Salivary alpha-amylase as a stress biomarker in diseased dogs

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ABSTRACT

Salivary alpha-amylase (sAA) is a stress biomarker in human diseases, but there are no reports of sAA measurements in diseased dogs. This study measured the sAA and serum alpha-amylase (AA) levels in 16 healthy dogs and 31 diseased dogs using a kinetic enzyme assay to assess the stress status. The sAA and serum AA levels were significantly higher in the diseased dogs than in healthy dogs (p < 0.05), but there was no correlation between the 2 groups (r = 0.251, p = 0.089). This suggests that sAA can be useful as a stress biomarker in diseased dogs.

Keywords: Alpha-amylase; dogs; saliva; serum; stress

The serum alpha-amylase (AA) and sAA levels were measured in 16 healthy Beagle dogs and 31 diseased dogs visiting the Veterinary Medical Teaching Hospital College of Chungnam National University and Daegu Small Animal Medical Center from May to October 2017. Thirty-one dogs with a range of diseases were selected randomly for this study, and the owners were informed. All dogs underwent physical examinations, complete blood counts (Advia 2120; Siemens Healthcare Diagnostics, USA), and biochemistry profiles (Mindray BS-300; Mindray Bio-Medical Electronics, China). The healthy dogs were housed individually in 1 m³ cages under a 12-h light/dark cycle and fed commercial canine food with water available ad libitum. The sampling procedures were performed within one day.
The dogs were fasted for more than 1 h prior to sampling. Saliva was collected using small cotton rolls around the mouth for over 2 min, which were then placed in Eppendorf tubes and centrifuged at 1,500 × g for 15 min. The saliva samples were transferred into new Eppendorf tubes, and stored at −70°C. Blood samples were collected through venipuncture of the jugular vein and centrifuged at 3,000 × g for 10 min at room temperature. The serum was transferred into Eppendorf tubes, and aliquots were stored as described. Almost all samples were collected between 10 AM and 3 PM to minimize the errors caused by the change in collection time. The sample collection protocol was approved by the Institutional Animal Care and Use Committee at Chungnam National University (approval No. CNU-00950).

The sAA and AA activities were measured using a commercial enzyme kit (Salivary α-Amylase; Salimetrics, USA) that uses 2-chloro-p-nitrophenol linked with maltotriose as the substrate. All aliquots were thawed, mixed thoroughly, and assayed in duplicate. The analysis was performed manually according to the manufacturer’s instructions. The absorbance was detected at a wavelength of 405 nm (Infinite M200; Tecan, Austria).

Statistical analyses were performed using SPSS (SPSS statistics 22; IBM, USA). Both parametric and non-parametric variables were analyzed. Therefore, the median (25th percentile and 75th percentile) was set to the representative values. The Kolmogorov-Smirnov and Shapiro-Wilk tests determined the normal distribution of the data. The sAA activity between the 2 groups was evaluated using the Mann-Whitney U test. The Spearman correlation was used to analyze the associations between sAA and serum AA. A p value < 0.05 was considered significant.

The sAA activity was evaluated in 47 dogs, including 16 healthy Beagles and 31 diseased dogs. Of these, 20 dogs were male, 17 of which were castrated, and 11 were females, 9 of which were spayed. In the diseased group, 16 dogs were purebreds of different breeds and 1 dog was a mixed-breed. The underlying conditions were tumors (n = 7), renal diseases (n = 5), pancreatitis (n = 4), cardiovascular diseases (n = 3), post-surgery (n = 3), infectious diseases (n = 2), dermatologic diseases (n = 2), endocrine diseases (n = 2), respiratory disease (n = 1), neurologic disease (n = 1), and immune-mediated disease (n = 1). Significant difference in serum and salivary AA activity were observed between the healthy and diseased dogs (p < 0.05) (Table 1). No correlation was observed between the sAA and serum AA activities (r = 0.251, p = 0.089) (Fig. 1).

Overall, the sAA activity increased in dogs with various diseases compared to that in healthy dogs. To the best of the authors’ knowledge, this is the first report confirming a high sAA concentration in diseased dogs. In a human salivary biomarker study, stress increased the sAA levels rapidly within 15 min and was moderately correlated with the State-Trait Anxiety Inventory score, whereas the other biomarkers, including chromogranin-A and cortisol, did not [9]. Therefore, the sAA activity is a better indicator of stress. In the present study, the median sAA activity (72.71 U/L) in diseased dogs was similar to that of dogs after sympathetic
activation in an earlier study (89.5 U/L) [7]. Based on this, the sAA levels increased in the diseased dogs due to stress. Earlier human studies reported that the sAA activity increased due to stress, occurring in a range of diseases, such as pulmonary disease and diabetes [10,11]. Therefore, the sAA activity may be a potential disease biomarker. Further studies will be needed to calculate the difference between the range of sAA concentrations for disease-free and disease-induced stressful conditions.

Salivary sampling has several distinct advantages in veterinary medicine. The disadvantages, however, are that the sAA activity is lower than the blood amylase activity and it is difficult to collect saliva from aggressive or dehydrated patients [12]. Needle injections can induce stress and increase sAA activity [6]. Because saliva can be collected without a needle, its sampling is easier and less stressful than blood or urine collection and it can be performed outside the hospital with minimal technical training [1,12]. Moreover, saliva can be multi-sampled and is delivered easily [12], making it ideal for owners unable to visit the hospital and for animals that are too small or anemic for blood sampling. Therefore, measurement of the sAA activity might be an effective, noninvasive, and minimally stressful screening test prior to blood testing.

The serum AA activity was significantly higher in the diseased group compared to that in the healthy group. This result is similar to that of humans with pancreatitis, mumps, and pancreatic cancer [13], and may be due to the AA produced in the pancreas and salivary glands. The serum AA levels have not been investigated in other diseases. In the present study, the serum AA was high in diseased dogs. The cause is yet unknown, but it is estimated that animals with diseases can easily become dehydrated and the pancreas is susceptible to hypoperfusion. On the other hand, there was no significant correlation between the sAA and serum AA levels \( r = 0.251, p = 0.089 \). Thus, the increased serum AA activity could be due to other unknown pathways in dogs.

This study had some limitations. First, the sAA activity was not compared with that of other stress hormones, such as norepinephrine and cortisol. Second, a larger study population is required to establish the normal range of sAA activity and assess the sAA sensitivity in diseased dogs. Third, the factors affecting the composition of saliva, such as
psychological stress, degree of hydration, body composition, and circadian rhythm, could not be controlled, and validation of the sAA enzymatic assay was not validated. Continual measurements from the same dogs are recommended if the sAA levels are to be used as a biomarker for monitoring the treatment and disease prognosis.

In conclusion, the AA activity increases in both canine serum and saliva due to disease-induced stress, even though there was no significant correlation between them. Therefore, sAA can be considered a less invasive, convenient, and relatively reliable method to detect stressful disease conditions in dogs.

REFERENCES

1. Rohleder N, Nater UM. Determinants of salivary α-amylase in humans and methodological considerations. Psychoneuroendocrinology 2009;34:469-485. 
PUBMED | CROSSREF
2. Baum BJ. Principles of saliva secretion. Ann N Y Acad Sci 1993;694:17-23. 
PUBMED | CROSSREF
3. McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, Nasca C. Mechanisms of stress in the brain. Nat Neurosci 2015;18:1353-1363. 
PUBMED | CROSSREF
4. Thoma MV, Kirschbaum C, Wolf JM, Rohleder N. Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. Biol Psychol 2012;91:342-348. 
PUBMED | CROSSREF
5. Takai N, Yamaguchi M, Aragaki T, Eto K, Uchihashi K, Nishikawa Y. Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. Arch Oral Biol 2004;49:963-968. 
PUBMED | CROSSREF
6. Fuentes-Rubio M, Fuentes F, Otal I, Quiles A, Hevia ML. Validation of an assay for quantification of alpha-amylase in saliva of sheep. Can J Vet Res 2016;80:197-202. 
PUBMED | CROSSREF
7. Contreras-Aguilar MD, Tecles E, Martinez-Subiela S, Escribano D, Bernal LJ, Cerón JJ. Detection and measurement of alpha-amylase in canine saliva and changes after an experimentally induced sympathetic activation. BMC Vet Res 2017;13:266. 
PUBMED | CROSSREF
8. Koh D, Ng V, Naing L. Alpha amylase as a salivary biomarker of acute stress of venepuncture from periodic medical examinations. Front Public Health 2014;2:121. 
PUBMED | CROSSREF
9. Noto Y, Sato T, Kudo M, Kurata K, Hirota K. The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: a pilot study. Anesth Analg 2005;101:1873-1876. 
PUBMED | CROSSREF
10. Yigla M, Berkovich Y, Nagler RM. Oxidative stress indices in COPD--Broncho-alveolar lavage and salivary analysis. Arch Oral Biol 2007;52:36-43. 
PUBMED | CROSSREF
11. Aydin S. A comparison of ghrelin, glucose, alpha-amylase and protein levels in saliva from diabetics. J Biochem Mol Biol 2007;40:29-35. 
PUBMED
12. Vangipuram S, Jha A, Bhashyam M. The diagnostic applications of saliva- a review. IOSR J Dent Med Sci 2016;15:96-101. 
CROSSREF
13. Mandal N, Bhattacharjee M, Chattopadhyay A, Bandyopadhyay D. Point-of-care-testing of α-amylase activity in human blood serum. Biosens Bioelectron 2019;124:125:75-81. 
PUBMED | CROSSREF