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Cytogenetic analysis of leukemic cells in a dog

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ABSTRACT

The dog is a potential model to study chromosome aberrations in leukemic cells. Similar to man, dog with leukemia frequently have chromosomal abnormalities and analysing these changes may provide diagnostic prognostic therapeutic and aetiopathogenic information in a way not possible in other species. The chromosomal analysis in leukemic dogs and the evaluation of dog as an animal model for the study of leukemia have been discussed by Carol *et al*.,(1986).

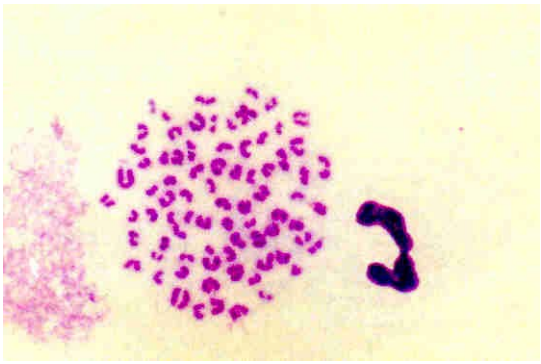
Key words : Leukemic cells, dogs and Cytogenetic analysis.

INTRODUCTION

In the chronic form of this disease a wide range of myeloid elements are differentiated. A typical mature and immature cells, stimulating in varying degree all the developemental forms of normal granulopoiesis, are found ordinarily in increased numbers in the blood and in excessive numbers in the bone marrow. It was stated by Fitzer *et al*., (1964) that the chromosomal abnormalities occur in acute leukemia and he could not report consistent anomaly of the karyotype. It has been found that the most human malignant neoplasms have abnormal chromosome patterns (Sandberg *et al*.,1980) and the abnormal karyotype could be the cause or the result of the cancerous conditions and the genetic sequences similar to known oncogenes have been found at break points in chromosomes from leukemic patients (Rowley,1983).As it was reported earlier that the cancerous condition could reflect in abnormal chromosomal DNA patterns, the present study has been undertaken to analyse the chromosomal DNA of a dog in acute leukemic stage in the small animal clinic, Madras Veterinary College. Blood collected from dog positive for leukemia has been subjected to analysis of the mitotic DNA and results were discussed.

The dog blood sample collected was processed for chromosomal DNA analysis. The short term lymphocyte culture was set up using 200µl blood in RPMI 1640 (Invitrogen) medium with 15% Fetal Calf Serum (Gibco) supplementation 2 mM L glutamine was added. 100ml mitogen (PHA-Phytohaemagglutinin) was added and incubated for 72 hours at 37^{0}C at 5% CO_2. Around 69 hours of the culturing the mitotic arrestant colchine (10µg/ml) was added and incubated at 37^{0}C CO_2 incubator for 90 minutes. The cell pellet after centrifugation was given hypotonic saline (0.56% KCl) treatment for 20-30 minutes. Then the cell pellet obtained after centrifugation was fixed in freshly prepared Carnoys fixative. After three washings in Carnoy's fluid, chromosomal spreads were made on clean precooled microscopic slides. Then the spreads were stained with 4% Giemsa in phosphate buffered saline and viewed under photomicroscope.

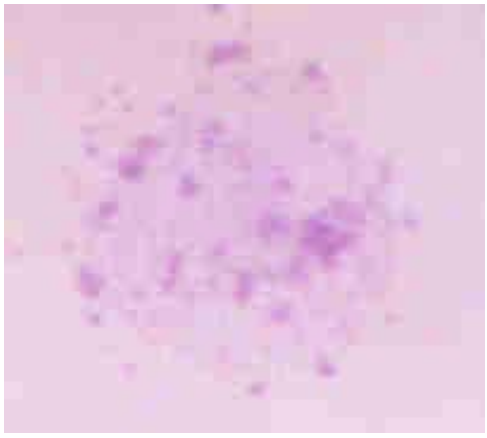
Dog cells normally have 78 chromosomes (Fig.) all of which are telocentric chromosomes except for the sex chromosomes. The sex chromosomes are large submetacentric X chromosome and small submetacentric Y chromosome (Mellink and Bosma.,1999).The chromosomal DNA abnormalities found normally in leukemic dogs could be aneploidy especially



38 autosome pair-Telocentric

One pair sex chromosome-Metacentric

Fig. -1: Dog chromosomes –Diploid Karyotype 2n-78



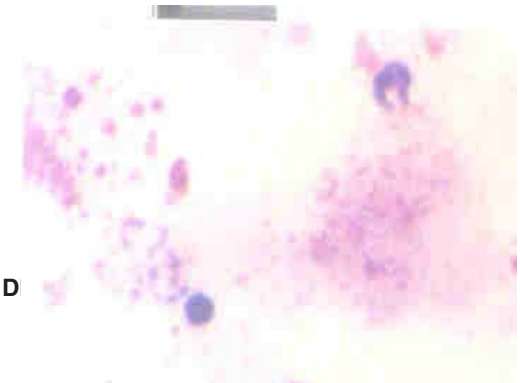


Fig. -2: Chromosomal spreads with D

hyperploidy, extrametacentric chromosomes, double minute chromosome, distortion in chromosomal DNA and tetraploidy (Carol *et al*.,1986).

The chromosomal DNA as spreads on microscopic slides from the dog in acute leukemia showed complete pulverization of the DNA strands and distorted chromosomal DNA contents (Fig). These findings can be compared with the studies of Tong *et al*., (2002). Authors reported that the fine needle aspiration of a lymphoma processed for cytogenetic analysis revealed histiocytes, freely lying karyorectic debries, mitotic figures which were readily identified and the molecular studies confirmed the genetic rearrangement (Tong *et al*.,2002).This distorted structures of DNA were observed by Tsitol and his colleagues,(1996) during their studies on the pied cattle which were exposed to genotoxic agents. These authors have also reported the presence of cytogenetic variabilities and anomalies in the cell division and the metaphase chromosomal DNA revealed distorted DNA structures. Such distorted structures could be correlated to the present report. Also the abnormal chromosomal DNA could be the cause or the result of the cancerous condition as reported by Sand berg (1980). It was also reported that the genetic

sequences similar to known oncogenes have been found at breakpoints in chromosomes from leukemic patients (Rowley,1983)

Hence, it can be concluded that the deformities in the DNA synthesis predisposes the animal for leukemic changes in the blood and could be recorded as an aetiopathogenic phenomenon in the conditions like leukemia in the dogs. With additional research and improvements, detailed studies on the chromosomal DNA of canine leukemia provide diagnostic and prognostic information.

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