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Investigation of the association between serum protein concentrations and concurrent chronic kidney disease in hyperthyroid cats

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ABSTRACT

Our objective was to identify if changes in serum protein concentrations occur in hyperthyroidism and to assess their association with the development of azotaemia following treatment.

Initially non-azotaemic hyperthyroid cats and healthy older cats were included. Serum concentrations of protein fractions were determined by agarose gel electrophoresis and compared between; hyperthyroid and control cats, initially non-azotaemic hyperthyroid cats which developed azotaemia in a 4 month follow up period (masked-azotaemic) and those which remained non-azotaemic, and hyperthyroid cats before and at the time of restoration of euthyroidism. Data are presented as median [25th, 75th percentiles].

Hyperthyroid cats ($n = 56$) had higher serum α_2 globulin concentrations (12.5 [10.9, 13.1] g/L vs. 9.8 [3.0, 11.4] g/L; $P < 0.001$) and lower serum γ globulin concentrations (11.4 [9.1, 13.3] g/L vs. 14.0 [12.4, 16.8] g/L; $P = 0.001$) than control cats ($n = 26$). Following treatment, serum total globulin concentration increased (from 38.6 [35.4, 42.8] g/L to 42.3 [39.0, 45.7] g/L; $P < 0.001$), serum α_2 globulin concentration decreased (from 12.5 [10.9, 13.9] g/L to 11.5 [10.1, 12.6] g/L; $P < 0.001$) and serum γ globulin concentration increased (from 11.4 [9.0, 13.3] g/L to 14.0 [12.4, 16.8] g/L; $P < 0.001$). Serum concentrations of total globulin or globulin fractions were not significantly different between masked-azotaemic and non azotaemic groups.

In conclusion, hyperthyroidism is associated with altered serum concentrations of the α_2 and γ globulin fractions, however these changes were not associated with the development of azotaemic chronic kidney disease following treatment.

1. Introduction

Chronic kidney disease (CKD) is a common co-morbidity in hyperthyroidism, however hyperthyroidism will complicate the diagnosis of CKD due to the consequent increase in glomerular filtration rate (GFR) (Adams et al., 1997) and decrease in body muscle mass (Shiel and Mooney, 2007). Hence, hyperthyroidism decreases serum creatinine concentrations, which 'masks' concurrent azotaemic CKD in these patients. Some hyperthyroid cats with concurrent CKD only develop azotaemia after treatment, once GFR and body muscle mass have returned to normal (for the cat). Identification of hyperthyroid cats with concurrent, but masked, CKD prior to treatment of hyperthyroidism would be beneficial because it would allow the institution of appropriate treatment strategies for CKD at an earlier time point.

Several studies have attempted to identify biomarkers of CKD in hyperthyroidism (Lapointe et al., 2008; Riensche et al., 2008; van Hoek et al., 2009a; van Hoek et al., 2009b; Williams et al., 2010; Williams et al., 2016), however, no single reliable test has been reported. In one previous study of 300 hyperthyroid cats, only (higher) plasma creatinine concentrations and (lower) plasma globulin concentrations were independent predictors of the development of azotaemia within 240 days of diagnosis of hyperthyroidism (Williams et al., 2010). These data suggest that plasma total globulin concentration, or the plasma concentration of a component of the globulin fraction, could be a marker of concurrent, but masked, azotaemic CKD in hyperthyroidism.

Agarose gel electrophoresis (AGE) is a technique which separates serum proteins into 4–6 major groups of one or more bands, based on the ability of the proteins to migrate through the agarose gel when an electrical field is applied. The distance of migration is dependent on the electrical charge, mass and shape of the protein. By utilising this technique, the serum concentrations of individual globulin fractions can be elucidated, which could help to determine which serum protein is associated with the presence of concurrent, but masked, azotaemic CKD in hyperthyroid cats.

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The first aim of this study was to establish if the results of our previous study were repeatable in an independent group of cats, and if so, to identify if any individual globulin fraction associated with the presence of concurrent, but masked, azotaemic CKD in hyperthyroid cats, since this could provide a novel biomarker for CKD in these cases. The second aim was to investigate if changes in serum protein concentrations occur in hyperthyroid cats (by comparison of serum concentrations of individual globulin fractions between hyperthyroid cats and healthy older cats, and before and after treatment of hyperthyroidism), since changes in serum concentrations of some proteins have been reported in animal models of hyperthyroidism previously (Farthing et al., 1960; Griffin and Miller, 1973).

2. Materials and methods

Hyperthyroid cats seen between March 2010 and June 2013 at two first opinion practices in London were included in the study. All were non-azotaemic (plasma creatinine concentration < 177 μmol/L) at the time of presentation. Cats with other known significant systemic disorders except hypertension (based on clinical examination, serum biochemistry and urinalysis), including significant systemic inflammatory disease (for example inflammatory bowel disease) were excluded. Included cats were not, however, screened for FeLV or FIV infection. Following informed consent by the owner, blood and urine samples were taken from the cats as part of a geriatric screening programme (Royal Veterinary College Ethics and Welfare Committee approval number and date; URN 20131258, 2nd December 2013). Blood was obtained by jugular venepuncture, placed in heparinised or non-anticoagulated tubes, and stored at 4 °C until sample processing (within 6 h). Urine samples were taken by cystocentesis. Biochemical analysis was performed by IDEXX Laboratories (Wetherby, UK) using heparinised plasma. Residual serum was stored at − 80 °C and subsequently submitted to Central Diagnostic Services (Cambridge, UK) for batch measurement of serum TT4 by enzyme immunoassay (Williams and Archer, 2016), serum total protein by biuret reaction, and AGE analysis. Urine specific gravity (USG) determination (by refractometry), urine dipstick, and urine sediment analysis was also performed in-house. The presence of bacteriuria or pyuria (> 5 white blood cells per 1000 × field) was an exclusion criterion for the study.

All hyperthyroid cats were treated with the aim of attaining a plasma TT4 < 40 nmol/L. Cats were initially treated with anti-thyroid medication (usually methimazole), with some animals also undergoing thyroidectomy following initial stabilisation. Cats were re-examined and plasma TT4 repeated every 4 weeks until restoration of euthyroidism was documented (defined as a plasma TT4 < 40 nmol/L). Once euthyroidism had been achieved, the cats were monitored for a 4 month period, with further blood and urine samples taken at the end of this monitoring period. Cats were defined as masked-azotaemic if they had a plasma creatinine concentration > 177 μmol/L with a concurrent USG < 1.035 at the end of this four month monitoring period. Cats with persistent azotaemia without obvious evidence of dehydration (pre-renal azotaemia) were also classified as masked-azotaemic. All other hyperthyroid cats were classified as non-azotaemic. Samples taken at the time of diagnosis of hyperthyroidism and at the time of first documentation of euthyroidism were used for AGE analysis.

Healthy older cats that presented to three first opinion practices in the South-East of England between March 2013 and April 2015 were also included. These cats all had no clinical history of disease (except the presence of tartar with or without associated gingivitis, or degenerative joint disease), no significant haematological or biochemical abnormalities, were on no long term medications (except anti-parasitic medications) and were at least 8 years old. Included cats were not, however, screened for FeLV or FIV. Blood and urine samples were obtained by practitioners following informed consent as part of a geriatric screening programme (Ethics and Welfare Committee of the Department of Veterinary Medicine at the University of Cambridge approval number and date; CR56, 4th August 2012) and submitted to Central Diagnostic Services within 3 days of sampling. Full haematology, serum biochemistry (including TT4 by enzyme immunoassay) and urinalysis (including urine protein:creatinine ratio [UPC]) was performed, and any animals with azotaemia (defined as a serum creatinine concentration > 153 μmol/L), proteinuria (defined as UPC > 0.4), borderline or overt hyperthyroidism (defined as a serum TT4 > 40 nmol/L), bacteriuria, pyuria (defined as > 5 white blood cells per 1000 × field) or other significant haematological or biochemical abnormalities were excluded from the cohort. Residual serum was stored at −80C until batch AGE analysis.

2.1. Agarose gel electrophoresis

AGE was performed with agarose gels (HydraGel 7 β1β2, Sebia), according to the manufacturer's instructions. A normal cat control sample was run on each gel and paired samples from each individual hyperthyroid cat (hyperthyroid and euthyroid time points) were run on the same gel. The electrophoretograms were read using a densitometer and Phoresis software (Sebia). Densitometric readings from each lane of the gel were displayed as a curve, and the various bands (represented by peaks on the curve) were resolved manually by one investigator (TW) with the percentage of albumin and the individual globulin fractions calculated based on the area under each part of the curve divided by the total area under the curve. Absolute concentrations of the proteins were determined by multiplication of the percentage of that protein by the serum total protein concentration (determined by biuret reaction).

2.2. Statistical analysis

Statistical analysis was performed with SPSS v21.0 (IBM). The Mann–Whitney *U* test was used to compare serum concentrations of total protein, albumin, total globulin and the individual globulin fractions between; hyperthyroid and healthy older cats, and masked-azotaemic and non-azotaemic hyperthyroid cats. The Wilcoxon signed rank test was used to compare serum concentrations of proteins in hyperthyroid cats before treatment and at the time of establishment of euthyroidism. Correlations between baseline serum concentrations of total protein, albumin, globulin and the individual globulin fractions and age, serum concentrations of creatinine and TT4, were assessed by Spearman's correlation co-efficient. Correlations were classified as weak if r_s < 0.5, moderate if r_s was 0.5–0.7, and strong if r_s > 0.7. The proportion of cats in the hyperthyroid and healthy older cat groups which had serum concentrations of the various protein fractions outside of the reference intervals reported in a previous study (Taylor et al., 2010) were compared using the Fisher's Exact test. Data are presented as median [25th, 75th percentiles] and statistical significance was defined as $P < 0.05$.

3. Results

Fifty six hyperthyroid cats and 26 healthy older cats were included in the study. In the hyperthyroid group, 21 cats developed azotaemia during the follow up period (masked-azotaemic group) and 35 cats were classified as non-azotaemic. Selected clinicopathological data for the hyperthyroid and healthy older cat groups are shown in Table 1.

The comparison of serum concentrations of total protein, albumin, total globulins and globulin fractions between the masked-azotaemic and non-azotaemic hyperthyroid groups are shown in Table 2. Serum total protein concentration was higher in masked-azotaemic hyperthyroid cats compared to those which remained non-azotaemic ($P = 0.049$), however no significant differences in the serum concentrations of α_1, α_2, β, γ or total globulin were evident between the masked-azotaemic and non azotaemic groups.