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Preliminary Evaluation of Protein Carbonyl Group in Canine Serum Using a Western Blotting Technique

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In human medicine, protein carbonyls (PCOs) are widely used biomarkers to detect oxidized proteins in serum and are significantly higher in septic patients.

The aim of this study was to detect PCOs in canine serum by using a western blotting technique and to assess their possible role as a novel marker of sepsis.

The technique described by Levine was used. After the separation by SDS-page of serum, samples were blotted onto nitrocellulose membrane and labelled with 2, 4-dinitrophenylhydrazine (DNPH). DNPH can react with PCOs by producing the dinitrophenyl group (DNP), that can be detected and quantified; a two-step incubation with anti-dinitrophenyl-KLH antibodies (rabbit IgG fraction) and secondary goat anti-rabbit IgG, HRP conjugate, was followed by PCO quantification with western blotting. The signal developed with enhanced chemoluminescence.

Oxidized sera from healthy, fasted, and privately-owned dogs were analysed in order to evaluate the linearity and the coefficient of variation (CV) of this method and to build a calibration curve.

Then, PCOs were evaluated in serum of 13 privately-owned dogs divided in 3 groups based on clinical and laboratory findings: 4 healthy dogs (A), 5 septic dogs with pyelonephritis (B), and 4 septic dogs with pyometra (C). The differences between the three groups were statistically evaluated and the possible practical utility of the method was estimated on the basis of the CV previously obtained.

The western blot technique showed an evident band of apparent MW of 69 kDa, consistent with carbonylated dog serum albumin. The obtained CV varied between 24% and 36%.

Results of sick dogs (groups B + C = 2.063 ± 0.719 mmol/L, min-max: 0.710–2.920) were significantly higher ($p < 0.05$) than those of healthy dogs (0.975 ± 0.440 mmol/L, min-max: 0.320–1.260) while no significant differences were found between dogs of group B (2.052 ± 0.614 mmol/L, min-max: 1.320–2.920) and C (2.078 ± 0.935 mmol/L, min-max: 0.710–2.970).

The western blotting method employed in this study may identify PCOs in canine serum but has a high intrinsic variability in terms of CVs. Hence, despite statistically significant differences found between healthy and septic dogs this high variability suggests that misinterpretation of

values close to the maximum values of the healthy dogs may occur in routine practice. Further studies on a higher caseload are needed to confirm PCOs as a reliable marker of sepsis.

DISCLOSURES

No disclosures to report.

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