Periodontal disease is associated with cognitive dysfunction in aging dogs: A blinded prospective comparison of visual periodontal and cognitive questionnaire scores

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Abstract

Background: Periodontal disease has been linked to the development of Alzheimer's disease in people. It is theorized that the chronic inflammatory condition characteristic of oral dysbiosis in patients with periodontal disease leads to disruption of the blood–brain barrier, cytotoxin- and pathogen-induced brain damage, and accumulation of neurotoxic β-amyloid. In this inflammatory theory of Alzheimer's disease, β-amyloid—a known antimicrobial protein—accumulates in response to oral pathogens. Canine cognitive dysfunction (CCD) is considered a naturally occurring animal model of human Alzheimer's disease. Like humans, periodontal disease is quite common in dogs; however, a link between periodontal disease and cognitive dysfunction has not been identified in this species.

Aim: The purpose of this prospective investigation was to compare visual periodontal scores (from digital oral photographs) with numerical (0–54) cognitive assessment questionnaire forms in aging dogs with and without a clinical diagnosis of CCD.

Methods: A visual analogue scale (0–4) was used to score the severity of periodontal disease in 21 aging dogs: 11 dogs with a clinical diagnosis of presumptive CCD and 10 dogs without a clinical history of cognitive decline. Individuals scoring the dental photographs were blinded to all case information, including cognitive assessment scores. Cognitive assessment scores were compared with periodontal disease scores for all dogs.

Results: There was a significant ($p < 0.05$) association between periodontal and cognitive scores, with higher cognitive impairment scores being more likely in dogs with more severe periodontal disease and vice versa. No associations were identified between age and either periodontal disease or cognitive impairment.

Conclusion: Although a cause-and-effect relationship between periodontal disease and cognitive impairment cannot be ascertained from this preliminary study, we established a link between these two disorders that warrants further investigation using more stringent criteria for evaluating both periodontal disease and cognitive dysfunction.

Keywords: Dog, Periodontal, Cognitive, Amyloid, Alzheimer's.

Introduction

Periodontal disease, a common chronic inflammatory disease of dogs and humans, increases in incidence and severity with advancing age (Singhrao *et al*., 2015; Wallis *et al*., 2019). Many factors contribute to periodontal disease, most notably colonization of the mouth with pathogenic Gram-negative bacteria. The altered oral microbiome (dysbiosis) in periodontal disease leads to tissue destruction and disruption of the normal gingival barrier, ultimately allowing bacteria and their toxins entry into the systemic circulation. Various researches have implicated systemic inflammation and bacteremia stemming from periodontal disease in several disorders (Kouki *et al*., 2013; Polkowska *et al*., 2014; Bui *et al*., 2019; Wallis *et al*., 2019), and with heart, liver, and kidney damage (Kouki *et al*., 2013; Bui *et al*., 2019; Wallis *et al*., 2019). Furthermore, periodontal disease has been hypothesized to be linked

to the development of Alzheimer's disease in people (Singhrao *et al*., 2015).

Comprehensive evaluation of periodontal disease in dogs requires anesthesia, probing of gingiva and dental radiographs (Kouki *et al*., 2013; Polkowska *et al*., 2014; Bauer *et al*., 2018; Wallis *et al*., 2019). However, one study provided evidence that a visual examination of the teeth correlated moderately with more comprehensive evaluations and could allow stratification of dogs into those requiring immediate attention and those with milder periodontal disease (Bauer *et al*., 2018).

There is evidence that pathogenic oral bacteria in people with chronic periodontal disease can eventually cause disruption of the blood–brain barrier and incite inflammation of the central nervous system. This inflammation, and the subsequent response to it, has been implicated as contributory and/or causal factors in the pathogenesis of Alzheimer's disease. Deposition

of neurotoxic β-amyloid protein in brain parenchyma might represent an immune response to microbial pathogens and/or their constituents rather than a primary disease process (Gaur and Agnihotri, 2015; Singhrao *et al*., 2015; Ide *et al*., 2016; Aragon *et al*., 2018; Maurer *et al*., 2018; Bui *et al*., 2019; Choi *et al*., 2019; Dioguardi *et al*., 2020).

Canine cognitive dysfunction (CCD) is the canine analog of human Alzheimer's disease having remarkably similar clinical and pathological features (Landsberg *et al*., 2012; Chapagain *et al*., 2018; Dewey *et al*., 2019). As with periodontal disease, the likelihood of a dog developing cognitive dysfunction increases with age. To the authors' knowledge, an association between periodontal disease and CCD has not been identified in aging dogs.

We hypothesized that dogs diagnosed with cognitive dysfunction would have significantly worse periodontal disease, based on visual evaluation of dentition from digital photographs, than similarly aged dogs without obvious cognitive impairment, which we based on numerical cognitive dysfunction owner questionnaires.

Materials and Methods

We conducted a prospective case-control study. We recruited aging dogs (>9 years) with a clinical diagnosis of cognitive dysfunction. We based the diagnosis of cognitive dysfunction on historical complaints and neurologic examination findings. A board-certified neurologist (CD) conducted all neurologic examinations. We also recruited similarly aged dogs presenting for disorders not associated with cognitive dysfunction (e.g., joint disease, disk extrusion/protrusion) to serve as controls. Clients consented to allowing their pets' dental photographs and cognitive scores to be used for the study. All dogs were fully awake for dental photography (no chemical restraint was used). Each client filled out a cognitive dysfunction questionnaire used in a previously published prospective research investigation (Table 1) (Pan *et al*., 2018). Briefly, this short questionnaire examines six variables: Disorientation, Interactions-social, Sleep/wake cycles, House soiling/learning/memory, Activity, and Anxiety (DISHAA) to produce a cumulative score that determines the degree of cognitive impairment or dysfunction (Table 1). We then obtained digital photographs of the dentition in each dog, consisting of frontal, right lateral, and left lateral views. One investigator (CD) also carried out a visual oral examination and photographed the dentition at the same time. We excluded dogs that received regular dental prophylactic care (at least yearly). A cohort of 11 general practice veterinarians and one co-author (MR), unassociated with the cases, evaluated the sets of dental photographs without any additional information. Each observer scored the photographic periodontal dental disease with values between 0 and 4; this scale was based on a previously published visual scoring system (Table 2) (Bauer *et al*., 2018).

Statistical analyses

First, we examined the agreement between observers in rating the periodontal disease using Fleiss' kappa (Real Statistics Using Excel Add-in for Microsoft Excel; https://www.real-statistics.com/). We did this in two ways – by examining the actual scores provided by each observer, and then by grouping periodontal disease into "less than moderate" (periodontal disease scores of 2 or less) versus "moderate to severe" (periodontal disease scores greater than 2).

We then examined the associations between several variables by Rank Sum tests (Hamm*er et al*., 2001). We used the modal periodontal disease score for these comparisons when comparing periodontal disease scores – analysis of the scores showed that the modal values and the median values in every case but one were identical. First, we examined the differences in DISHAA scores between dogs with "less than moderate" and "moderate to severe" periodontal disease. Next, we compared the periodontal disease scores between dogs diagnosed with CCD and dogs without the diagnosis. Third, we compared DISHAA scores between dogs diagnosed with CCD and dogs without the diagnosis. Finally, we examined the association between DISHAA scores and periodontal disease scores using Kendall's Tau.

We created the dot plots using a free online software (Potsma and Goedhart, 2019).

Results

Our study included 21 aging dogs: 10 control dogs and 11 dogs with a presumptive diagnosis of CCD. The two groups did not differ in age ($p = 0.08$) or body weight ($p = 0.31$). Breeds represented in the control group included mixed breed (4) and one each of Brittany Spaniel, Standard Poodle, Rat Terrier, Cavalier King Charles Spaniel, Golden Retriever, and Labrador Retriever. There were five female spayed and five male castrated control dogs. Breeds represented in the group of dogs with presumptive CCD included mixed breed (4), Shih Tzu (2), Beagle (2), and one each of Toy Poodle, Lhasa Apso, and Coonhound. There were three female spayed dogs and eight castrated male dogs with CCD.

Observers ranking periodontal disease showed moderate agreement for the 21 cases (kappa = 0.39; 95% CI = 0.36–0.42). When cases were dichotomized into "less than moderate" and "moderate to severe", observers showed better agreement (kappa = 0.56, 95% CI = 0.51–0.61). The cases showing most disagreement were those that observers scored as either "mild" or "moderate" – i.e., the marginal cases. Cases considered "severe" showed substantial agreement (kappa = 0.7).

Dogs with "less than moderate" periodontal disease had lower DISHAA scores than dogs with "moderate to severe" periodontal disease (Fig. 1, $p = 0.027$). Dogs with a diagnosis of CCD had worse periodontal disease (Fig. 2, $p = 0.001$) and higher DISHAA scores (Fig. 3, $p = 0.012$) than dogs without CCD. DISHAA scores

Table 1. DISHAA (Disorientation; Interactions-social; Sleep/wake cycle; House soiling, learning and memory; Activity; and Anxiety) scoring system used to assess cognitive ability in dogs.

Disorientation
Gets stuck, difficulty getting around objects, goes to hinge side of door
Stares blankly at walls, floor, or into space
Does not recognize familiar people/familiar pets
Gets lost in home or yard
Less reactive to visual (sights) or auditory (sounds) stimuli
Interactions (social)
More irritable/fearful/aggressive with visitors, family, or other animals
Decreased interest in approaching, greeting, or affection/petting
Sleep/wake cycles
Pacing/restless/sleeps less/waking at night
Vocalization at night
House soiling, learning and memory
Less able to learn new tasks or respond to previously learned commands/name/work
Indoor soiling of urine or stool/decreased signaling to go out
Difficulty getting dog's attention/increased distraction/decreased focus
Activity
Decrease in exploration or play with toys, family members, other pets
Increased activity including aimless pacing or wandering
Repetitive behaviors, e.g., circling, chewing, licking, stargazing
Anxiety
Increased anxiety when separated from owners
More reactive/fearful to visual (sights) or auditory (sounds) stimuli
Increased fear of places/locations (e.g., new environments/going outdoors)

Each subcategory (bullet point) is scored as 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Total scores are assessed as 4–15 = mild impairment, 16–33 = moderate impairment, and >33 = severe impairment (Landsberg *et al.*, 2012).

Table 2. Scoring system for periodontal disease based on dental photographs.

Value	Definition
0	No plaque or calculus. Gums are normal
1	Mild amount of plaque. Gums can be mildly red.
2	Moderate amount of plaque. There is likely redness and swelling of the gums.
3	Tartar is present. Gums are inflamed, receding or hyperplastic.
4	Heavy tartar is present. Gums are severely inflamed. There may be evidence of infection or tooth loss.

showed a modest correlation with periodontal scores (Fig. 4, tau = 0.42, *p* = 0.008).

Discussion

Our study suggests that older dogs with CCD tend to exhibit worse periodontal disease, as assessed by examination of dental photographs, than similarly aged dogs without cognitive dysfunction. Scores of cognitive dysfunction (DISHAA) tended to correlate positively, but modestly, with periodontal disease. Unsurprisingly, DISHAA scores correlated, albeit imperfectly, with a diagnosis of CCD. It is important to interpret our findings in light of the limitations of the study design and not necessarily indicative of a cause-and-effect relationship. Nonetheless, the establishment of this association between the two disorders warrants further investigation of the existence and potential nature of such a relationship.

The relationship between periodontal disease and Alzheimer's disease in humans is well established, with
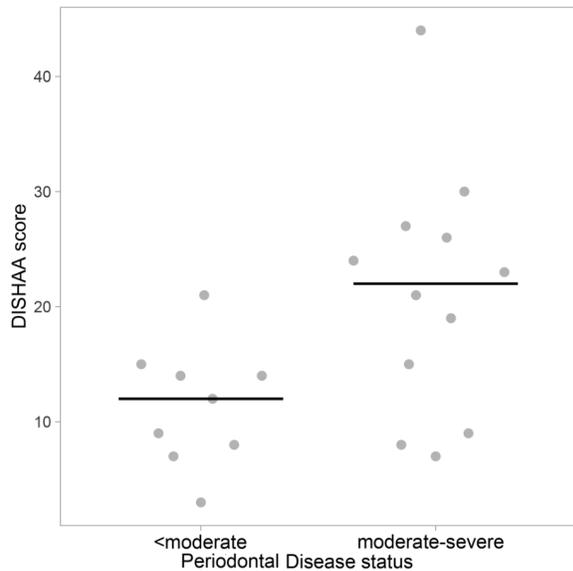
Fig. 1. Scatterplots with medians (bars) comparing DISHAA scores in dogs with less than moderate and moderate to severe periodontal disease. DISHAA scores (*y* axis) are plotted against periodontal disease scores (*x* axis). DISHAA = Disorientation; Interactions-social; Sleep/wake cycle; House soiling, learning and memory; Activity; and Anxiety.
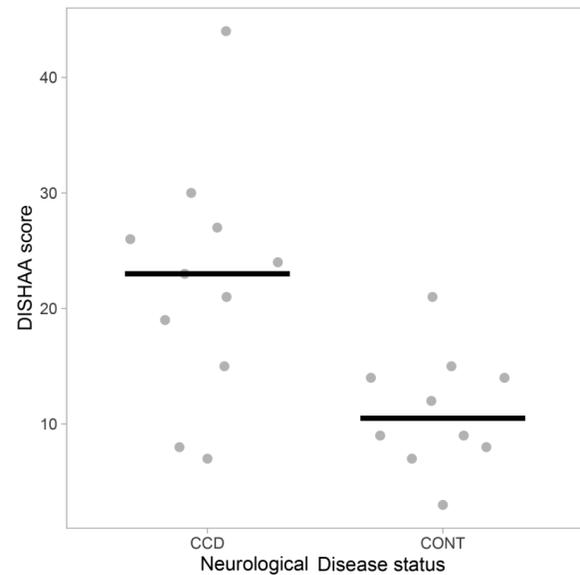
Fig. 3. Scatterplots with medians (bars) comparing DISHAA scores in CCD and control dogs. DISHAA scores (*y* axis) are plotted against CCD dogs and control dogs (*x* axis). CCD = canine cognitive dysfunction; DISHAA = Disorientation; Interactions-social; Sleep/wake cycle; House soiling, learning and memory; Activity; and Anxiety.
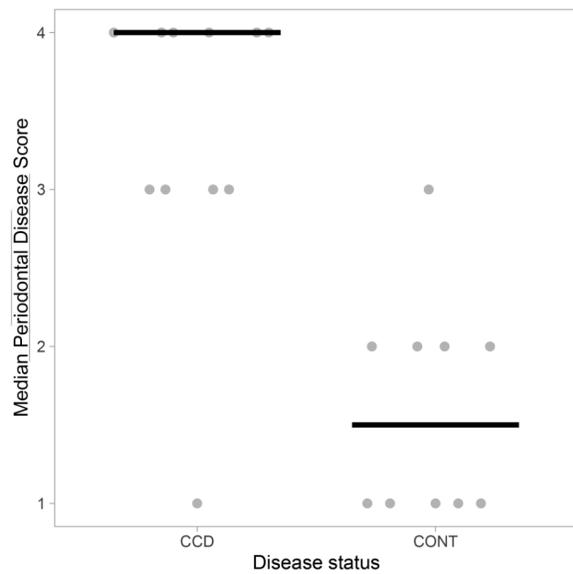
Fig. 2. Scatterplots with medians (bars) comparing periodontal disease scores in CCD dogs versus control dogs. Median periodontal disease score (*y* axis) is plotted against CCD or control status (*x* axis). CCD = canine cognitive dysfunction.

periodontal disease being recognized as a risk factor for the development of Alzheimer's disease. Whether periodontal disease is causal versus contributory in

human Alzheimer's disease remains unknown, but evidence suggests that gastrointestinal dysbiosis might play a role in the pathogenesis of Alzheimer's disease (Cerajewska *et al*., 2015; Gaur and Agnihotri, 2015; Singhrao *et al*., 2015; Ide *et al*., 2016; Teixeira *et al*., 2017; Aragon *et al*., 2018; Maurer *et al*., 2018; Bui *et al*., 2019; Choi *et al*., 2019; Kowalski and Mulak, 2019; Liu *et al*., 2019; Sochocka *et al*., 2019; Dioguardi *et al*., 2020; Sun *et al*., 2020). The gut–brain axis describes an anatomic and physiologic communication pathway between the gastrointestinal system and the central nervous system that is mediated via the systemic circulation, peripheral nerve pathways, and the immune system. The oral cavity is one contributor to the gut–brain axis (Ambrosini *et al*., 2019; Kowalski and Mulak, 2019; Liu *et al*., 2019; Sochocka *et al*., 2019; Sun *et al*., 2020). Oral pathogens commonly implicated in severe human periodontal disease include *Porphyromonas gingivalis*, *Treponema denticola*, and *Tanerella forsythia*, among others (Cerajewska*, et al*., 2015; Gaur and Agnihotri*,* 2015; Singhrao *et al*., 2015; Harding *et al*., 2017; Teixeira *et al*., 2017; Bui *et al*., 2019; Dioguardi *et al*., 2020). Dogs with periodontal disease harbor similar oral bacteria (Polkowska *et al*., 2014; Golynska *et al*., 2017; Nomura *et al*., 2020). Some of these pathogens have been directly or indirectly linked to the development of Alzheimer's disease (Cerajewska *et al*., 2015; Gaur and Agnihotri, 2015; Singhrao *et al*., 2015; Harding *et al*., 2017; Teixeira *et al*., 2017; Bui *et al*., 2019; Dioguardi *et al*., 2020). It is theorized that the
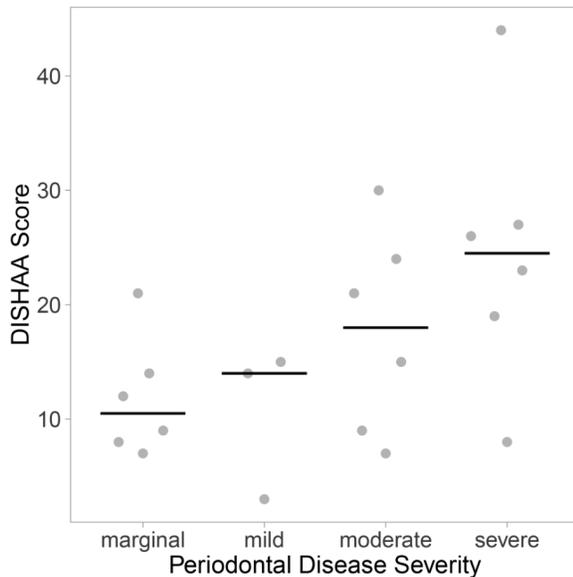
Fig. 4. Scatterplots with medians (bars) comparing DISHAA scores in dogs with marginal, mild, moderate, and severe periodontal disease, based on the median periodontal disease scores ranging from 1 and 4. DISHAA scores (*y* axis) plotted against periodontal disease severity (*x* axis). DISHAA = Disorientation; Interactions-social; Sleep/wake cycle; House soiling, learning and memory; Activity; and Anxiety.

pathogens themselves, some of their constituents (e.g., lipopolysaccharide) and/or the inflammation induced by the presence of these factors induces and promulgates central nervous system inflammation. The subsequent inflammation leads to direct parenchymal damage as well as promotion of neurotoxic β-amyloid deposition (Poole *et al*., 2013; Noble *et al*., 2014; Cerajewska *et al*., 2015; Gaur and Agnihotri, 2015; Singhrao *et al*., 2015; Ide *et al*., 2016; Teixeira *et al*., 2017; Maurer *et al*., 2018; Bui *et al*., 2019; Kowalski and Mulak, 2019; Sochocka *et al*., 2019; Dioguardi *et al*., 2020; Sun *et al*., 2020).

We examined the association between periodontal disease and cognitive dysfunction scores for all dogs and compared results between dogs diagnosed with cognitive dysfunction and those considered cognitively normal by their owners and showing no strong evidence of cognitive dysfunction on a neurological evaluation (control dogs). Based on the questionnaire scores, only one of the control dogs achieved a score indicating no cognitive impairment, and one scored as moderately cognitively impaired; the remainder scored as mildly cognitively impaired, despite owner assessments that their dogs did not exhibit cognitive dysfunction. This phenomenon likely reflects a degree of imprecision of the DISHAA questionnaire. Owners might attribute some signs of mild cognitive impairment as aspects of normal aging or certain findings on the DISHAA questionnaire might actually reflect normal aging –

without additional examination of the questionnaire, we cannot determine why most of our control dogs had scores consistent with mild cognitive dysfunction. On the other hand, none of the dogs with cognitive dysfunction scored as not cognitively impaired and only three scored as mildly impaired on the client questionnaire; the remainder scored as moderately or severely impaired.

There are several limitations to this small, prospective, case-control study, most of which relate to the methods of quantifying both periodontal disease and severity of cognitive dysfunction. The clinical standard of scoring the severity of canine periodontal disease involves full dental radiographs and a full oral examination under general anesthesia that incorporates gingival probing (to assess depths of gingival pockets) (Kouki *et al*., 2013; Polkowska *et al*., 2014; Bauer *et al*., 2018; Wallis *et al*., 2019). A visual assessment scale as used in our study is a crude approximation of this standard. Nonetheless, one study demonstrated that visual assessment scores agree – albeit moderately – with more stringent periodontal disease severity assessments that include dental radiographs and full oral examination with patients under anesthesia (Bauer *et al*., 2018). Also, in that study, it was found that disagreement was rarely extended by more than one level of disease and did not show bias with the visual method (i.e., the visual examination did not consistently over- or underestimate the severity of the periodontal disease). The authors commented that a visual examination might overcome some of the requirements of comprehensive evaluations when screening dogs for periodontal disease. We found similar degrees of agreement between observers in evaluating periodontal disease from photographs as did the previous study, given that Fleiss' Kappa produces more conservative estimates of agreement than a simple Cohen's Kappa. Furthermore, regardless of the method of periodontal evaluation, our study identified a difference in periodontal disease between dogs presenting with or without cognitive dysfunction. We used a more crude measure of periodontal disease, ultimately classifying dogs as "less than moderate" or "at least moderate" for purposes of analysis. Importantly, none of the dogs in our study presented primarily for evaluation of their dentition – therefore, our study was not biased by pre-existing knowledge or suspicion of dental disease. Whether similar results to ours would be obtained with more comprehensive periodontal evaluation remains undetermined.

Similarly, although prior investigators have successfully used the same questionnaire we used for investigating cognitive dysfunction (Pan *et al*., 2018), the questionnaire is subject to owner-dependent variability. More stringent and non-biased methods of cognitive status would entail objective behavioral testing of cognitive ability such as delayed nonmatching to positioning memory tasks, attention tasks, food searching tasks, and problem-solving tasks (Landsberg

et al., 2012; Gonzalez-Martinez *et al.*, 2013; Chapagain *et al.*, 2018).

Our study is the first to identify an association between visually categorized periodontal disease and CCD. Future investigation into the link between periodontal disease and cognitive dysfunction in dogs should include more detailed and objective dental evaluations and cognitive testing. Because such investigations can be both time-consuming and expensive, our initial study results justify moving forward with more extensive research into this apparent association.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

Drs. Dewey and Rishniw were involved in the study's inception and design. Dr. Dewey procured all digital photographs and questionnaire results. Dr. Rishniw carried out statistical analyses. Both Dr. Dewey and Dr. Rishniw were involved in manuscript preparation.

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