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ESTIMATION AND CORRELATION OF UREA AND CREATININE LEVELS IN SALIVA AND SERUM OF DOGS WITH RENAL FAILURE

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Abstract: A study was conducted to estimate and correlate the levels of salivary creatinine and urea in dogs with renal failure, in comparison to apparently healthy dogs. Dogs brought to Small Animal Clinic, Outpatient Medical Unit in Teaching Veterinary Clinical Complex, Madras Veterinary College, was chosen for this study. Thirty apparently healthy dogs of different age groups, sex and breed brought for routine health check up and vaccination were randomly selected to obtain normal parameters which formed the Control group. The experimental group consisted of thirty four dogs with clinical signs suggestive of renal failure. The selected animals were classified into the four groups based on International Renal Interest Society (IRIS) staging system for kidney disease. Saliva and blood samples were collected from these dogs as per the standard protocol. Biomarkers such as creatinine and urea were estimated using commercially available kits. Experimental data was statistically analyzed by general linear model with interaction in one – way analysis of variance (ANOVA) and post hoc analysis were carried out using Duncan's test for multiple comparisons using SPSS software version 20 for windows. The results showed that the serum and salivary urea / creatinine were significantly higher ($p < 0.001$) in stage IV, when compared to the control groups. The concentration of serum and salivary urea/creatinine reflects the renal damage and serves to the monitor the kidney function in dogs.
Keywords: Dogs, kidney disease, saliva, serum, urea, creatinine.

Introduction

Renal diseases are important clinical problems encountered in dogs and are frequent causes for illness and death. Renal disorders are more common in aged dogs. But recent report show that dogs of any age could be affected. The overall occurrence of renal disorder in dogs is 0.5 – 7 per cent and 15 percent in dogs aged about 10 years and above (Lund *et al.,* 1999, Polzin, 2010). Renal disease may regress, persist, or advance. Many renal diseases are not detected until they become generalized, leading to serious impairment of renal function. The condition may progress due to late diagnosis. The final stage renal failure is associated with high rate of fatality and a high financial expenditure for therapy (Mrudula *et al.,* 2005).

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Despite the relative clinical importance of kidney diseases, their early diagnosis is challenging. Measurement of Glomerular filtration rate (GFR) is considered the gold standard method to evaluate kidney function, but measurement is time-consuming and is not routinely used. Most common laboratory investigations used to diagnose renal disease are haematology and urine analysis.

Urine analysis and collection of blood for serum analysis proves to be an invasive technique that causes anxiety and relative discomfort to animals. Hence, a simple diagnostic test that provides a reliable evaluation of disease status/ stages and is of value to both clinicians and patients is required. As blood is the most common sample in clinical chemistry for identification of diseases and to follow progress of affected individuals under medical treatment, similar use has been envisioned for saliva (Madalli *et al*. 2013). The potential use of saliva as a diagnostic aid has attracted the attention because of its virtue of being non-invasive in nature, relative simplicity of collection, economic procedure that can be performed by the patient with minimal involvement of medical personnel. Saliva acts as an alternative route of excretion by the body in compromised renal function state. Therefore, saliva a multi-constituent biologic fluid secreted by salivary glands with hundreds of components serves to detect systemic diseases and provide biomarkers of health and disease status. Hence, an attempt has been made to study the merit of the salivary markers of kidney function and comparison with that of blood with the following objectives:

1. To Estimate and correlate the level of salivary creatinine and urea in dogs with renal failure in comparison to apparently healthy dogs.
2. To assess the reliability of diagnostic potential of saliva.

Materials and Methods

The study was conducted in dogs brought to Small Animal Clinic, Outpatient Medical Unit in Teaching Veterinary Clinical Complex and at Department of Veterinary Physiology, Madras Veterinary College, Chennai. Thirty apparently healthy dogs of different age groups, sex and breed brought for routine healthy check up and vaccination were randomly selected to obtain normal parameters which formed the Control group. The dogs with clinical signs suggestive of renal failure were subjected to clinical examination, haematology, serum biochemistry and urinalysis. Based on these parameters, minimum of thirty four cases that were diagnosed as suffering from renal disease were selected. Saliva and blood samples were collected from apparently healthy and renal disease dogs as per the standard protocol.

The selected animals were classified into the following groups based on International Renal Interest Society (IRIS) staging system for kidney disease.

SERUM CREATININE LEVEL	STAGE	NUMBER OF ANIMALS
Serum creatinine < 1.4 mg per cent	I	4
Serum creatinine 1.4 - 2mg per cent	II	6
Serum creatinine 2.1 – 5 mg per cent	III	9
Serum creatinine > 5 mg per cent	IV	15
TOTAL		**34**

Saliva collection

Saliva was collected from the dogs in the morning hours using human pediatric dental cotton rolls mixed with sialagogue. The cotton rolls were placed under the tongue/ and in the cheek pouches of the patient for 30 seconds. The rolls were immediately transferred to the labelled centrifuge tubes. Saliva was extracted from the cotton roll by centrifugation at 5000 rpm for 5 minutes and the extract was stored at – 20°C in aliquots until further analysis (German *et al*., 1998).

Blood collection

About five millilitres of blood was collected in a clot retracting vacutainer from saphenous / cephalic vein for bio-chemical study. The serum was separated by centrifugation at 3000 rpm for 15 minutes and the separated serum sample was stored in aliquots at – 20°C until further analysis.

Statistical analysis

The experimental data were statistically analysed by general linear model with interaction in one – way analysis of variance (ANOVA) and post hoc analysis were carried out using Duncan's test for multiple comparisons using SPSS software version 20 for windows.

Results and Discussion

The mean ± SE Values of serum and salivary urea/ creatinine levels are presented in Table 1 and 2.

Urea levels in the serum of dogs

The mean ± SE values of serum urea level in control and renal disease dogs are presented in Table 1.A highly significant ($P < 0.001$) increase in serum urea values were observed in stage I, stage II, stage III and stage IV when compared to normal control group.

Urea levels in the saliva of dogs

The mean ± SE values of salivary urea level in control and renal disease dogs are presented in Table 1. There was a significant increasing ($p < 0.001$) trend in the levels of salivary urea during different stages when compared to the control group of dogs. The level of salivary urea was significantly higher ($p < 0.01$) in the stage IV group of dogs.

The present study revealed a significant elevation of urea values in dogs with renal disease when compared with apparently healthy dogs which was in agreement with earlier observations (Forterre *et al.* 2004, Bradea *et al.* 2013 and Rusenov *et al.* 2014). Doxey (1983) reported that the elevated blood urea levels above 10 mmol/L was regarded as indicative of some impairment of renal function and levels above 30 mmol/L were extremely serious. The raised urea and BUN levels in renal diseased dogs may be due to retention of nitrogenous substances normally excreted by healthy kidneys (Cowgill *et al.*, 1998 and Polzin *et al.*, 2000). Previous studies have showed a significant elevation of salivary urea levels in human apparently healthy and patient with renal failure which was in accordance with the observation of Zuniga *et al.* 2012, Anuradha *et al.* 2015, Yajamanam *et al.* 2016 and Bagalad *et al.* 2017. The present study showed that the dogs with renal disease had elevated salivary urea concentration when compared to normal dogs. This observation is in accordance with the findings of Suresh *et al*., (2014) in human. Whenever there is an increase in the blood urea there is concomitant increase in salivary urea also, because the kidneys are unable to excrete urea in renal failure and its concentration in blood increases with increased concentration in saliva. This increased concentration of serum urea creates an increased concentration gradient, which in turn increases the diffusion of urea from serum to saliva. Hence, an increased salivary urea concentration was observed in this study

Creatinine levels in the serum of dogs

The mean ± SE values of serum creatinine level in control and renal disease dogs are presented in Table 2. A highly significant ($p < 0.01$) increase in serum creatinine values was observed in stage III and stage IV dogs when compared to normal control dogs. No significant difference was observed between control, stage I and stage II dogs.

Creatinine levels in the saliva of dogs

The mean ± SE values of salivary creatinine level in control and renal disease dogs are presented in Table 2. The level of creatinine in the saliva was found to be significantly high ($p < 0.01$) in the stage I group of dogs, than the control group of dogs. The level of creatinine was found to be increased in stage III and stage IV group of dogs and was significantly higher in stage IV group of dogs.

The present study revealed a significant elevation of serum creatinine values in dogs with renal disease when compared to apparently healthy dogs which agreed with the observations of Vaden *et al*., 1997, Cowgill *et al.*, 1998 and Mrudula *et al.*, 2005 in dogs. The creatinine levels are used to measure kidney dysfunction and are the basis for International Renal Interest Society (IRIS) staging system (Lefebvre, 2011). The increase in serum creatinine concentration is as a result of the progression of kidney disease and decline of GFR (Dibartola *et al.*, 1983). The present study showed a significant elevation of salivary creatinine levels, when compared to control, which was an accordance with the findings of Venkatapathy *et al.* 2014, Yajamanam *et al.* 2016 and Bagalad *et al.* 2017 in human. Under normal healthy conditions creatinine cannot diffuse easily across the cells and tight intercellular junction of the salivary gland, but in diseased state, its value increases in the saliva due to an alteration in the permeability of salivary gland cells, hence increased serum creatinine levels in CKD patients create a concentration gradient that facilitates diffusion of creatinine from serum in to saliva (Venkatapathy *et al.* 2014).

The study showed a positive correlation between blood and salivary urea and creatinine which was in accordance of the observations of Akai *et al*., 1983 in human.

Conclusion

The concentration of salivary urea and creatinine reflects the renal damage, serve to monitor kidney function of renal failure dogs and help in the diagnosis of middle stage to late-stage renal disease. Salivary estimation of renal biomarkers is a simple, noninvasive technique, which can be used as an alternate diagnostic tool for assessing renal dysfunction in dogs.

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Table 1. Level of urea in serum and saliva of dogs in control and experimental groups

GROUP	Urea (mg/dl)	
	Serum	Saliva
CONTROL	25.87 ± 1.22[a]	9.86 ± 0.42[a]
STAGE I	82.32 ± 1.66[b]	49.80 ± 1.00[b]
STAGE II	115.14 ± 3.58[c]	68.22 ± 2.34[c]
STAGE III	124.63 ± 6.29[c]	73.59 ± 1.97[c]
STAGE IV	148.04 ± 5.25[d]	95.61 ± 4.20[d]
F- values	265.11**	293.18**

Mean values having same superscript within a column do not differ significantly.
** - Highly significant (p<0.01)

Table 2. Level of creatinine in serum and saliva of dogs in control and experimental groups

GROUP	Creatinine (mg/dl)	
	Serum	Saliva
CONTROL	0.81 ± 0.04[a]	0.64 ± 0.02[a]
STAGE I	1.32 ± 0.04[a]	1.02 ± 0.02[b]
STAGE II	2.02 ± 0.02[ab]	1.24 ± 0.01[b]
STAGE III	3.41 ± 0.28[b]	1.72 ± 0.08[c]
STAGE IV	10.05 ± 0.96[c]	2.91 ± 0.08[d]
F- VALUE	65.151**	279.77**

Mean values having same superscript within a column do not differ significantly.
** - Highly significant (p<0.01)

Table 3. Correlation between serum and salivary urea and creatinine levels in dogs with renal failure and healthy controls

Correlation between serum and salivary parameters	Cases				Controls n=30
	Stage I n=4	Stage II n=6	Stage III n=9	Stage IV n=15	
Urea	+ 0.999**	+ 0.996**	+ 0.842*	+ 0.686**	+ 0.641**
creatinine	+ 0.833**	+1.000**	+0.966**	+0.901**	+0.903**

Correlation between serum and salivary urea and creatinine
** - Highly significant (p < 0.01)