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Hematobiochemical and serum iron profile in dogs with anemia due to chronic or renal disease

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Abstract
Iron is vital mineral for all the living organisms and is essential to multiple metabolic functions, oxygen transport in haemoglobin being the most important function. There exist limited uses of serum iron profile parameter study in anemic dogs with chronic or renal azotemia. The present study was carried out to observe the alteration in iron metabolism disturbance occurring in anemic dogs with chronic or renal azotemia (n=6). The mean ± standard error (SE) of serum iron analytes [serum iron, serum total iron binding capacity (TIBC) and percent transferrin saturation (%TSAT)] were measured by calorimetric analyser to estimate the iron profile status in the clinical cases. Among the serum iron profile, dogs with anemia of chronic or renal disease showed depletion of iron store that might be due to decrease in erythropoiesis which required therapy of iron and exogenous erythropoietin hormone.

Keywords: Anemia, serum iron, serum total iron binding capacity, percent transferrin saturation, dogs

Introduction
Anemia can be caused by a number of factors such as hemoprotozoal parasites, gastrointestinal parasites, immune-mediated diseases, chronic diseases like renal disease, neoplasms, endocrinopathies and genetic abnormalities etc (Fry, 2010) [5]. Routine clinical examination of animal and blood analysis will also not reveal the exact cause of anemia in most of the cases. Identification of the underlying cause of anemia is the proper approach for prompt recovery. Clinically anemic dogs are weak, have lack of stamina, pale to icteric mucous membranes and hemoglobinuria (Tvedten, 2010) [16]. Cowgill (2004) observed that low packed cell volume, erythrocyte count and hemoglobin are characteristics of dogs with moderate to advanced (stages 3 to 4) CKD. The principal cause is hypoplasia of erythroid elements of the bone marrow secondary to inadequate renal production of erythropoietin. Shortened erythrocyte lifespan, erythropoietic inhibitor substances in plasma, chronic gastrointestinal blood loss, nutritional abnormalities (e.g. iron deficiency) and bone marrow fibrosis may contribute to anemia in some patients with CKD.
Iron being essential mineral for living organisms play integral role to multiple metabolic functions with oxygen transport in hemoglobin being the most important function. The use of serum iron profile parameter for assessment of anaemia in dogs is limited. (Velayudhan JM *et al*. 2015) [18]. 60-70% of total body iron is available in hemoglobin (McCown and Specht, 2011; Harvey, 2008) [10, 8]. Clinical manifestations observed due to iron imbalance in body can lead to severe complications and be life threatening too (McCown and Specht, 2011) [10]. Monitoring iron status of animal is carried out through serum analytes estimation (serum iron, serum total iron binding capacity (TIBC), serum ferritin and percent transferrin saturation (%TSAT) (Wians *et al*., 2001) [19]. Serum ferritin concentration has limited use in veterinary medicine since reagents are species-specific and the assay is not widely available (Schaefer and Stokol, 2015) [13]. Hence alteration in serum analytes of iron is observed in anemic patients thus helping in classification of iron disturbance and monitoring the therapy.
The present study was intended to observe the type of iron metabolism disturbance occurring in anemic dogs with chronic or renal disease since very little data is available regarding iron metabolism disturbance in dogs with chronic or renal disease.

Material and Methods
The present study was carried out in dogs of any age group and breed affected with chronic or renal disease with clinical signs suggestive of anemia with alteration in complete blood count

(CBC) profile value. Clinical examination of the dogs was performed as described by Ettinger and Feldman (2005) [4]. The serum iron profile analytes (serum iron, serum TIBC, % TSAT) was also measured. The study included 6 apparently healthy dogs as control groups which had a proper history of vaccination, deworming and were negative for any presence of diseases.

Out of 1247 cases examined at Department of TVCC Bombay Veterinary College, Parel, 20 clinically anemic cases showed hemoglobin less than 10g% and PCV less than 37 per cent. The overall prevalence of anemia was 1.60%. Out of which 6 cases showed anemia due to acute blood loss anemia.

Blood was collected in sterile vacuttainers (LS ECOTAINER EDTA K3). Serum was separated by using the LS ECOTAINER Plain vacuttainer tubes. Hematological parameters under study that included hemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), reticulocyte count, platelet count, white blood corpuscular count (WBC), differential leucocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) was evaluated along with clinical materials like peripheral blood smear, fecal sample were collected and detailed examinations were carried out to determine the cause of anemia by the method as described by Benjamin (2007) [1].

Serum biochemistry parameters under study that included Alkaline phosphatase(ALP), serum glutamate pyruvate transaminase(SGPT), serum glutamic-oxaloacetic transaminase(SGOT), total serum protein, albumin, globulin, Albumin/Globulin ratio (A/G ratio), Blood Urea Nitrogen (BUN), urea, creatinine, total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB) were estimated by spectrophotometry in robonik prietest touch auto biochemistry analyser using the supplied reagent (M/s Span diagnostics, Kolkata). SGPT and SGOT were dertermined by IFFC method (Reitman and Frankel, 1957) [12], total protein by biuret method (Gornall *et al*. 1949) [6], albumin by BCG method (Gustaffson, 1978) [7], total and direct bilirubin by diazo method (Ertingshausen *et al*., 1973) [3], urea by GLDH-urease method (Tietz, 1995) and creatinine by jaffe's reaction (Vasiliades, 1976) [17]. Indirect bilirubin was calculated as [IB= TB- DB]. Albumin/Globulin ratio (A/G ratio) was calculated as [A/G ratio= Albumin/Globulin]

Serum iron profile study parameters that such as serum iron, serum total iron binding capacity (TIBC) and percent transferrin saturation (%TSAT) were estimated by I-lab 600 accute auto analyser using the supplied reagent (Sigma Diagnostics, St. Louis, USA). Serum iron was determined by ferene method (Smith *et al*. 1981) [14], serum TIBC by biochemical method (Henery, 1984) [9]. Transferrin saturation was calculated by the following formula

% TSAT= (Serum iron/ TIBC) x 100

Normally distributed data are reported as mean ± standard error. Student's t-test was used for statistical analysis. Statistical significance was set at P≤0.05. This was performed by using the analysis tool pack software of Microsoft excel 2007.

Clinical cases were managed and treated as per standard treatment protocol hence ethical approval was not necessary for the study. Tentative diagnosis and therapeutic management of dogs having acute blood loss anemia are tabulated in Table 1.

Result and Discussion

The hemato-biochemical alterations when compared to the apparently healthy control dogs (Table 2) showed statistically significant difference regarding in parameters Hb, TEC, reticulocyte count and platelet count. Statistical significant difference was also observed in the TP, albumin, A/G ratio and BUN parameters when compared with the apparently healthy control group. Hypochromasia and microcytosis were the major cellular changes, however anisocytosis was the other cellular changes observed. Giger (2005) stated low values of Hb, T.E.C and P.C.V in the cases may be credited to deficiency of iron, red blood corpuscles destruction and/or excess blood loss and decreased erythropoietic activity.

Serum iron profile between the apparently healthy and clinical cases showed statistical significant difference between the serum iron parameter and non-significant difference between serum TIBC and %TSAT. The same is depicted in table 3.

Therapeutic management of clinical cases is shown in table 1. The hemato-biochemical and serum iron profile parameters were re-evaluated in the affected cases after clinically recovery and is summarized in table 4, 5, 6 and 7. No Statistical significant difference was observed in CBC, LFT and KFT parameters before and after therapy. The anemia in dogs with renal azotemia/chronic disease might be due to decrease in the erythropoietin production. The mean ± SE values of serum iron, TIBC and per cent TSAT before treatment were 64.97 ± 23.08 µg/dl, 332.77 ± 51.35 µg/dl and 29.56 ± 14.71 per cent respectively and after treatment values were 73.18 ± 26.22 µg/dl, 386.97 ± 26.77 µg/dl, 18.93 ± 6.35 per cent respectively and is depicted in There was no statistical significant difference ($P \geq 0.05$) observed. The cause for decrease in mean level of TSAT per cent after treatment may be due to the increase in the per cent TSAT levels observed in the two cases of jaundice. The serum iron profile level in the renal azotemic patients were in accordance to the findings of Cowgill (1995) [2] who opinioned that iron deficiency exists in renal azotemic patients with low level of serum iron and per cent TSAT. The mean serum iron profile levels in animals with anemia of chronic/renal disease were found to be similar to Radostits *et al*. (2007) [11].

In conclusion the results from this study, clinical cases with anemia of chronic disease resulted in disturbance of iron metabolism subsequently leading to anemia. Dogs with anemia of chronic /renal disease showed depletion of iron store that might be due to decrease in erythropoiesis, required therapy of iron and exogenous erythropoietin hormone. Hence serum iron profile studies are a valuable aid in the diagnosis of iron metabolism disturbances and should be included as a routine diagnostic parameter in the anemic dogs.

Table 1: Provisional diagnosis and therapeutic management of clinical cases with anemia due to chronic or renal disease

Case no	Provisional diagnosis	Treatment given
1	Acute Renal Azotemia	Inj NS 500ml, Inj RL 500ml, Inj Lasix 2mg/kg, Inj Renocel 2000 IU total dose and supportive therapy.
2	Acute Renal Azotemia	Inj NS 500ml, Inj RL 500ml, Inj Lasix 2mg/kg, Inj Renocel 2000 IU total dose and supportive therapy.
3	Chronic Renal Azotemia	Inj NS 500ml, Inj RL 500ml, Inj Lasix 2mg/kg, Inj Renocel 2000 IU total dose and supportive therapy.
4	Acute Renal Azotemia	Inj NS 500ml, Inj RL 500ml, Inj Lasix 2mg/kg, Inj Renocel 2000 IU total dose and supportive therapy.

5	Toxic Jaundice	Inj Fructodex 500ml, Inj Renocel 2000 IU total dose and supportive therapy including liver protectants.
6	Obstructive Jaundice	Inj Fructodex 500ml, Inj Renocel 2000 IU total dose and supportive therapy including liver protectants.

Table 2: The Mean±SE values of CBC, LFT, and KFT in apparently healthy and clinical cases before treatment

Parameters	Apparently Healthy	Clinical Cases
Hb (g%)	13.38 ± 0.37	8.02 ± 1.04*
T.E.C($\times10^6$/µl)	5.76 ± 0.13	3.59 ± 0.42*
P.C.V (%)	40.15 ± 1.12	24.52 ± 2.73*
Reticulocyte count (%)	1.67 ± 0.33	0.55 ± 0.14*
W.B.C($\times10^3$/µl)	11450 ± 1393.26	27200 ± 4778.08
Platelets($\times10^5$/µl)	283666.70 ± 25935.39	235666.7 ± 43664.2*
M.C.V (fL)	69.67 ± 1.09	68.97 ± 4.70
M.C.H (pg)	23.18 ± 0.37	22.27 ± 1.90
M.C.H.C (g/dl)	33.25 ± 0.07	32.53 ± 0.97
T.B(mg/dl)	0.32 ± 0.048	2.66 ± 1.4
D.B(mg/dl)	0.17 ± 0.03	1.61 ± 0.92
I.B(mg/dl)	0.15 ± 0.03	1.05 ± 0.49
S.G.P.T(IU/L)	81.33 ± 3.13	80.72 ± 14.93
S.G.O.T(IU/L)	53.52 ± 2.89	109.94 ± 80.68
A.L.P(IU/L)	94.87 ± 3.65	607.88 ± 137.88
T.P(g/dl)	8.10 ± 0.15	6.44 ± 0.53*
Albumin(g/dl)	3.90 ± 0.20	1.64 ± 0.29*
Globulin(g/dl)	4.37 ± 0.16	4.8 ± 0.74
A/G	0.89 ± 0.06	0.42 ± 0.11*
BUN(mg/dl)	8.99 ± 0.35	49.14 ± 8.98*
Urea(mg/dl)	19.21 ± 0.761	105.2 ± 19.24
Creatinine(mg/dl)	0.84 ± 0.07	2.89 ± 0.48

*Significant ($P\leq0.05$) at t-crit (2.06)

Table 3: Mean ±SE value of serum iron profile of apparently healthy and clinical cases before treatment

Parameters	Apparently Healthy dogs	Clinical cases
Serum iron(µg/dl)	141.83 ± 7.91	64.97 ± 23.08*
Serum TIBC(µg/dl)	319.33 ± 31.89	332.77 ± 51.35 ^{NS}
TSAT (%)	47.53 ± 6.54	29.56 ± 14.71 ^{NS}

*Significant ($P\leq0.05$) at t-crit (2.06)
NS- Non Significant

Table 4: Mean ± SE values of CBC in clinical cases before and after treatment

Parameters	Before treatment	After treatment	t-stat	t-crit
Hb (g%)	8.02 ± 1.04	8.9 ± 0.99	1.48	2.57
T.E.C ($\times10^6$/µl)	3.59 ± 0.42	4.33 ± 0.54	1.43	
PCV (%)	24.52 ± 2.73	28.48 ± 2.78	2.12	
WBC ($\times10^3$/µl)	27200 ± 4778.08	17516.67 ± 2413.22	2.56	
Reticulocyte count (%)	0.55 ± 0.14	0.8 ± 0.27	1.54	
Platelets ($\times10^5$/µl)	235666.7 ± 43664.38	239333.3 ± 49134.96	0.05	
MCV(fL)	68.97 ± 4.7	67.65 ± 4.54	0.18	
MCH(pg)	22.27 ± 1.9	21.08 ± 1.82	0.49	
MCHC(g/dl)	32.53 ± 0.97	31.05 ± 0.93	3.31	

* Significant ($P\leq0.05$)

Table 5: Mean ± SE values of LFT in clinical cases before and after treatment

Parameters	Before treatment	After treatment	t-stat	t-crit
T.B.(mg/dl)	0.27 ± 1.40	1.37 ± 0.83	1.46	2.57
D.B.(mg/dl)	1.61 ± 0.92	0.82 ± 0.56	1.42	
I.B.(mg/dl)	1.05 ± 0.49	0.55 ± 0.28	1.50	
SGPT(IU/L)	80.72 ± 14.93	64.77 ± 8.66	1.54	
SGOT(IU/L)	109.64 ± 80.68	37.48 ± 5.99	0.96	
ALP(IU/L)	607.88 ± 137.88	542.83 ± 239.51	0.47	
TP(g/dl)	6.44 ± 0.53	6.73 ± 0.19	0.57	
Albumin(g/dl)	1.64 ± 0.29	2.23 ± 0.29	1.88	
Globulin(g/dl)	4.80 ± 0.74	4.50 ± 0.29	0.48	
A/G	0.42 ± 0.11	0.52 ± 0.09	0.89	

*Significant ($P\leq0.05$)

Table 6: Mean ± SE values of KFT in clinical cases before and after treatment

Parameters	Before treatment	After treatment	t-stat	t-crit
BUN (mg/dl)	49.14 ± 8.98	30.22 ± 16.04	1.19	2.57
Creatinine (mg/dl)	2.89 ± 0.48	1.76 ± 0.55	1.76	
Urea (mg/dl)	105.20 ± 19.24	64.72 ± 34.35	1.19	

*Significant ($P \leq 0.05$)

Table 7: Mean ± SE values of serum iron profile in clinical cases before and after treatment

Parameters	Before treatment	After treatment	t-stat	t- crit
S. iron (µg/dl)	64.97 ± 23.08	73.18 ± 26.22	2.01	2.57
S. TIBC (µg/dl)	332.77 ± 51.35	386.97 ± 26.77	1.34	
TSAT (%)	29.56 ± 14.71	18.93 ± 6.35	1.22	

*Significant ($P \leq 0.05$)

Conflict of interest

There is no conflict of interest among all or any of the authors.

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