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Clinical, haemato-biochemical and oxidative changes associated with chronic kidney disease (CKD) in dogs

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Abstract
Chronic kidney disease (CKD) is a commonly encountered problem in canine practice and associated with significant haematological and biochemical abnormalities. The aim of this investigation was to identify the clinical signs, haemato-biochemical and oxidative alterations in dogs affected with chronic kidney disease. Twenty-two dogs affected with CKD were evaluated. All affected animals had microcytic hypochromic and non-regenerative anaemia. Haemoglobin (7.96±0.33g/dl), PCV (26.58±5.8%), TEC (4.39±0.16 x10^6 cells/µl) and platelet count (112±8.62 x10^3 cells/µl) were reduced significantly in CKD patients as compared to healthy animals. TLC count and neutrophil percentage were significantly increased in affected dogs. Serum creatinine (7.52±1.09 mg/dl) and BUN level (286.35±35.16 mg/dl) was increased significantly, whereas, serum total protein and albumin level were decreased, but statistically non-significant. Serum electrolyte estimation revealed hyperphosphatemia (6.99±0.04 mg/dl), hyperkalaemia, hyponatremia, and hypocalcaemia. There was a significant reduction in catalase, SOD, and GSH level and increase in LPO level (7.99±0.37 nmol MDA/mg of Hb) indicating severe oxidative injury to tissues and RBCs, and associated with severity of the disease. In conclusion, these serum and haematological markers provided important information regarding pathogenesis and disease progression of CKD, and should be evaluated in these patients while adopting appropriate treatment protocol

Keywords: CKD, anaemia, uremia, creatinine, hyperphosphatemia, oxidative stress

Introduction
The inability of kidneys to excrete the waste metabolic products or toxins, concentrate urine, and maintain normal fluid electrolyte homeostasis resulting in excess accumulation of waste products is known as renal failure, which is classified as acute and chronic renal failure depending on the onset of disease condition [1]. Chronic renal failure or chronic kidney disease (CKD) is defined as the presence of structural and functional abnormalities of one or both kidneys that have persisted for more than three months [2]. CKD is one of the common problems in small animal practice. Estimation of non-protein nitrogen substance especially serum creatinine (SCr) and blood urea nitrogen (BUN) remained as an outstanding marker to diagnose and classify renal failure cases. As per International Renal Interest Society (IRIS) staging system, serum creatinine is used for staging CKD, in which SCr >1.4 mg/dl is considered azotemic. CKD can be diagnosed by clinical examination, serum biochemical analysis and ultrasonographic evidences of renal parenchyma damage.
In dogs with CKD, anaemia is one of the factors, contributing to worsening clinical condition especially in advanced cases [3]. However, anaemic condition may not be solely due to suppression of RBC production but may be due to suppression of erythropoiesis by uremic toxins or increased hemolysis [4]. Several clinical renal diseases involve oxidant in their pathogenesis. During tissue hypoxia and reperfusion, free-radical mediated cellular damage occurs and the formation of these oxidants exceeds the cellular detoxification capacity. Increased free radicals enhances the activity of phospholipase which subsequently destroys lipid plasma membrane. Substantial data were also correlated oxidative stress and changes in RBC characteristics in renal failure patients, where osmotic fragility might have increased due to oxidative stress which may be a reason for anaemia [5]. Therefore, keeping view on the above-mentioned facts, this study was planned to assess the clinical signs, haemato-biochemical, and oxidative changes in dogs suffered from chronic renal failure.

Material and Method

Study area and animal selection

This study is carried out in Teaching Veterinary Clinical Complex, OUAT, Bhubaneswar, situated geographically at 20.27ºN 85.84ºE in Odisha, India. Out of 450 dog cases presented, 133 cases were suspected for renal failure and screened based on the history, clinical signs, serum biochemical analysis, and ultrasonographical evidences of renal failure. Twenty-two cases (diseased group) having serum creatinine value >1.4 mg/dl and showing ultrasonographic evidences of CKD were included in this study. Clinical signs exhibited by these animals were recorded. Ten healthy dogs were taken as control group for comparison of different parameters.

Sample collection and Processing

Five millilitres of whole blood with and without adding EDTA were collected from diseased (n=22) and control (n=10) group for haematological and serum biochemical analysis. Serum was separated by centrifuging 3 ml of blood at 3000 rpm for 10 minutes, and stored at -20 ºC until further use.

Preparation of RBC hemolysate and RBC suspension

About 3 ml blood was collected in non-vacuum tube containing heparin (10 IU/ml of blood) as anticoagulant and centrifuged at 3000 rpm for 10 min. The plasma and buffy coat were separated by pipetting. Then, RBCs were washed three times with isotonic ice-cold solution of sodium chloride (NSS). For preparation of 10% RBC hemolysate, RBC pellet was diluted with ice-cold distilled water in 1:10 ratio and rest of the RBC pellet was diluted with ice-cold NSS in 1:1 ratio to get RBC suspension.

Analysis of oxidative parameters

Reduced glutathione (GSH) was determined by DTNB method as described by Prins and Loos (1969) [6] using RBC suspension. Other oxidative parameters *viz.* lipid peroxides (LPO), catalase (CAT) and superoxide dismutase (SOD) were estimated as per the methods described by Placer (1967) [7], Bergmayer (1983) [8], and Madesh and Balasubramanian (1998) [9], respectively, using 10% RBC hemolysate.

Results and Discussions

Clinical manifestations

Observed clinical sign in chronic kidney disease in present study have been presented in the figure 1. The predominant clinical sign noticed were weakness (n=21, 95.5%), anorexia (n=20, 90.9%), and vomiting (n=19, 86.4%) etc., and the least observed clinical sign was nervous signs, in 2 cases (9.1%), which showed abnormal behaviour. These findings are similar to other report [10]. Weakness in CKD patients may be a consequence of dehydration or prolonged anorexia. In present study, anorexia was recorded in 90.9% of CKD patients which may be due to the poor taste sensation and nausea due to increased urea and ammonia level in blood [11]. Vomiting is a frequent finding resulting from the effect of uremic toxin on medullary emetic chemoreceptor trigger zone and uremic gastroenteritis. Renal dysfunction results in reduced gastrin clearance and elevated gastric acid production, which progress to development of gastric lesion and vomiting [2]. In present study halitosis and oral ulcer was also observed. The oral lesions may arise from the caustic effect of ammonia produced locally by the action of bacterial ureases [11]. Diarrhoea recorded in the present study might be occurred due to degradation of urea to ammonia by bacteria urease. Polyuria in CKD patients occurs due to glomerular hyper filtration and systemic hypertension which reduces the ability to concentrate urine [10]. Nervous sign (i.e., abnormal behaviour) observed in this study may be due to the retention of nitrogenous substance in the blood, electrolyte disturbances and anaemia.

Haematological parameters

The mean values of different haematological parameters are mentioned in table 1. All the affected dogs were suffering from microcytic hypochromic, non-regenerative anaemia, which might be due to reduced erythropoietin production [12] or blood loss in different forms such as gastro-intestinal bleeding or haematuria [13], or due to uremic hemolysis [14]. Haemoglobin (7.96±0.33g/dl) and total erythrocyte count (TEC) level (4.39±0.16 x10^6 cells/µl) in CKD patients were decreased significantly as compared to healthy animals, which can be attributed to lack of erythropoiesis and reduced RBC survival because of uremia [1]. Similar reduction in haemoglobin and TEC values were also recorded by Pradhan and Roy (2012) [15] and Devipriya *et al.* (2018) [16]. The mean PCV values in affected dogs was found to be (26.58±5.8%) which is significantly lower than the healthy animals (43.66±8.74%). Similar findings were also recorded by Pradhan and Roy (2012) [15], Sharma *et al.* (2015) [17] and Patil (2011) [18], which might be due to reduced thrombopoietic activity in uremic dogs [1]. The platelet count in affected animals were significantly lower than the control animals which might be due to reduced thrombopoiesis [17] or due to uremic intoxication [19]. This finding in our study is also corroborated with Sharma *et al*. (2015) [17] and Tilley and Smith (2000) [20].

Total leucocyte count (TLC) in CKD dogs were higher significantly than healthy dogs because of inflammation of organs of urinary system [1]. Neutrophil count (85.20±2.25%) was significantly higher and lymphocyte count (15.68±2.12%) was significantly lower in renal failure patients as compared to control group. Similar haematological findings were also observed by Mrudula *et al.* (2005) [21] and Kandula and Karlapudi (2015) [22]. Mean eosinophil count in healthy and affected dogs were found to be 0.98±0.01% and 0.0±0.0%, respectively.

Serum biochemical parameters

The mean serum creatinine (SCr) value in dogs suffering from renal failure and healthy dogs were 7.52±1.09 and 0.79±0.08 mg/dl, respectively, and the mean blood urea nitrogen (BUN) level in affected and healthy dogs were 286.35±35.16 and 11.76±1.69 mg/dl, respectively (table 2). This significant increase in SCr and BUN values in renal failure patients can be attributed to reduced glomerular filtration rate (GFR), thus reduced excretion of these toxins in the affected kidneys. Similar elevation in SCr and BUN was also observed by Devipriya *et al.* (2018) [16], Sumit *et al.* (2018) [23], Lefebvre (2011) [24]. The total protein (TP) level in renal failure patients was decreased non-significantly as compared to control animals (table 2). Similarly, non-significant reduction was also found in serum albumin level in between these two groups of animals. This hypoproteinemia and hypoalbuminemia in CKD patients might be due to protein loss in urine due to kidney failure [23] or loss due to gastro-intestinal bleeding in uremic patients [1]. This finding is in

accordance with the other works carried out by Pradhan and Roy (2012) [15] and Devipriya *et al.* (2018) [16].

Mean serum calcium level in renal failure patients were significantly lower as compared to healthy dogs (table 2). Similar findings were also reported by Ross *et al.* (2007) [25] and Kumar (2013) [26]. There is less production of calcitriol from the affected kidneys as a result calcium absorption reduces, which might be the reason for getting hypocalcemia in CKD patients in this study. However, our findings contradict the results obtained by Sonu *et al.* (2019) [1], which reported significantly higher calcium level in renal compromised dogs. All the renal failure patients in our study had significantly higher mean serum phosphorus level (6.99±0.04 mg/dl) as compared to control animals (4.3±0.23 mg/dl) which was also found in other studies Vidyadhar (2012) [27] and Sonu *et al.* (2019) [1]. Kidney is the primary route of phosphorus excretion. Therefore, hyperphosphatemia in renal compromised patients occurs mainly because of reduced phosphorus excretion by the affected kidneys. Hyperphosphatemia is positively correlated with renal lesions and mortality rates in azotemic patients [28]. This study observed statistically non-significant (P>0.05) reduction in the mean serum sodium level in affected dogs (142±1.02 mmol/L) as compared to healthy dogs (148±1.25 mmol/L), which might be due to decreased GFR in renal failure patients which causes activation of renal angiotensin-aldosterone system (RAAS) and increased antidiuretic hormone (ADH) release. This leads to increased water retention causing dilution of blood, causing low sodium concentration [29]. This finding is in accordance with Kumar (2013) [26] and Patil (2011) [18]. All the diseased dogs evidenced higher potassium level with overall mean of 5.31±0.07 mmol/L as compared to 4.01±0.31 mmol/L in control group. This finding is in accordance with Patil (2011) [18] and Lefebvre (2011) [24].

Oxidative parameters

Fig 2. shows the lipid peroxide level and antioxidant enzyme activity in erythrocytes from diseased and control group animals. Oxidative stress increases in uremic patients due to either increased oxidative stress or decreased anti-oxidant activity. Significant increase in LPO was found in diseased animals (7.99±0.37 nmol MDA/mg of Hb) as compared to control group animals (3.37±0.39 nmol MDA/mg of Hb), whereas, GSH, CAT and SOD activity was reduced significantly (P<0.05) in diseased animals as compared to control animals. Previous studies in canines affected with chronic renal failure also demonstrated similar alterations in oxidative parameters [30, 31]. However, another study did not find any significant changes in oxidative parameters and reported no oxidative damage to RBCs in azotemic dogs [5].

Table 1: Haematological parameters in healthy and CKD dogs

Parameters	Diseased group (n=22)	Control group (n=10)
Haemoglobin (gm/dl)	7.96±0.33*	12.46±0.23
Packed Cell Volume (%)	26.58±5.8*	43.66±8.74
Total Erythrocyte Count (×10^6 Cell/µl)	4.39±0.16*	6.88±0.30
Total Leukocyte Count (×10^3 Cells/µl)	17.49±0.43*	8.15±0.62
Neutrophil (%)	85.20±2.25*	71.30±3.45
Lymphocyte (%)	15.68±2.12*	20.15±3.62
Eosinophil (%)	0.98±0.01	0.0±0.0
Platelet count (×10^3 Cells/µl)	112±8.62*	258±32.88

*Indicates values are significantly altered at p<0.05.

Table 2: Serum biochemical parameters in healthy and CKD dogs

Parameters	Diseased group (n=22)	Control group (n=10)
Creatinine (SCr) (mg/dl)	7.52±1.09*	0.79±0.08
Blood Urea Nitrogen (BUN) (mg/dl)	286.35±35.16*	11.76±1.69
Total Protein (TP) (mg/dl)	4.86±0.15	5.93±0.23
Albumin (mg/dl)	3.15±0.18	3.75±0.06
Calcium (Ca) (mg/dl)	8.1±0.11*	10.53±0.19
Phosphorus (P) (mg/dl)	6.99±0.04*	4.3±0.23
Sodium (Na) (mmol/L)	142±1.02	148±1.25
Potassium (K) (mmol/L)	5.31±0.07*	4.01±0.31

*Indicates values are significantly altered at p<0.05.

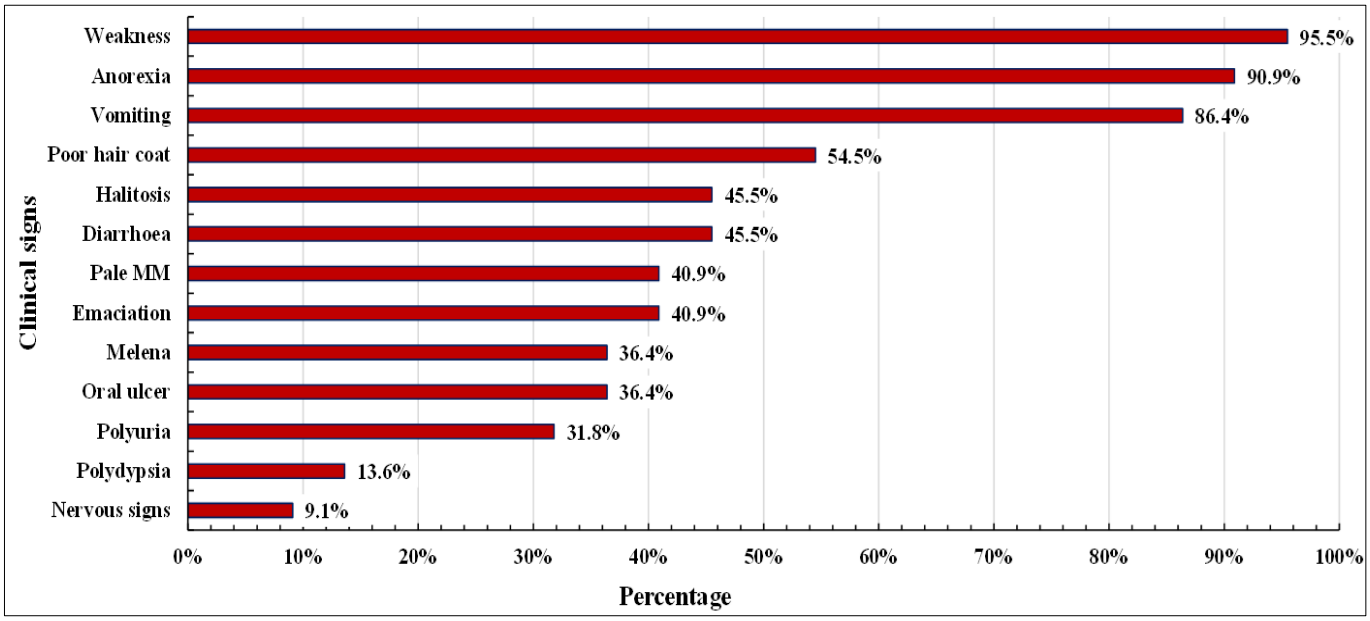

Fig 1: Clinical signs exhibited by dogs suffering from chronic kidney disease

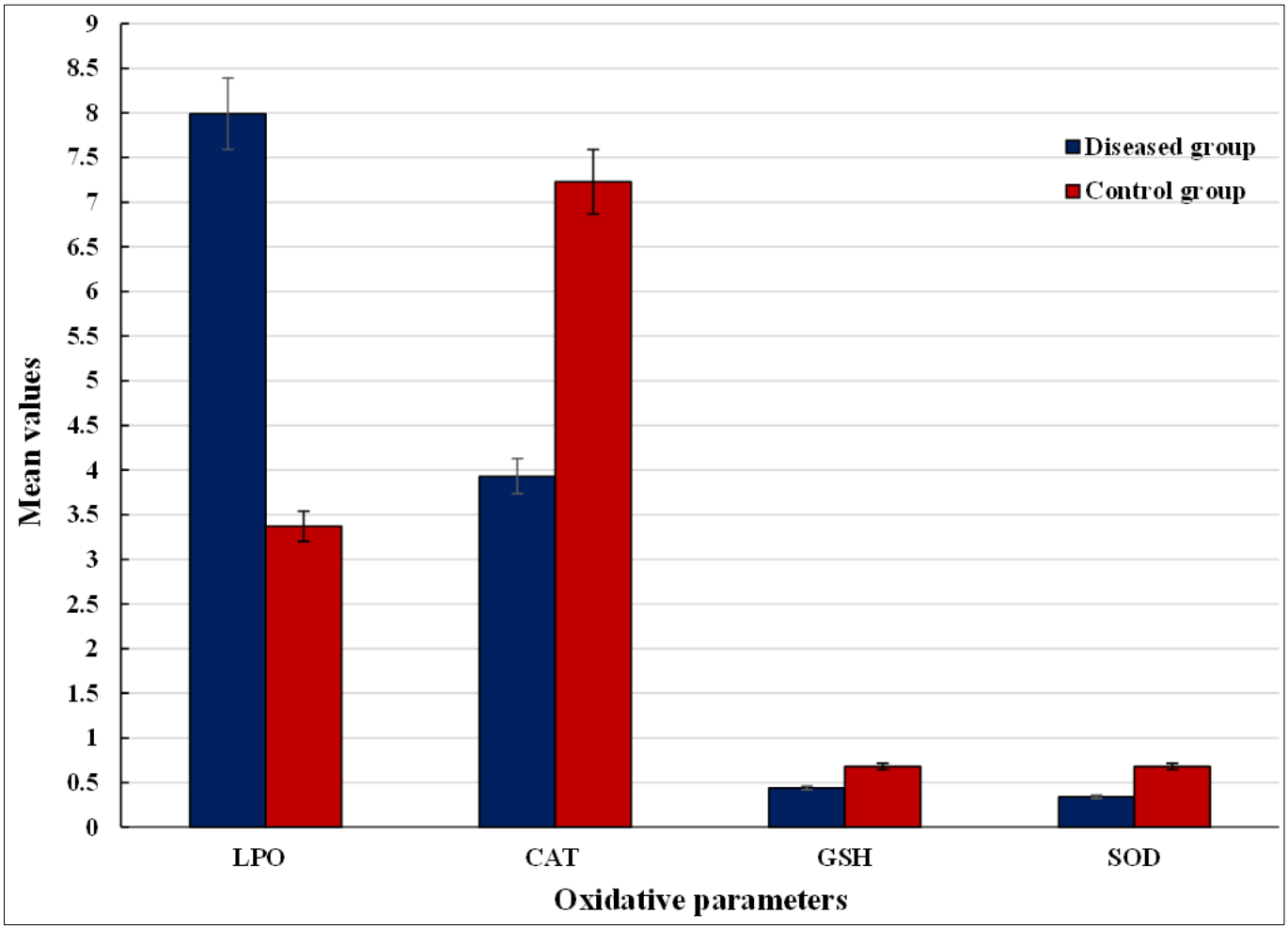

Fig 2: Lipid peroxide level and anti-oxidant enzymes activity in erythrocytes from dogs suffering from chronic kidney disease. LPO- lipid peroxides (nmol MDA/mg of Hb), CAT- Catalase (Units/mg of Hb), GSH- Reduced glutathione (µmol/ml of packed RBCs), SOD- Superoxide dismutase (Units/mg of Hb).

Conclusion

Dogs affected with chronic renal failure had altered haematological parameters and non-regenerative anaemia mostly due to reduced erythropoietin level in kidney and bone marrow suppression. Alteration in serum biochemical parameters in these uremic patients were also associated with various clinical signs like melena, vomiting, weakness, diarrhoea and nervous signs etc. Changes in different oxidative markers indicated oxidative stress on tissue and RBCs in dogs with renal failure. These haemato-biochemical and oxidative alterations are associated with the severity of the disease and loss of kidney function. These parameters should always be evaluated in renal failure patients, so that appropriate management protocol can be adopted.

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Conflict of interest

Authors declare there is no conflict of interest.

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