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Comparative cytological evaluation of vincristine sulfate and ivermectin-vincristine combination therapy in canine transmissible venereal tumour

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Abstract

Canine transmissible venereal tumour is a contagious neoplasm affecting dogs through physical contact with injured skin or mucous tissue. The present study comparatively evaluated the cytological response to vincristine sulfate alone and ivermectin-vincristine combination therapy in dogs affected with transmissible venereal tumour. Twelve client owned dogs diagnosed cytologically as plasmacytoid type were randomly allotted to two groups of six animals each. Group I received vincristine sulfate intravenously at 0.025 mg/kg body weight weekly, while Group II received ivermectin subcutaneously at 500 µg/kg body weight (biweekly) followed 24 hrs later by vincristine sulfate. Mitotic index and apoptotic index were recorded weekly. Both groups showed progressive reduction in mitotic index and increased apoptotic activity to a greater extent in Group II. The mean treatment duration was significantly shorter in Group II (22.17 ± 1.17 days) compared to Group I (28.00 ± 1.81 days) suggesting that combination chemotherapy enhances cytological and treatment response.

Keywords: Canine transmissible venereal tumour, vincristine sulfate, ivermectin, mitotic index, apoptotic index, cytology

1. Introduction

Canine transmissible venereal tumour (CTVT) is a naturally occurring, contagious round cell neoplasm of dogs (Spugnini *et al.*, 2008) [24], commonly affecting the external genitalia and occasionally extragenital sites (Das and Das, 2000) [7]. The tumour is primarily transmitted during coitus and is characterised clinically by friable, cauliflower-like, haemorrhagic masses that bleed easily on manipulation (Aprea *et al.*, 1994) [4].

Based on the predominant tumour cell population, CTVT is classified into lymphocytoid, plasmacytoid, and mixed subtypes (Amaral *et al.*, 2004 and Lima *et al.*, 2013) [3, 18]. From a cytomorphological perspective, the plasmacytoid subtype is characterised by the presence of 60 per cent or more tumour cells exhibiting an ovoid shape, abundant cytoplasm, multiple clear cytoplasmic vacuoles, and eccentrically placed nuclei (Amaral *et al.*, 2004) [3]. Plasmacytoid TVTs follow a more aggressive biological behaviour and possess a higher malignant potential than the lymphocytoid or mixed forms (Gaspar *et al.*, 2009) [11]. Gaspar *et al.* (2010) [10] further reported that plasmacytoid cells exhibit increased P-glycoprotein expression, which may contribute to the development of multidrug resistance. In addition, these tumours are less responsive to vincristine sulphate chemotherapy when compared to the lymphocytoid and mixed subtypes (Amaral *et al.*, 2004; Greatti *et al.*, 2004 and Fonseca *et al.*, 2012) [3, 13, 9].

Clinical evidence suggests that the combination of ivermectin and vincristine may result in faster remission of CTVT than vincristine used alone (Lapa *et al.*, 2012) [16]. Recent studies have indicated that ivermectin may enhance chemotherapeutic response by modulating P-glycoprotein (P-gp) activity, a membrane associated protein, thereby reducing their intracellular concentration and therapeutic efficacy (Pouliot *et al.*, 1997; Griffin *et al.*, 2005;

Lespine *et al.*, 2006; Jiang *et al.*, 2019)^[21, 14, 17, 15]. Since both vincristine and ivermectin are known substrates of P-gp, ivermectin has been proposed as a potential adjuvant anticancer agent capable of improving the effectiveness of chemotherapy (Jiang *et al.*, 2019 and Markowska *et al.*, 2019)^[15, 19].

Beyond its role in reversing multidrug resistance, ivermectin has also been shown to possess direct antitumour activity (Crump, 2017 and Domínguez-Gómez *et al.*, 2018)^[6, 8]. Supporting this, Bulhosa *et al.* (2020)^[5] reported successful tumour remission in dogs treated with 1 per cent ivermectin administered subcutaneously at 0.5 mg/kg, 24 hours prior to vincristine and repeated biweekly until complete tumour regression was achieved. He also reported that ivermectin could inhibit P-gp activity in tumour cells at the time of vincristine administration thereby allowing greater intracellular accumulation of vincristine and enhancing its antitumour effect. In addition, Tang *et al.* (2021)^[25] demonstrated that ivermectin can induce multiple forms of programmed cell death including apoptosis, autophagy, and pyroptosis, in different tumour cell types.

The present study was therefore undertaken to comparatively evaluate the cytological changes induced by vincristine sulfate alone and ivermectin-vincristine combination therapy in dogs with plasmacytoid type CTVT, with special emphasis on mitotic index, apoptotic index and treatment duration.

2. Materials and Methods

A total of twelve client owned dogs above one year of age, presented with genital tumour masses suggestive of CTVT, were included in the study. Diagnosis was confirmed by cytological examination and only dogs diagnosed with plasmacytoid type CTVT were considered for this study. The 12 dogs were randomly allotted to two treatment groups comprising six animals each. Group I (n = 6) was treated with vincristine sulfate intravenously at a dose of 0.025 mg/kg body weight once weekly until complete tumour regression and Group II (n = 6) was treated with ivermectin subcutaneously at a dose of 500 µg/kg body weight on the day of presentation and repeated biweekly. 24 hours after the first ivermectin administration, vincristine sulfate was administered intravenously at 0.025 mg/kg body weight, followed by weekly administration until complete tumour regression.

Complete tumour regression was confirmed by the absence of tumour cells in consecutive cytological examinations. Impression smears were collected from the tumour mass for cytological evaluation. The smears were air dried, fixed in methanol for two minutes and stained using Giemsa stain (HiMedia Laboratories Pvt. Ltd) for 15 minutes. After air drying, the stained smears were examined under a 40x lens of light microscope for cytological evaluation.

Mitotic index was determined by counting the number of mitotic figures (fig.1) observed per 100 tumour cells at high-power magnification. Apoptotic index was assessed by enumerating the number of apoptotic cells (fig.2) per 100 tumour cells and expressing the values as a percentage of positive cells. Treatment duration was recorded as the number of days required for complete tumour regression in each dog. The data obtained were tabulated and analysed statistically using SPSS version 24.0 and the differences were considered statistically significant at $p < 0.05$.

3. Results

The mean mitotic index declined progressively in both treatment groups during the course of therapy. In Group I, the mean mitotic index decreased from 4.50 ± 0.43 on day 0 to

2.17 ± 0.31 on day 7 and 0.50 ± 0.22 on day 14, reaching 0 by day 21 and remaining absent post-treatment. In Group II, the mitotic index decreased from 5.17 ± 0.40 on day 0 to 1.50 ± 0.34 on day 7 and reached 0 by day 14, which was maintained through day 21 and post-treatment (Table 1). A statistically significant decline ($p < 0.05$) in mitotic index was observed within both groups over time however, no statistical significant difference was observed between the two treatment groups.

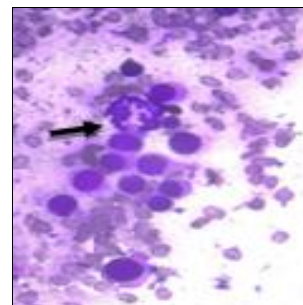


Fig 1: Mitotic figure (black arrow)

The mean apoptotic index increased significantly during treatment in both groups. In Group I, the apoptotic index increased from 1.17 ± 0.17 on day 0 to 3.33 ± 0.42 on day 7 and 4.33 ± 0.71 on day 14, followed by a decline to 1.00 ± 1.00 on day 21 and 0 post-treatment. In Group II, the apoptotic index increased from 1.17 ± 0.17 on day 0 to 5.50 ± 0.76 on day 7 and 6.83 ± 0.75 on day 14, and thereafter declined to 0 by day 21 and post-treatment (Table 2). A statistically significant increase ($p < 0.05$) in apoptotic index was observed within both groups during the initial phase of treatment. Between groups, Group II showed a significantly higher apoptotic index ($p < 0.05$) on days 7, 14 and 21 compared to Group I.

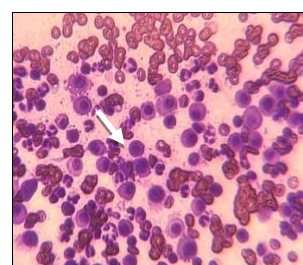


Fig 2: Apoptotic cell (white arrow)

The mean duration required for complete tumour regression was 28.00 ± 1.81 days in Group I and 22.17 ± 1.17 days in Group II (Table 3). The difference between the two groups was statistically significant ($p < 0.05$), indicating that dogs treated with ivermectin and vincristine combination therapy required a shorter treatment period for complete tumour regression compared to dogs treated with vincristine alone.

4. Discussion

The present study demonstrated that both vincristine sulfate alone and ivermectin-vincristine combination therapy were effective in inducing regression of canine transmissible venereal tumour. However, the combination therapy exhibited a comparatively better cytological response and shorter treatment duration.

A progressive and statistically significant decline in mitotic index was observed in both groups following initiation of treatment, which was in agreement with the findings of Aja (2023)^[11], who reported a statistically significant decline in MI

during the treatment period. A comparable finding was reported by Santos *et al.*, (2005) [23], who recorded a mean MI of 8.10 ± 3.47 in growing TVTs and 2.80 ± 1.80 in regressive TVTs. The absence of mitotic figures by day 14 in Group II, compared to day 21 in Group I, suggests a strong therapeutic and anti-proliferative effect of ivermectin and vincristine combination treatment.

The apoptotic index showed statistically significant increase in both groups during the regression phase of the tumour, with Group II consistently exhibiting higher apoptotic index than Group I. The apoptotic index observed in both treatment groups was markedly higher than the values reported in experimentally transplanted CTVTs by Santos *et al.* (2005) [23], where progressive tumours had an apoptotic index of 0.38 ± 0.08 and regressing tumours showed 0.62 ± 0.11 . Ozalp *et al.* (2012) [20] proposed that vincristine induces tumour regression by stimulating apoptosis and at the same time suppressing the tumour cell proliferation. Likewise, González *et al.* (2000) [12] reported a marked difference in apoptotic activity between the progressive and regressive phases of the tumour, with the regressive phase showing a substantially higher proportion of apoptotic cells (57.75 per cent) compared to the progressive phase (8.73 per cent). In the present study, Group II exhibited a higher apoptotic index than Group I, which may be due to the additional apoptotic effect of ivermectin. This observation was consistent with the findings of Tang *et al.* (2021) [25], who demonstrated that ivermectin can activate multiple forms of programmed cell death, including apoptosis, autophagy, and pyroptosis. Therefore, the inclusion of ivermectin may have further enhanced tumour cell death, resulting in faster tumour regression in the combination therapy group.

In the present study, the apoptotic index recorded in Group II was 6.83 ± 0.75 , which was higher than the peak value of 5.11 ± 1.01 reported by Aja (2023) [1] and this difference could be due to variations in the dose and frequency of ivermectin administration, as the present study used a higher dose of 0.5 mg/kg administered biweekly, whereas Aja (2023) [1] administered a single dose of 0.2 mg/kg on day 0. The mean treatment duration was significantly shorter in Group II (22.17 ± 1.17 days) compared to Group I (28.00 ± 1.81 days), indicating a higher therapeutic response in the combination treated dogs and are consistent with the findings of Prasanth *et al.* (2024) [22], who reported that vincristine monotherapy required nearly six weeks to achieve complete tumour regression, whereas the vincristine and ivermectin combination therapy (0.2 mg/kg) shortened the treatment period to four weeks. Similarly, Akudari (2022) [2] reported that dogs treated with vincristine (0.025 mg/kg) in combination with ivermectin (500 µg/kg, SC, at two-week intervals) showed 83.33 per cent complete clinical regression within just two weeks of treatment. In addition, dogs receiving vincristine with a lower dose of ivermectin (200 µg/kg) exhibited noticeable clinical improvement within three weeks following weekly vincristine administration.

Comparable findings were also reported by de Oliveira Lima *et al.* (2013) [13], who observed that vincristine was effective in all CTVT cases, with 93 per cent of cases achieving remission within four to five weekly sessions. In contrast, Aja (2023) [1] reported that the mean duration required for complete tumour regression was 23.10 ± 1.49 days in vincristine treated dogs and 22.00 ± 1.00 days in dogs treated with the vincristine and ivermectin combination (0.2 mg/kg on day 0), with no significant difference between the two groups. Similar findings were also reported by Bulhosa *et al.* (2020) [5]. The variation between those studies and the present findings may be due to differences in the dose and

administration interval of ivermectin, as well as the lack of cytomorphological classification in earlier studies.

Faster tumour regression in the ivermectin and vincristine group may be explained by the combined antimitotic effect of vincristine and the enhanced apoptotic activity. The findings suggest that ivermectin may act as a useful adjunct to vincristine in improving therapeutic response in plasmacytoid CTVT. Overall, the cytological findings of the present study indicate that serial monitoring of mitotic and apoptotic indices can serve as useful indicators of therapeutic response in canine TVT.

Table 1: Comparison of mitotic index between groups (Mean $\pm$ SE)

Treatment days	Group I (n=6)	Group II (n=6)
Day 0	4.50 ± 0.43^a	5.17 ± 0.40^a
Day 7	2.17 ± 0.31^b	1.50 ± 0.34^b
Day 14	0.50 ± 0.22^c	0 ± 0^c
Day 21	0 ± 0^c	0 ± 0^c
Post treatment	0 ± 0^c	0 ± 0^c

Different superscripts (a, b, c) within a group indicate significant differences between days ($p < 0.05$).

Table 2: Comparison of apoptotic index between groups (Mean $\pm$ SE)

Treatment days	Group I (n=6)	Group II (n=6)
Day 0	1.17 ± 0.17^a	1.17 ± 0.17^a
Day 7	$3.33 \pm 0.42^{b,x}$	$5.50 \pm 0.76^{b,y}$
Day 14	$4.33 \pm 0.71^{b,x}$	$6.83 \pm 0.75^{b,y}$
Day 21	$1.00 \pm 1.00^{a,x}$	$0 \pm 0^{c,x}$
Post treatment	$0 \pm 0^{b,x}$	$0 \pm 0^{c,x}$

Different superscripts (x, y) between group indicate significant differences between days ($p < 0.05$).

Different superscripts (a, b, c) within a group indicate significant differences between days ($p < 0.05$).

Table 3: Comparison of treatment duration between groups (Mean $\pm$ SE)

Treatment days	Group I (n=6)	Group II (n=6)
Days	28.00 ± 1.81^a	22.17 ± 1.17^b

Different superscripts (a, b) within a group indicate significant differences between days ($p < 0.05$).

5. Conclusion

Both vincristine sulfate alone and ivermectin-vincristine combination therapy were effective in the treatment of canine transmissible venereal tumour. However, the addition of ivermectin resulted in earlier reduction in mitotic activity, significantly higher apoptotic response and shorter treatment duration. The present study demonstrated that both vincristine monotherapy and the ivermectin and vincristine combination were effective in the treatment of plasmacytoid CTVT, as all treated dogs showed complete tumour regression and survived until the completion of therapy. However, the combination therapy was associated with shorter treatment duration, higher apoptotic activity, and good overall clinical stability without major adverse effects. These findings indicate that ivermectin, when used alongside vincristine, may enhance antitumour activity and can be considered a promising alternative therapeutic protocol for the management of dogs affected with CTVT. This study suggests that ivermectin and vincristine combination therapy may provide a better therapeutic response than vincristine monotherapy in dogs with plasmacytoid CTVT.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Aja TN, Becha BB, Jayakumar C, Unnikrishnan MP, Devi SS. Occurrence of canine transmissible venereal tumour. *Journal of Veterinary and Animal Sciences*. 2023;54(2):382-387.
2. Akudari A. Studies on transmissible venereal tumour (TVT) in canines. PhD Thesis. P V Narsimha Rao Telangana Veterinary University, Hyderabad. 2022.
3. Amaral AS, Gaspar LFJ, Bassani-Silva S, Rocha NS. Cytological diagnostic of transmissible venereal tumour in the Botucatu region, Brazil: Descriptive study. *Portuguese Journal of Veterinary Sciences*. 2004;99(551):167-171.
4. Aprea AN, Allende MG, Idiard R. Intrauterine transmissible venereal tumor: A case description. *Veterinaria Argentina*. 1994;11(103):192-194.
5. Bulhosa LF, Lima AE, Goncalves GSD, Larangeira DF, Pinho FA, Melo SMB. Vincristine and ivermectin combination therapy in dogs with natural transmissible venereal tumour of different cytomorphological patterns: A prospective outcome evaluation. *Animal Reproduction Science*. 2020;216(1):106-358.
6. Crump A. Ivermectin: Enigmatic multifaceted 'wonder' drug continues to surprise and exceed expectations. *Journal of Antibiotics*. 2017;70(7):495-505.
7. Das U, Das AK. Review of canine transmissible venereal sarcoma. *Veterinary Research Communications*. 2000;24(8):545-556.
8. Dominguez-Gomez G, Chavez-Blanco A, Medina-Franco JL, Saldivar-Gonzalez F, Flores-Torrontegui Y, Juarez M, *et al.* Ivermectin as an inhibitor of cancer stem-like cells. *Molecular Medicine Reports*. 2018;17(2):3397-3403.
9. Fonseca LS, Mota LS, Colodel MM. Spontaneous canine transmissible venereal tumor: Association between different phenotypes and the insertion line-1/c-myc. *Revista Colombiana de Ciencias Pecuarias*. 2012;25(3):402-408.
10. Gaspar LFJ, Ferreira I, Colodel MM, Brandao CVS, Rocha NS. Spontaneous canine transmissible venereal tumor: Cell morphology and influence on P-glycoprotein expression. *Turkish Journal of Veterinary and Animal Sciences*. 2010;34(5):447-454.
11. Gaspar LF, Amaral AS, Bassani-Silva S, Rocha NR. Immunoreactivity to P-glycoprotein in the different cytomorphological types of canine transmissible venereal tumor. *Veterinary in Focus*. 2009;6(2):140-146.
12. Gonzalez CM, Griffey SM, Naydan DK, Flores E, Cepeda R, Cattaneo G, *et al.* Canine transmissible venereal tumour: A morphological and immunohistochemical study of 11 tumours in growth phase and during regression after chemotherapy. *Journal of Comparative Pathology*. 2000;122(4):241-248.
13. Greatti WF, Amaral AS, Silva SB. Proliferation indexes determination by CEC and Ki-67 in fine needle aspiration cytology of transmissible venereal tumor. *Archives of Veterinary Science and Medicine*. 2004;9(1):53-59.
14. Griffin J, Fletcher N, Clemence R, Blanchflower S, Brayden DJ. Selamectin is a potent substrate and inhibitor of human and canine P-glycoprotein. *Journal of Veterinary Pharmacology and Therapeutics*. 2005;28(1):257-265.
15. Jiang L, Wang P, Sun YJ, Wu YJ. Ivermectin reverses drug resistance in cancer cells through EGFR/ERK/Akt/NF- κ B pathway. *Journal of Experimental and Clinical Cancer Research*. 2019;38(1):1-18.
16. Lapa FAS, Andrade SF, Gervazoni ER, Kaneko VM, Sanches OC, Gabriel Filho LRA. Histopathological and cytological analysis of transmissible venereal tumor in dogs after two treatment protocols. *Colloquium Agrariae*. 2012;8(1):36-45.
17. Lespine A, Dupuy J, Orlowski S, Nagy T, Glavinas H, Krajcsi P, *et al.* Interaction of ivermectin with multidrug resistance proteins (MRP1, 2 and 3). *Chemico-Biological Interactions*. 2006;159(3):169-179.
18. Lima CRDO, Rabelo RE, Vulcani VAS, Furtado AP, Helrigel PA, Brito LAB, *et al.* Morphological patterns and malignancy criteria of transmissible venereal tumor in cytopathological and histopathological exams. *Brazilian Journal of Veterinary Research and Animal Science*. 2013;50(3):238-246.
19. Markowska A, Kaysiewicz J, Markowska J, Huczynski A. Doxycycline, salinomycin, monensin and ivermectin repositioned as cancer drugs. *Bioorganic and Medicinal Chemistry Letters*. 2019;29(13):1549-1554.
20. Ozalp GR, Zik B, Bastan A, Peker S, Ozdemir-Salci ES, Bastan I, *et al.* Vincristine modulates the expression of Ki67 and apoptosis in naturally occurring canine transmissible venereal tumor (TVT). *Biotechnic and Histochemistry*. 2012;87(7):325-330.
21. Pouliot JF, L'Heureux F, Liu Z, Prichard RK, Georges E. Reversal of P-glycoprotein-associated multidrug resistance by ivermectin. *Biochemical Pharmacology*. 1997;53(1):17-25.
22. Prasanth Kumar M, Murugavel K, Hemalatha H, Kantharaj S. Comparative efficacy of two different treatment protocols for treatment of canine transmissible venereal tumour. *International Journal of Advanced Biochemistry*. 2024;8(9 Suppl):1400-1402.
23. Santos FGA, Vasconcelos AC, Nunes JES, Cassali GD, Paixao TA, Moro L. The canine transmissible venereal tumor: General aspects and molecular approach. *Journal of Biosciences*. 2005;21(3):41-53.
24. Spugnini EP, Dotsinsky I, Mudrov N. Biphasic pulses enhance bleomycin efficacy in a spontaneous canine genital tumor model of chemoresistance: Sticker sarcoma. *Journal of Experimental and Clinical Cancer Research*. 2008;27(1):58-58.
25. Tang M, Hu X, Wang Y, Yao X, Zhang W, Yu C, *et al.* Ivermectin, a potential anticancer drug derived from an antiparasitic drug. *Pharmacological Research*. 2021;163(2):105-207.

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