Cytomorphological, molecular diagnosis and evaluation of insertion of the LINE-1 elen[illegible] MYC gene in canine transmissible venereal tumor: Applicability in veterinary clinical routine.

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Introduction and Purpose

Canine transmissible venereal tumor (CTVT) is the oldest cancer in the world, which is transmitted during the mating process. Cytomorphologically, they are classified into three types:lymphocytoid, mixed or plasmocytoid, the latter being chemoresistant, and important from a predictive perspective. Previous studies have shown that the insertion of transposon LINE-1 in the C-MYC gene has diagnostic importance, but the role of this process in the distinct behaviors observed in different cytomorphological types is still unknown. Therefore,we analyzed the presence of this insertion and cytomorphological diagnosis in CTVT samples in Belém, Brazil.

Materials and Methods

Samples were collected from animals treated at the Veterinary Hospital of the Universidade Federal Rural da Amazônia. The diagnosis and cytomorphological classification were made according to the predominant cell type. The DNA of the samples was extracted and the molecular diagnosis was made by PCR using specific primers to detect insertion as well as the sequencing of the samples.

Results and Discussion

We found most of the samples were of the lymphocytoid type (75%) and we observed on molecular diagnosis a fragment of approximately 400 bp (Figure 1 and Figure 2), which corresponds to the insertion of the transposon to the gene, which was confirmed in the sequencing of the samples. In the three cytomorphological types, the insertion of transposon is in the same region of DNA.

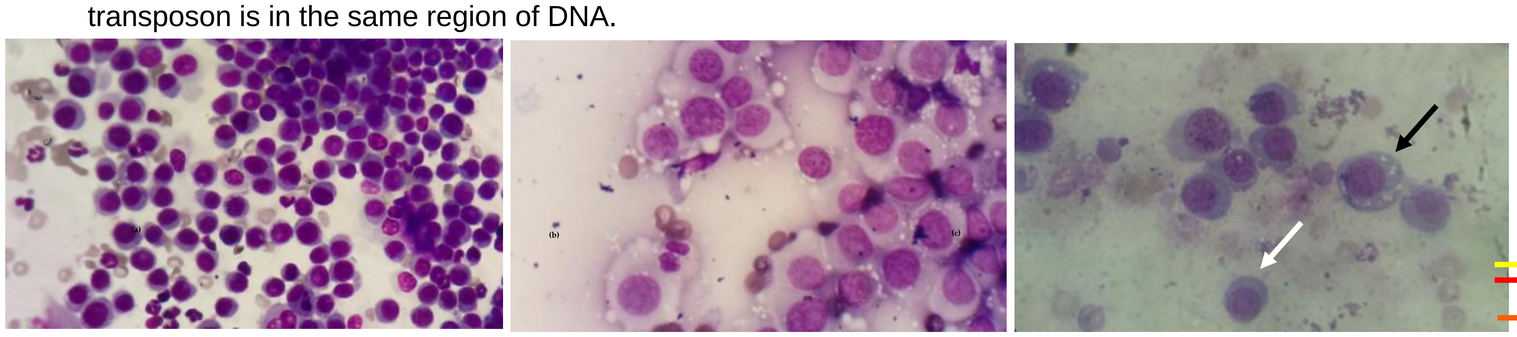

Figure 1: Cytology images of CTVT of different cytomorphological types: A: lymphocytic type - predominance of more than 60% of round cells, with little presence of cytoplasmic vacuoles and centralized nuclei. B: Plasmacytic type - predominance of more than 60% of ovoid cells with more abundant cytoplasms and eccentric nuclei. C: mixed type - there is the presence of both cell subtypes, however neither type exceeds 59% of the total cells analyzed in the slide. Black arrow - plasmacytoid cell; White arrow - lymphocytoid cell.

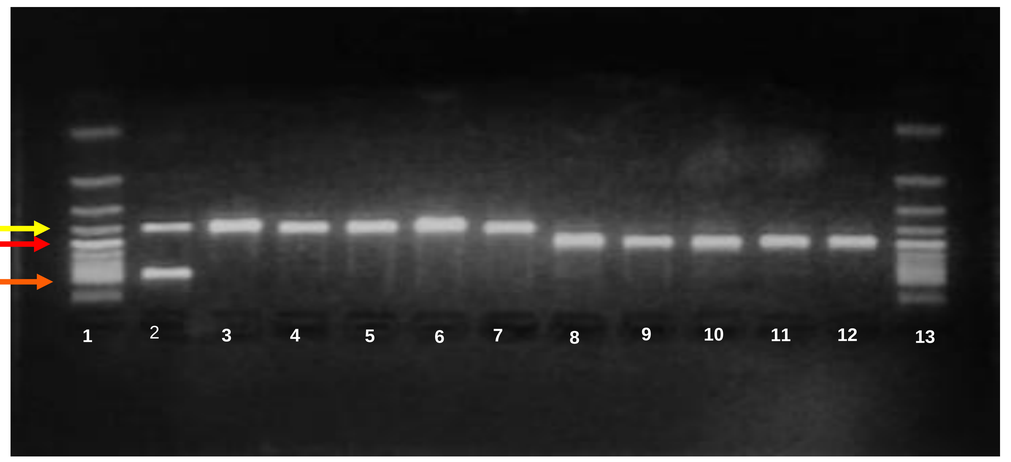

Figure 2: Molecular diagnosis of CTVT. Yellow arrow - 400bp; Red arrow - 500bp; Orange arrow - 900bp; 1 and 13- Molecular weight of 1kb; 2- Dog testicle DNA as negative control of CTVT. There are two fragments of different sizes (900bp and ~ 400bp), respectively, transposon and MYC gene; 3- Sample of positive CTVT according to histopathological report; 4 to 7- CTVT samples showing the insertion of the transposon Line-1 (900bp) in the MYC gene (~ 400bp), in which there is only 1 fragment; 8 to 12- Positive internal sample controls (~ 480pb).

PCR results prove the high sensitivity of the test for the detection of rearrangement of the LINE-1 element in the MYC gene in CTVT, since all the samples of the transmissible tumors studied amplified a fragment of 400pb, which characterizes such rearrangement, corroborating the observations by O'Neill (2011). Some studies have shown different insertion sizes in CTVT samples, but generally close to the 400bp size (Lima et al., 2016; Park, 2006; Spin, 2010, Fonseca 2012) this is justified that the LINE-1 element has a sequence of 1,378pb inserted into the C-MYC gene, the differences in amplifications are justified by virtue of the use of primers that configure diferente gene regions in this rearrangement (Murchison, 2009).

Conclusions

Hence, considering that the insertion is in the same region in the three cytomorphological types, we can conclude that even though it is important for the oncogenesis process, this insertion has no influence on the cytomorphological type and its clinical differences.

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