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Treatment of Canine Transmissible Venereal Tumour using Different Surgico-chemotherapeutic Protocols

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ABSTRACT

Background: Canine transmissible venereal tumour (CTVT) also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or sticker tumour is usually transmitted through coitus and mainly affects the external genitalia of young sexually matured dogs. Surgery, chemotherapy, radiotherapy and immunotherapy are considered as effective treatment protocols. Therefore, depending upon the availability present study was designed to investigate the efficacy of different surgico-chemotherapeutic protocols for treatment of canine transmissible venereal tumour.

Methods: The study was conducted during January 2018 to July 2018 at the Teaching Veterinary Clinical Complex (TVCC) and Department of Veterinary Surgery and Radiology, College of Veterinary Science and A.H., Anjora, Durg (C.G.) on 18 canines of various breed, irrespective of age, sex and divided into three groups consisting 6 animals in each group. Group A was treated with surgical excision of tumour only where as Group B and Group C were treated with surgical excision of tumour followed by administration of Doxorubicin (30 mg/m^2) BSA and Vincristine sulphate (0.025 mg/kg) intravenously alongwith DNS at 7th and 14th post-operative days respectively. Different physiological and haemato-biochemical parameters (Hb, PCV, TLC, TPC, DLC, serum glucose, TSP, SUN, SC, ALT, AST and ALP) were recorded preoperatively, postoperatively and after chemotherapy at 10th, 30th and 60th days intervals.

Result: The present investigation showed transient changes in physiological and haemato-biochemical parameters before, post surgery and post chemotherapeutic management and was within normal range. Histopathological examination revealed confluent sheet of tumour cells arranged in large round oval or polyhedral shaped distributed in tight clusters or cords. Group A showed mild to moderated reoccurrence while Group B showed minimum reoccurrence. Group C showed no reoccurrence. Thus, surgery combined with vincristine therapy is most effective for treating dogs suffering with transmissible venereal tumour.

Key words: Canine, Chemotherapy, Doxorubicin, Transmissible venereal tumour, Treatment, Vincristine.

INTRODUCTION

Among the commonly encountered affections of dogs, transmissible venereal granuloma (TVT) is a naturally occurring, coitally-transmitted neoplastic disorder but may also be transmitted through licking, biting and sniffing of tumour affected areas (Das and Das, 2000). The lesions are confined to mucous membrane of external genitalia of both sexes, vulva and vagina in females; penis and prepuce in male dogs but metastasis less frequently to skin, oral cavity, ocular and nasal passage. Initially the tumour mass is small, subsequently progressing and become large, ulcerated with contaminated mass. The lesions are friable, hyperemic, multilobular, cauliflower like masses and haemorrhagic discharge produces offensive odour. Canine transmissible venereal tumour (TVT) or commonly known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma is a tumour that infects the outer genital organs of male and female dogs (Behera *et al*., 2012).

Though surgery is the treatment of choice for TVT (Hoque, 2002), but frequent recurrence following surgery occurs due to the growth of the residual tumour warrants which carry high risk of metastasis. In such cases, radical surgery is advocated followed by chemotherapy to arrest the recurrence from the remnants as suitable alternative to further surgery however, accompanied by various side effects such as vomition, diarrhoea, anorexia, alopecia, anaemia *etc*. Several treatment protocols have been established for TVT but chemotherapy with vincristine sulphate is the most widely used protocol (Hantrakul *et al*., 2014). However, combination with chemotherapy can yield a satisfactory degree of success using cyclophosphamide, methotrexate and vincristine in clinical cases of CTVS without major ill effects or recurrence (Hoque *et al*., 1995). Vincristine Sulphate belong to a group of medications known as vinca alkaloids and is obtained from the plant *Vinca rosea*

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Linn which act as ant imicrotubule agents that block mitosis by arresting cells in the metaphase (Tella *et al*., 2004). Doxorubicin is an anthracycline glycoside antibiotic originally produced by *Streptomyces peucetius var. caesius* and used in cancer chemotherapy (Lori *et al*., 2010). It exerts its cytotoxic effect as a DNA-intercalating agent to inhibit further DNA and RNA biosynthesis. Since, the report regarding the use of doxorubicin and vincristine sulphate in adjunct chemotherapy for treatment of canine transimissible venereal tumour in clinical cases are limited. Therefore, the present study was designed to investigate the efficacy of three different protocols for treatment of canine transimissible venereal tumour including surgical excision, combination of surgical excision with chemotherapy (doxorubicin and vincristine) drug used in cancer chemotherapy.

MATERIALS AND METHODS

The present study was conducted during January 2018 to July 2018 at the Teaching Veterinary Clinical Complex (TVCC) and Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Anjora, Durg (C.G.) on 18 clinically suspected cases of tumour in dogs (9 females and 9 males) with the clinical history of vaginal bleeding, cauliflower like growth in both vulva and vagina, bleeding from penis, cauliflower like growth on penis and prepuce in males of various breed, irrespective of age, sex and divided into three groups consisting 6 animals in each group.

Treatment protocols

The 18 animals were divided randomly into three groups *viz*., Group A, Group B and Group C, each comprising 6 animals. In Group A, dogs were treated with surgical excision of tumourous mass only where as in Group B and Group C, dogs were subjected to combination protocol of surgical excision of tumourous mass followed by administration of Doxorubicin (30 mg/m^2) BSA and Vincristine sulphate (0.025 mg/kg) intravenously alongwith DNS at 7th and 14th post-operative days respectively. Supportive therapy of Tribivet @ 0.05-0.2 mg/kg b.wt. I/M was given for 5 days followed by Polybion syrup one tsf bid and Tab Liv-52® every day to dogs of Group B and C during course of chemotherapeutic protocol. Surgical excision was performed in all dogs under general anaesthesia using atropine sulphate @ 0.65 mg (total dose) I/M, xylazine @ 1 mg/kg and ketamine @ 5 mg/kg I/V. After aseptic preparation of dogs, the surgical de-bulking of all the nodules of tumour and in some dogs, episiotomy was done to expose the deep-seated tumours growth on vulva and vagina by incising dorsal vulvar orifice. Episiotomy wound was repaired in two rows suturing pattern and complete dressing was done afterwards.

Physiological parameters

Rectal temperature (°F), Heart rate (beats/minute) and Respiration rate (breaths/minute) were recorded before and during surgery at 10, 30, 60, 90 and 120 minutes and on 10th, 30th and 60th day postoperative and after chemotherapeutic protocol.

Haematological parameters

Blood samples were collected from the dogs before and at 10th, 30th and 60th day after surgery and post-chemotherapeutic protocols. Two ml of blood was withdrawn from cephalic or saphenous vein in a vacutainer containing ethylene diamine tetra acetic acid (EDTA) for estimation of haematological parameters before and 10th, 30th and 60th day after surgery and post-chemotherapeutic protocols. Complete haematological parameters were estimated with help of automated haematology blood cell counter (B.C- 2800 Vet, Mindray). Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), total platelet count (TPC) were analyzed. Differential leucocyte count (DLC) was done manually by making blood smear from fresh blood and stained with Leishman stain.

Biochemical parameters

Three ml of blood was collected from the dog before and at 10th, 30th and 60th day after surgery and post-chemotherapeutic protocols for biochemical examination in clean sterilized vials and allowed to clot at room temperature. After 2 hr serum was separated and following parameters were estimated *viz*., Serum glucose (mg/dl), total serum proteins (gm/dl), serum urea nitrogen (mg/dl), serum creatinine (mg/dl), aspartate aminotransferase (U/L), alanine aminotransferase (U/L) and alkaline phosphatase (U/L) with Semi-Automated Biochemistry Analyzer (Diasil-100 Systronics make).

Histopathological examination

After surgery, the tissue specimens were collected and were rinsed with normal saline to wash off excess blood and other debris. The specimens taken were then cut into 2 mm cubes and fixed in 10% neutral buffered formalin as per standard protocol and then embedded in paraffin wax. The fixed tissues were sectioned at 3-5 µm thickness and stained with Haematoxylin and Eosin (H&E) as per standard method (Luna, 1968) for microscopic examination under light microscope for histopathological details.

Surgico-chemotherapeutic evaluation of protocols

Evaluation was done on the basis of wound examination and recurrence of the nodules after 2 weeks of treatment protocols. Wound examination was done at 0 day, 3rd and 10th day post-operative protocols for nature of the wound (normal/oedematous) and nature of discharge (bloody/serosanguinous/foetid/pussy). Side-effects or unwanted effects of protocols, if any were recorded.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) to compare mean values among different groups at same time intervals and within groups at different time intervals in each group. All the data were analyzed by using SPSS v17.0 statistics

software program and data were presented as mean± Standard Error. The level of significance was set at P<0.05.

RESULTS AND DISCUSSION

The present study was under taken to investigate the efficacy of different surgico-chemotherapeutic protocols for treatment of canine transimissible venereal tumour with particular reference to their clinico-physiological, haemato-biochemical alteration post-surgery and after chemotherapeutic agent (doxorubicin and vincristine) administration.

Examination of animals before treatment

On clinical examination of animals the growth appeared round to diffused swelling, lobulated and cauliflower like growth varied from red to grayish-pink. In females the growth was extending 2-4 cm in to vagina, pendulous valve with serosanguinous fluid discharging from vulva orifice (Fig 1). Whereas in males, growths were located on the base and tip of penis with bloody discharge (Fig 2).

Physiological parameters

There was non-significant decrease in rectal temperature and respiration rate upto 60 min. in group A, B and C respectively. Whereas, heart rate showed a significant (P<0.05) increase at 30 min. However, the values were within physiological limits in all the three groups. Decrease in temperature and respiration rate after administration of anaesthetic drugs might be due to combined effect of xylazine and ketamine. There was no significant difference in rectal temperature, heart rate and respiration rate between groups at various time intervals.

Fig 1: Cauliflower like growth seen in retracted vagina before treatment.

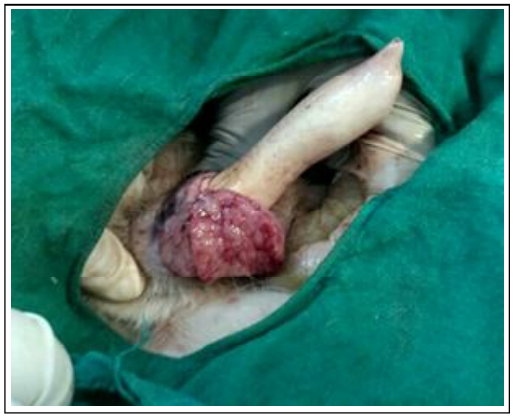


Fig 2: Cauliflower like growth seen at base of penis after retraction of prepuce before treatment.

Haematological parameters

The mean value of some haematological parameters in canine transmissible venereal tumour before, post-surgery and after surgico-chemotherapeutic protocols in different groups at various time intervals did not show much deviation from reference value. Haemoglobin showed non-significant decrease in group B and C, upto 30th day which was less than 0 day value and indicated anaemia. Thereafter, value showed increasing trend upto 60th day of observation period. Packed cell volume and TEC showed non-significant decrease upto 30th days in group A, B and C, respectively which further increased non-significantly at 60th day. There was non-significant decrease in TEC in group B and C, which could be due to chemotherapy induced erythrocytopenia due to myelosuppression. Yadav *et al*. (2017) reported consistent decreasing (P>0.05) trend of TEC in dog affected with TVT which was treated with vincristine and doxorubicin during post-operative period. There was significant (P<0.05) decrease in TLC in group B and C, as these cytotoxic drugs suppress the replicating precursor cells of bone marrow, thus, resulting in reduced production of leucocytes. Total platelet count showed non-significant changes in group A and B during the observation period but in group C, platelet count increased (thrombocytosis) upto 30th day and then declined which was statistically non-significant. Similarly, Upadhye (2007) reported significant (P<0.05) thrombocytosis on 28th and 35th day in surgical removal of venereal granuloma with vincristine therapy after 5 cycles in dogs. Whereas, Kumar *et al*. (2018) observed a significant (P<0.01) decline in platelet count after vincristine therapy when compared to before therapy in dog affected with TVT. Therefore, it could be stated that chemotherapeutic agent vinca alkaloid vincristine sulphate is a safe drug since thrombocytosis was evident with minimum haematological alterations. The neutrophils count in group B and C showed a significant (P<0.05) decrease at 30th day whereas, in group A, neutrophils count did not show much change during period of observation. Whereas, lymphocyte count in group B and C showed significant (P<0.05) increase at 30th day which decreased significantly (P<0.05) on 60th day whereas in group A there was no much variation in lymphocyte count during period of observation. The significantly (P<0.05) reversible leukopenia and neutropenia observed following chemotherapy in the present study was probably due to the action of cytotoxic drugs which suppressed the replicating precursor cells of bone marrow and created myeloid toxicity. There was non-significant variation in the values of eosinophils and monocytes before and after surgico-chemotherapeutic protocol and all the haematological values were remained within normal physiological range.

Biochemical parameters

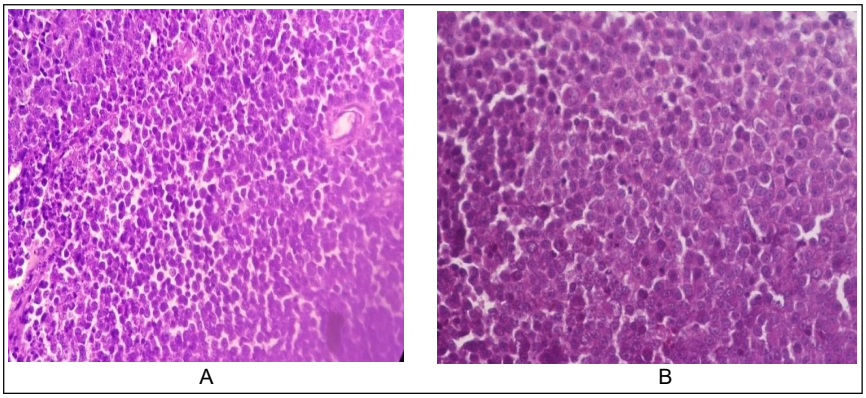
Serum glucose levels in group B showed significant (P<0.05) increase upto 60th day after doxorubicin therapy and non-significant decrease upto 60th day in group C after vincristine therapy. This increase in glucose level might be due to stress created on body by chemotherapeutic agent doxorubicin which caused the release of glucocorticoid and mineralocorticoid due to stimulation of adrenal cortex along with epinephrine and non-epinepherine due to stimulation of the medulla. But serum glucose level in animals of group A showed irregular variation throughout the observation period and values remained within normal physiological limits. Similar pattern of alteration in glucose level was observed by Upadhye (2007) with vincristine and doxorubicin therapy administered after surgical removal of venereal granuloma in dogs. Total serum proteins levels in the animals of group B showed significant (P<0.05) decrease at 10th day which further decreased significantly (P<0.05) upto 60th day. However, the TSP levels in group A and C showed marginal changes during period of observation. Yadav *et al*. (2017) reported that serum total proteins showed slight drop on day 7 followed by rise (P>0.05) on day 14 and elevated profiles continued during post vincristine and doxorubicin therapy in TVT affected dogs. In group B, serum urea nitrogen and serum creatinine levels showed significant (P<0.05) increase upto 30th day which furthers significantly (P<0.05) decrease at 60th day whereas, in group A and C, non-significant variation was observed upto 60th day of observation period. Blood urea nitrogen is the most common test to assess the renal function in dogs. Increase in BUN may be linked to glomerular filtration rate or increased protein catabolism caused by necrosis of tumour or metabolic side effects of neoplasia and increase in serum creatinine was attributed to the increase in catabolic activity (Kumar *et al*., 2018). In group B and C, Aspartate amino transferase (AST) and Alanine amino transferase (ALT) levels showed significantly (P<0.05) increasing trend upto 30th day which further decreased on 60th day after doxorubicin and vincristine therapy respectively. However, group A, showed non-significant variation after surgery. In the present study, the increase in AST and ALT levels following chemotherapy in both groups may probably be due to the result of detoxification of vincristine and doxorubicin in the liver. Doxorubicin hydrochloride gets detoxified by liver and the liver gets loaded much for secretion of transaminase. Similarly, Gandhimati *et al.* (2011) observed non-significant difference in glucose, blood urea nitrogen, mean creatinine level whereas SGOT/SGPT levels were significantly (P<0.01) elevated in both vincristine treated and doxorubicin treated groups in dog affected with TVT. The mean serum values of alkaline phosphatase (ALP) were towards the higher limit of normal range in all the three groups at time of presentation. Alkaline phosphatase (ALP) levels in the animals of groups A, B and C showed significantly (P<0.05) decreasing trend upto 60th day. However, all biochemical parameter variation were within normal physiological limits. This indicates that increase in the alkaline phosphatase level could be attributed to presence of malignancy and addition of chemotherapy (vincristine and doxorubicin) induced stress, which is intensely reflected by rise in alkaline phosphatase activity. Similarly, Swamy *et al*. (2012) documented that level of alkaline phosphatase remained high in all eighteen TVT affected dogs and concluded that level of ALP can be used to indicate the presence of tumour.

Histopathological examination

The section of tumour mass was stained with Haematoxylin and Eosin and histopathological examination was carried which revealed confluent sheet of tumour cells arranged in large round oval or polyhedral shape distributed in tight clusters or cords. Sheets of neoplastic round cells separated by thick fibrous connective tissue with numerous mitotic fig were observed (Fig 3 A and B). These findings were in agreement with Da Silva *et al*. (2014) that confluent sheets of neoplastic round cells growing in cords were infiltrating into the submucosa and muscle, confirming it as TVT.

Response to treatment in different groups

In the present study, the nature of wound healing was normal in all the three groups without any complications or discharge. In all the three groups, complete wound healing



A. Neoplastic cells are arranged in large, round, oval or polyhedral shape distributed in tight clusters or cords (20X).
B. Round cells with vesicular nuclei arranged in sheets (40X)

Fig 3: A and B: Showing histopathology of Venereal granuloma (H&E stain).

was observed within 10 days and followed for three months after surgery and post chemotherapeutic protocols (doxorubicin and vincristine sulphate). No recurrence (0) of venereal granuloma in group C (surgical excision following vincristine therapy) was observed during the study period. Whereas, minimum recurrence (+) venereal granuloma was seen in one case in group B (surgical excision following doxorubicin therapy) and group A (surgical excision only) showed mild to moderate recurrence (+ to ++) in two cases. This might be due to incomplete removal of tumour nodules or inaccessibility of tumour sites which led to metastasis. The symptoms associated with administration of chemotherapeutic drug were inappetence, vomition, anaemia and alopecia. These above symptoms were comparatively more in animals of group B as compared to group C. However, the condition of the animals was managed by the supportive therapy with administration of intravenous fluids, antaacids and liver tonics as a palliative measure. These findings are in agreement with Srivastava *et al*. (2009), Awan *et al*. (2014) and Premsairam *et al*. (2018) who observed that surgery combined with Vincristine therapy has proved to be excellent therapeutic regimen for canine TVT which leads to complete regression of the neoplasm without relapse. However, surgical excision following doxorubicin therapy was also effective in management of venereal granuloma in present study but showed minimum recurrence in one case. Gandhimati *et al.* (2011) also reported complete regression of canine TVT following treatment with doxorubicin. The overall response of treatment using surgical excision alongwith administration of chemotherapeutic drug (doxorubicin and vincristine) indicates that vincristine sulphate has minimum side effect on haematological and biochemical parameters, which proves its superiority over doxorubicin. Therefore, combined treatment protocol, surgery followed by chemotherapy is the optimal management for Canine TVT as it reduces the recurrence rate.

CONCLUSION

From investigation of present study, it was concluded that surgical excision followed by vincristine therapy is most effective protocol for management of canine transmissible venereal tumours as compared to doxorubicin therapy.

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