Investigating expression of proteins associated with glomerular endothelial cell fenestrations in diabetic cats and dogs.

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Introduction

Diabetic nephropathy (DN) is a progressive and irreversible process occurring in both type 1 and 2 diabetes. It is characterised by glomerular basement membrane thickening, mesangial expansion, podocyte foot process effacement and loss of glomerular endothelial cell (GEnC) fenestrations, resulting in proteinuria and reduced glomerular filtration rate (GFR)[1]. Diabetic nephropathy is not generally recognised in cats and dogs, however, proteinuria has been described[2] along with non-specific histopathological lesions in cats[3]. Reduced GFR has not been documented in diabetic cats and dogs[4][5]. Ultrastructural studies using electron microscopy required to visualise the characteristic changes associated with DN, have not been performed in cats and dogs. Research in human patients and rodent models suggest a role of certain proteins in GEnC fenestration regulation, and therefore may be involved in the pathogenesis of DN. Four such proteins are vascular endothelial growth factor [VEGF-A], moesin and Eps homology domain 3&4 [EHD 3&4]. Glomerular VEGF-A expression is upregulated in diabetic mice and humans in early DN before falling dramatically in late stage disease[6] and VEGF-A positively regulates GEnC fenestration formation[7]. EHD3&4 knockout mouse models demonstrate GEnC fenestration loss[8]. Moesin is involved in fenestration formation in other endothelial cell types[9]. Limited or no data is available regarding expression of these proteins in dog and cat kidney tissue.

Objectives

To evaluate expression of proteins that play a role in glomerular endothelial cell fenestration formation (vascular endothelial growth factor-A [VEGF-A], moesin and Eps homology domain 3&4 [EHD 3&4] in glomeruli from diabetic compared to control cats and dogs.

Method

Formalin fixed, paraffin embedded kidney sections were obtained from the University of Bristol pathology archives from diabetic dogs (DD, n=11), diabetic cats (DC, n=7), dogs with glomerulonephritis (GD, n=4), cats with glomerulonephritis (GC, n=3) and cats with normal kidney structure and function (NC, n=5). A standard immunohistochemistry protocol was followed trialling different primary antibodies and optimised for use in kidney sections from cats and dogs. Glomerular protein expression was evaluated manually by scoring staining intensity under light microscopy, using a 0-3 scale by two independent blinded scorers. Objective quantification was performed using image-processing software (FIJI). Staining intensity was compared between groups using the T-test. Significance was set at $P<0.05$.

Results

An immunohistochemistry protocol for VEGF-A, moesin and EHD 3&4 staining was successfully optimised for kidney sections from dogs and cats. Glomerular expression of VEGF-A was significantly increased in DC compared with NC ($P=0.016$, see Fig 1) and GC ($P=0.028$) based on manual scoring (see Fig 2). Glomerular moesin expression was also significantly increased in GC compared to NC ($P=0.038$) based on objective quantification. There was no significant difference in glomerular EHD 3&4 expression in cats (see Fig 2). There was no significant difference in VEGF-A, EHD 3&4 or moesin expression in glomeruli from DD compared to GC.

Fig 1. VEGF-A staining in normal and diabetic cat glomeruli.

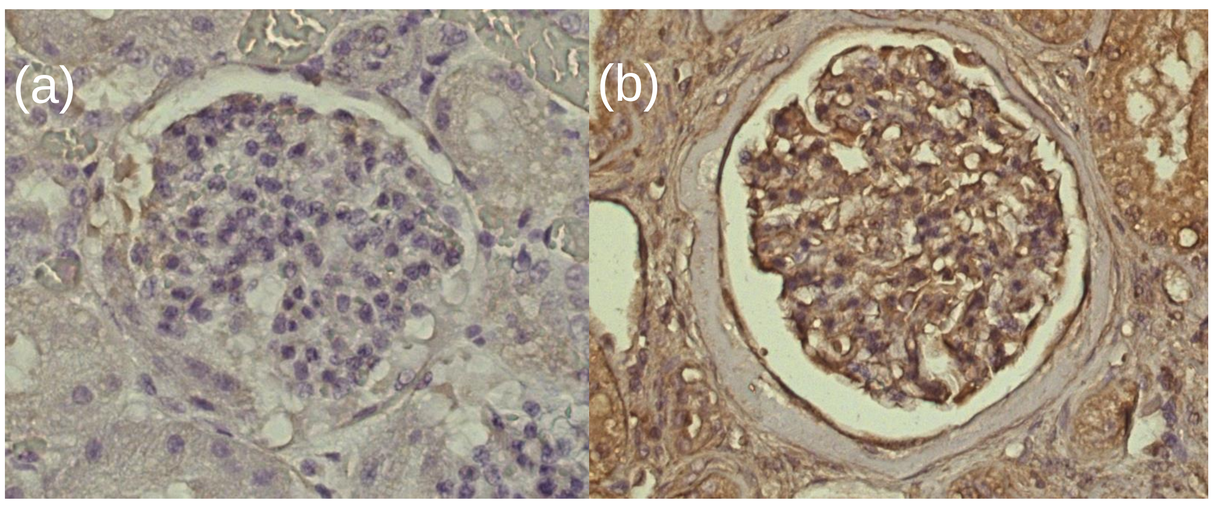

Figure 1. VEGF-A staining in the glomerulus of a normal cat (a) and diabetic cat (b). Diabetic cats had significantly higher staining intensity compared to normal cats.

Fig 2. Protein expression in cat kidney tissue using manual scoring for staining intensity.

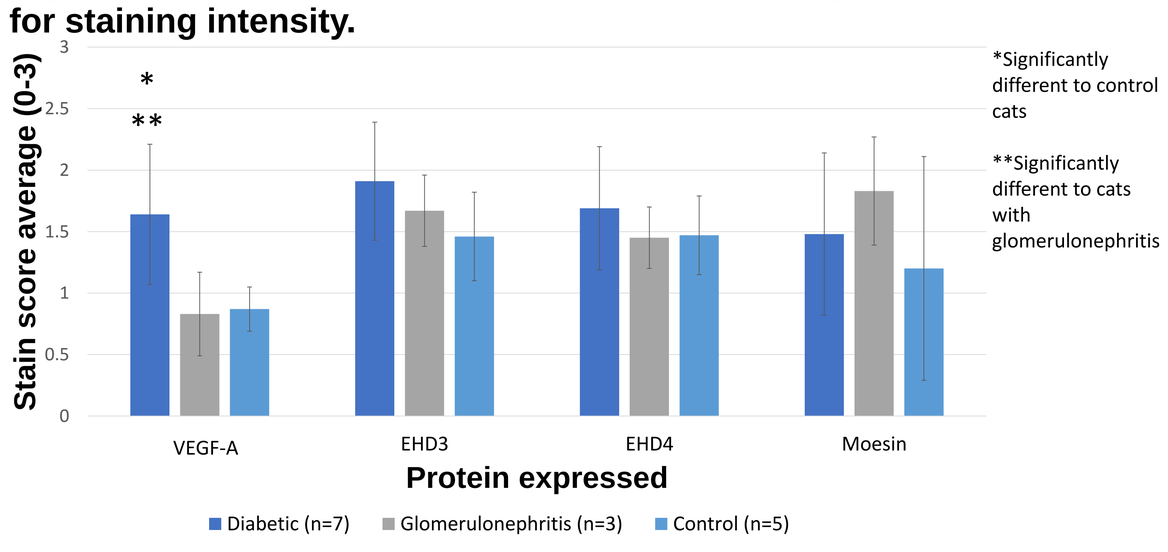

Figure 2. VEGF-A staining was significantly increased in glomeruli from diabetic cats compared with cats with glomerulonephritis and normal cats.

Conclusion

In diabetic cats, there was an increase in glomerular VEGF-A expression compared to normal cats and cats with glomerulonephritis that could suggest early DN. The significance of increased moesin expression in diabetic cats on objective staining evaluation is unclear. The study is limited by the small number of samples studied and this may explain lack of significant results. Furthermore, an optimal control group against which to compare diabetic dogs was not studied. Further studies using electron microscopy are required to evaluate whether GEnC fenestration loss occurs in diabetic cats and dogs. Knowledge of GEnC fenestration loss in diabetic cats and dogs and proteins associated with this may provide better understanding of whether DN develops in these species and offer potential therapeutic targets.

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