weekly intervals till complete tumour regression. The haemato-biochemical parameters like TEC, Hb, VPRC, TP, BUN, creatinine, albumin, globulin and ALT were within normal range. Significant differences ($p<0.05$) were observed in DLC, TC, MCV, AST, ALP and bilirubin ZI and group II, and thrombocytopenia in group III. Highly elevated AST, ALP and bilirubin were observed in all three groups. Combination therapy with ivermectin was more efficient with fewer adverse effects and decreased treatment duration.

Key words: Chemotherapy, Haematology, Biochemistry, Transmissible venereal tumour.

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INTRODUCTION

Canine transmissible venereal tumour (CTVT) is a contagious tumour in nature and commonly seen in sexually active young dogs. Transmission of tumour occurs by the transfer of viable tumour cells through exposed skin or mucosa (Strakova and Murchison, 2014). The CTVT has a world-wide distribution. Occurrence of CTVT among total clinical cases and total reproductive cases was found to be 0.15 and 0.88 per cent respectively (Nazer *et al.,* 2023). Diagnosis is based on clinical history, signalment, cytology, histology, cytogenetic analysis

*Corresponding author.
E-mail address: bibinbecha@gmail.com (Bahuleyan Bibin Becha)
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and molecular techniques (Jain *et al.,* 2002). Cytology is the method of choice for definitive diagnosis of CTVT. Treatment includes chemotherapy, surgery, radiotherapy, immunotherapy and combination therapy. Even though vincristine sulphate is effectively used as first line chemotherapeutic agent, various therapeutic modifications could reduce its side effects. In this study haematological and biochemical analysis was used to assess the response in three different chemotherapeutic regimens to CTVT.

MATERIALS AND METHODS

Thirty-one dogs presented to the University Veterinary Hospitals, Thrissur with symptoms suggestive of CTVT and confirmed by cytology, were randomly allocated into three chemotherapeutic regimens. The group I (n=13) dogs were administered with vincristine sulphate at a dose of 0.025 mg/Kg body weight intravenously at weekly intervals until tumour regression. The group II (n=9) dogs were administered with ivermectin at a dose of 200µg/Kg subcutaneously, followed by vincristine sulphate at a dose of 0.025 mg/Kg body weight intravenously after 24 h and repeated weekly until tumour regression. The group III (n=9) dogs were administered with oral cyclophosphamide (5mg/Kg body weight) for 10 days, along with vincristine at a dose of 0.0125mg/Kg body weight intravenously at weekly intervals till complete regression of the tumour.

Whole blood and serum samples of all the dogs were collected on the day of presentation and at weekly intervals till complete regression of the tumour. Blood samples were processed within 3-4 hours of collection and haematological parameters like total erythrocyte count (TEC), volume of packed red cells (VPRC), haemoglobin concentration (Hb), total leucocyte count (TLC), mean corpuscular volume (MCV), differential leucocyte count (DLC) and thrombocyte count (TC) were assessed using auto analyser (Mythic 18 Vet, Woodley, Switzerland). Serum samples were separated, centrifuged, aliquoted and used immediately for the analysis of albumin, globulin, serum total protein (TP), blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin using commercially available kits in a semiautomatic biochemical analyser (Erba Manheim, Chem-5 Plus V2, USA). The duration of treatment was assessed from the day of initiation of treatment till the complete regression of tumour.

The data obtained were tabulated and analysed statistically (Snedecor and Cochran, 1994) using SPSS version 24.0 by repeated measures ANOVA.

RESULTS AND DISCUSSION

On haematological examination, TEC, Hb and VPRC on different days of treatment in three different groups were within the normal range (Table 1) without significant difference ($p<0.05$) within and between different treatment groups. Similar findings in TEC values were reported by Albanese *et al.,* (2006) and Behera *et al.,* (2012). A significant decrease in Hb (11.59 ± 0.201 g/dL) was reported by Priyadarshini *et al.,* (2021). The normal TEC, Hb, and VPRC results showed that there was no obvious bone marrow suppression caused by the dose and frequency of chemotherapeutic drug administration in the presence of tumoral bleeding.

The mean MCV in all the groups were lower than normal range on day of presentation which became normal by day 28 (Table 1). A significant decrease ($p<0.05$) was observed in the MCV value in group I by day 14 which became normal by day 28. The lower MCV values in the present study could reflect a bone marrow response to compensate the short-term bleeding disorders.

On the day of presentation, the mean TLC in groups I and III were higher than usual, which could be due to concurrent tumour infection (Birhan and Chanie, 2015). A significant reduction in TLC was observed by day 7, but within normal limits in all three groups, which might be due to chemotherapeutic drug effects (Table 1).

The total neutrophil count in all three treatment groups was within normal range (Table 1). A significant decrease was observed in the total neutrophil count by day 14 in treatment Group I and day 7 in Group II when compared to day of presentation. No significant difference was observed in the neutrophil counts in group III during the treatment period. Lymphocytosis was observed in all the groups during the course of treatment, while in Group III, normal values were observed by day 21 (Table 1). A significant increase in lymphocyte count was observed in groups I by day 14 and group II by day 7. Monocytosis was observed in treatment Groups I and II, on day 14 and 21, respectively. In treatment group III, the monocyte count was within the normal limits during the treatment. Neutropenia reported in the current study was a sign of myelosuppression brought on by the cytotoxic effects of chemotherapy drugs on the bone marrow. Monocytosis and lymphocytosis could be a manifestation of an ongoing neutropenia. But, Kumar *et al.,* (2017) reported significant neutrophilia, significant lymphopenia and no variation in the monocyte count in CTVT affected dogs.

The mean TC was within normal range in groups I and II, while significant thrombocytopenia was

observed in group III on day 14 of chemotherapy (Table 1). Priyadarshini *et al.,* (2021) reported thrombocytopenia (25.76 ± 1.248) after vincristine chemotherapy. Cyclophosphamide metabolites promote increased platelet destruction or decreased platelet formation via megakaryocyte apoptosis.

On serum biochemical analysis, the TP, albumin, globulin, BUN, creatinine and ALT levels were within normal ranges until therapy was completed in all treatment groups (Table 2). Similar findings were reported by Albanese *et al.,* (2006). No significant difference in these variables between and within the treatment groups ($p>0.05$) indicated that the hepatic and renal parenchyma was not significantly damaged (Yadav *et al.,* 2018). The normal ALT levels (Braz and Marinho, 2021) indicated no significant injury to the hepatic parenchyma caused by cytotoxic drug metabolites.

The mean ALP, AST and bilirubin levels in all treatment groups were observed to be higher on all days of observations (Table 2). The levels of AST were significantly lower on day 14 compared to the day of presentation in group II dogs. There was significant increase in serum ALP levels by day 14 of treatment in group III ($p<0.05$). Similar observations of AST and ALP were made by Behera *et al.,* (2012) and high level of serum bilirubin was reported by Alkan *et al.,* (2017) in CTVT treated dogs. The increased values of AST, ALP and bilirubin revealed minor hepatocellular damage caused by chemotherapy-induced anorexia and subsequent cholestasis.

Table 1. Haematological parameters (Mean ± SE) in CTVT affected dogs on weekly intervals

Parameters		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
	GI	5.76±0.24	5.96±0.26	6.60±0.39	5.78±0.25	5.76±0.17	---
TEC (10^6/mm^3)	GII	5.93±0.28	6.14±0.30	6.15±0.36	6.19±0.28	4.82±0	---
	GIII	5.85±0.28	5.76±0.38	6.19±0.47	6.02±0.19	5.85±0.27	5.83±0.32
	GI	13.13±0.62	13.62±0.93	14.32±0.77	13.46±0.85	14.03±0.74	---
Hb (g/dL)	GII	14.91±1.24	13.91±0.77	14.09±0.77	13.73±0.63	11.70±0	---
	GIII	13.63±0.57	13.21±0.74	14.06±0.98	13.66±0.46	13.46±0.92	13.15±0.55
	GI	36.76±1.60	36.93±1.46	42.08±2.78	37.74±1.93	38.98±1.98	---
VPRC (%)	GII	38.27±1.71	38.92±1.92	39.27±2.01	38.71±1.48	33.40±0	---
	GIII	37.77±1.24	36.52±2.15	39.23±2.52	38.71±1.12	37.50±1.75	37.18±1.77
	GI	64.02^{a}±1.45	62.45^{ab}±1.47	63.66^{b}±1.49	65.20^{ab}±1.12	67.68±3.00	---
MCV (fL)	GII	64.61±1.17	63.48±1.36	64.13±1.82	58.31±3.92	69.30±0	---
	GIII	65.32±2.36	63.90±1.87	62.77±2.31	64.64±2.31	66.32±2.71	66.13±3.66
	GI	15.05^{a}±1.13	9.79^{b}±1.73	8.63^{bc}±0.84	8.14^{bd}±1.05	8.65±1.12	---
TLC (10^3/mm^3)	GII	11.99^{a}±1.52	8.00^{b}±0.96	6.89^{bc}±0.71	8.5^{ab}±1.37	7.7±0	---
	GIII	14.63^{a}±1.02	6.29^{b}±1.94	12.00^{ab}±2.36	9.58^{ab}±2.47	9.24±1.76	13.34±6.19
	GI	$73.51^{a,x}$±2.76	$63.67^{ab,x}$±4.04	$57.66^{b,x}$±3.53	$52.91^{b,x}$±3.42	59.35±6.17	---
Neutrophil (%)	GII	$74.37^{a,x}$±4.57	$53.73^{b,x}$±4.33	$54.86^{b,x}$±5.61	$60.86^{ab,x}$±5.93	58.90±0	---
	GIII	$66.17^{a,x}$±6.42	$59.97^{a,x}$±6.69	$64.46^{a,x}$±6.88	$64.16^{a,x}$±5.89	74.46±1.97	72.53±5.64
	GI	$19.48^{a,x}$±2.29	$27.94^{ab,x}$±3.90	$28.72^{b,x}$±2.79	$35.16^{b,x}$±3.99	31.00±4.63	---
Lymphocyte (%)	GII	$18.20^{a,x}$±3.09	$36.48^{b,x}$±3.61	$35.21^{b,x}$±4.92	$28.81^{ab,x}$±4.38	41.20±0	---
	GIII	$26.50^{a,x}$±5.13	$33.17^{a,x}$±3.61	$30.00^{a,x}$±6.82	$27.57^{a,x}$±6.23	18.88±2.13	16.22±2.46
	GI	$5.94^{a,x}$±0.53	$7.26^{a,x}$±0.62	$13.06^{ab,x}$±4.55	$10.70^{b,x}$±1.79	9.65±1.98	---
Monocyte (%)	GII	$7.43^{a,x}$±1.52	$9.79^{a,y}$±1.05	$9.93^{a,x}$±1.86	$10.33^{a,x}$±1.70	12.90±0	---
	GIII	$7.33^{a,x}$±1.81	$6.86^{a,x}$±0.89	$5.54^{a,x}$±0.89	$6.01^{a,y}$±0.88	6.66±0.46	6.88±0.35
	GI	331.15±45.32	360.38±40.31	$346.40^{a,x}$±50.56	348.63±35.73	380.75±27.08	---
Thrombocyte count (10^3/mm^3)	GII	244.89±34.85	298.89±38.70	$232.29^{a,xy}$±39.76	281.71±42.98	252.00±0	---
	GIII	263.33±23.78	244.44±43.95	$192.71^{a,y}$±45.20	244.71±59.14	200.40±30.51	287.50±57.74

^{abc}Values with different superscript in a row differ significantly ($p<0.05$), ^{xy}Values with different superscripts in a column differ significantly($p<0.05$), Values without any superscript in a column/row did not differ significantly

Major gastrointestinal complications were reported in Group III dogs, like anorexia (88.89%), vomiting (77.78%), diarrhoea (11.11%) and tenesmus (22.22%) followed by Group I dogs with anorexia (23.07%), vomiting (38.46%), diarrhoea (7.69%). In group II, 33.33 per cent dogs exhibited anorexia. Cunha *et al.,* (2017) reported vomiting (21%), inappetence (20%) and diarrhoea (20%) with cyclophosphamide and vincristine combination therapy. Other side effects observed were hair loss, dysuria and loss in body weight. Least gastrointestinal complications were exhibited by group II dogs, which highlight the advantage of combination therapy using ivermectin and vincristine. Metronomic therapy was practised in dogs treated with vincristine-cyclophosphamide combination, to reduce the side effects.

The treatment duration was recorded as 23.10 ± 1.49 days in group I, 22.00 ± 1.00 days in Group II and 30.00 ± 2.00 days in group III. A significant increase in the number of days taken for complete regression of tumour in vincristine-cyclophosphamide combination therapy compared to the other two treatment groups. No significant difference was observed between treatment duration in vincristine therapy and vincristine-ivermectin combination therapy. Treatment of CTVT using a combination of ivermectin and vincristine showed superior results when compared to other treatment protocols as the duration of treatment and side effects were least in these dogs.

CONCLUSIONS

The results of the study revealed that treatment of CTVT affected dogs using a combination of vincristine and

Table 2. Serum biochemical parameters (Mean ± SE) in CTVT affected dogs on weekly intervals

Parameters		Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
TP (g/dL)	GI	6.92±0.28	6.94±0.19	6.97±0.19	6.63±0.21	6.65±0.43	---
	GII	6.10±0.29	6.74±0.11	6.20±0.20	6.66±0.30	6.26±0	---
	GIII	6.82±0.24	6.80±0.25	6.62±0.22	6.77±0.23	6.84±0.22	5.99±0.54
Albumin (g/dL)	GI	2.56±0.09	2.80±0.09	2.74±0.12	2.86±0.12	2.92±0.20	---
	GII	2.59±0.11	2.66±0.10	2.70±0.11	2.81±0.14	2.95±0	---
	GIII	2.88±0.09	2.88±0.11	3.00±0.18	3.05±0.20	3.17±0.26	3.05±0.13
Globulin (g/dL)	GI	4.36±0.22	4.14±0.20	4.16±0.20	3.77±0.14	3.73±0.56	---
	GII	3.51±0.21	4.08±0.16	3.50±0.26	3.85±0.30	3.31±0	---
	GIII	3.93±0.26	3.92±0.32	3.62±0.34	3.71±0.08	3.66±0.36	2.94±0.66
BUN (mg/dL)	GI	13.70±1.20	12.76±0.95	11.71±1.51	11.13±1.52	10.95±1.47	---
	GII	10.96±1.23	13.77±1.89	12.13±2.51	13.31±2.50	12.44±0	---
	GIII	13.36±1.59	10.60±1.34	12.83±2.02	12.59±1.31	12.79±1.19	9.02±1.22
Creatinine (mg/dL)	GI	1.06±0.04	1.00±0.04	0.94±0.06	0.98±0.08	1.04±0.29	---
	GII	1.08±0.08	0.95±0.08	0.93±0.07	0.96±0.05	1.35±0	---
	GIII	1.10±0.07	0.95±0.08	0.98±0.06	1.04±0.08	0.97±0.08	0.97±0.16
ALT (IU/L)	GI	31.05±1.26	33.21±2.81	50.01±10.99	36.32±5.05	36.37±5.39	---
	GII	35.59±5.93	34.88±3.76	32.32±3.20	31.33±2.27	29.75±0	---
	GIII	37.03^{ab}±5.36	30.90^{a}±2.36	42.23^{ab}±5.71	65.60^{b}±16.65	39.80±13.64	40.18±12.75
AST (IU/L)	GI	$30.16^{a,x}$±2.02	$35.60^{a,x}$±3.98	$43.66^{a,x}$±7.49	$34.54^{a,x}$±6.10	24.13±2.37	---
	GII	$42.85^{a,y}$±6.37	$31.62^{ab,x}$±3.45	$25.63^{b,x}$±1.52	$28.38^{ab,x}$±3.48	29.75±0	---
	GIII	$34.50^{a,xy}$±5.39	$30.09^{a,x}$±4.54	$31.41^{a,x}$±4.62	$36.14^{a,x}$±6.93	29.40±3.78	31.25±6.72
ALP (IU/L)	GI	$162.25^{a,x}$±15.45	$201.88^{a,x}$±21.03	$173.59^{a,x}$±26.51	$175.47^{a,x}$±35.00	145.27±25.10	---
	GII	$194.44^{a,x}$±27.03	$213.97^{a,x}$±39.59	$165.39^{a,x}$±35.96	$198.39^{a,xy}$±35.98	153.00±0	---
	GIII	$181.96^{a,x}$±28.10	$236.28^{ab,x}$±23.58	$305.30^{b,y}$±33.01	$372.11^{b,y}$±83.22	260.94±61.71	373.10±71.23
Total bilirubin (mg/dL)	GI	0.53±0.09	0.47±0.08	0.52^{xy}±0.10	0.48±0.10	0.43±0.13	---
	GII	0.66±0.13	0.56±0.09	0.86^{x}±0.09	0.61±0.11	0.49±0.00	---
	GIII	0.45±0.08	0.50±0.08	0.55^{y}±0.08	0.66±0.12	0.54±0.15	0.52±0.21

^{ab}Values with different superscript in a row differ significantly ($p<0.05$), ^{xy}Values with different superscripts in a column differ significantly($p<0.05$), Values without any superscript in a column/row did not differ significantly.

ivermectin was more effective with shorter duration of treatment and fewer side effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest among themselves.

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