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Study of prognostic potential of Red Cell Distribution Width (RDW) in hepato-renal diseases of canines

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ABSTRACT

Background: Red blood cell distribution width (RDW) is a readily available and cost effective indicator of variation in erythrocyte size. The alteration in RDW has been found to be associated with several disease conditions thus could be used as potential biomarker. This study aimed to determine the RDW alterations in canine hepato-renal diseases and to evaluate association of RDW with other conventional hemato-biochemical parameters.

Methods: Twenty two canines suffering from hepato-renal diseases ($n = 11$ each) were enrolled for this study. The hepatic and renal diseases were confirmed by hemato-biochemical parameters along with ultrasonography. Six healthy canines were kept as control.

Results: RDW varied significantly ($P \leq 0.0021$) between hepatic and renal disease group. However, increase in the RDW was non-significant as compared to healthy control. The Hb concentration was significantly ($P \leq 0.001$) lower in both hepatic and renal disease groups as compared to the healthy control. Statistically significantly ($P \leq 0.0001$) higher serum SGPT concentration was found in the hepatic disease groups from renal and healthy groups however, the Alb, TB, DB and IB did not vary significantly in both the groups. There was moderate but significant positive correlation between RDW and SGPT ($r = 0.394$) and a moderate negative correlation was found between RDW and PCV ($r = -0.309$) and TP ($r = -0.323$). In the hepatic diseases group, the RDW has significant but negative correlation ($r = 0.610$, $P \leq 0.05$) with Hb. However, no significant correlation was found with RDW and other parameters in hepatic and renal disease groups. The findings of the present study suggested that the increased RDW could be used as useful supplemental information along with standard biochemical markers of hepatic and renal diseases. Therefore, the RDW can be used as reliable indicator of early disease diagnosis and can also be proved useful in predicting the response to therapy.

Keywords: Canine, RDW, Hepato-renal diseases, Haemato-biochemical, Association, Prognostic potential

Introduction

Red blood cell distribution width (RDW) is a quantitative measure of variation in erythrocyte size and is a reliable indicator of anisocytosis. RDW is the standard deviation (SD) of the red cell volume divided by the mean corpuscular volume (MCV). RDW reflects the heterogeneity in circulating erythrocyte volume. An alteration in RDW can be seen in variety of conditions that causes significant increased number of immature RBCs in circulation, such as regenerative anaemias or inflammatory processes in bone marrow stimulating the hematopoietic cells (Rogiers *et al.*, 1992; Neiger *et al.*, 2002; Zurauskaite *et al.*, 2018) [1, 2]. Numerous pathological conditions are associated with elevated RDW levels with or without anemia such as renal, hepatic, cardiorespiratory disease, pancreatic, vascular, neoplastic, endocrine, bone marrow disorders, nutritional deficiencies, chronic or acute systemic inflammation, sepsis, and the use of drugs like nebivolol and metoprolol (Salvagno *et al.*, 2015; Martinez *et al.*, 2019) [4, 3]. Furthermore, elevated RDW value could be used as reliable predictor of mortality in heart failure, hematological malignancies, acute pancreatitis, kidney disease, obstructive jaundice and community-acquired pneumonia induced by cancer, and sepsis or septic shock (Salvagno *et al.*, 2015) [4].

RDW has been extensively researched as a readily available quantitative marker of erythrocyte anisocytosis in human patients (Qin *et al.*, 2017) [5]. Higher RDW readings could be due to various physiological and pathological conditions (Martinez *et al.*, 2019) [3]. The prominent physiological causes includes ageing (Cheng *et al.*, 2004; Patel *et al.*, 2004) [6], pregnancy (Shehata *et al.*, 1998) [7], ethnicity (Saxena *et al.*, 1990) [8] and moderate physical exercise (Lippi *et al.*, 2012; Salvagno *et al.*, 2015) [9, 4]. The previous studies have demonstrated the direct association between high RDW and risk of liver disease, cardiovascular dis

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ease, kidney disease and metabolic syndrome (Montagnana *et al.*, 2011; Hu *et al.*, 2013; Laufer *et al.*, 2015) [11, 12, 10].

Besides, RDW, another most commonly used index to ascertain the degree of anisocytosis is the mean corpuscular volume (MCV), which is deduced using volume and globular erythrocyte counts. However, small changes in the volume of RBCs may not have significant alteration in MCV, therefore a large percentage of cells with modified volume are required to produce appreciable changes in the MCV and anisocytosis in blood picture (Ferreira *et al.*, 2009) [13]. The RDW along mean corpuscular volume (MCV) has also been used in sub-classification of anemias (Salvagno *et al.*, 2015) [4]. The findings of study suggested that the increased MCV with unaltered RDW could be due to aplastic anemia and chronic liver disease whereas the increased RDW with unaltered MCV are associated with iron, cobalamin, folate deficiency or chronic hepatobiliary disease.

The studies on RDW as tool of diagnosis and prognosis in canines are scarcely available. Further, the microcytosis with elevated RDW are common findings in healthy dogs of some breeds like Akita and Hokkaido, Japanese shiba. Similarly, elevated RDW with macrocytosis have been reported in toy and miniature poodles (Perret *et al.*, 2001; Aniolek *et al.*, 2017) [15, 14]. Breeds like Alaskan malamutes, Drentse partridge, and miniature schnauzers with hereditary symptomatic and asymptomatic stomatocytosis have been documented to have increased RDW (Bonfanti *et al.*, 2004) [16]. In another study, the higher RDW was found associated with a higher risk of mortality in dogs (Ludwik *et al.*, 2022) [17]. The limited availability of studies on RDW in canines, the present study was designed with objective to measure the RDW alteration in canine patients suffering from hepatic and renal diseases, and to evaluate if any prognostic association exists with RDW.

Materials and methods

Animals

Canines patients presented to the Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh with the symptoms suggestive of liver and kidney disease were enrolled in the present study. The sampling and other diagnostic modalities used in this study has prior client consent.

Sampling: The 2 ml of whole blood was aseptically collected from cephalic venipuncture. The 500 µl was placed in EDTA tubes and 1.5 ml in serum collection tube. The serum tube was allowed to clot followed by centrifugation @ 3000 rpm and serum were stored at -20°C until used for serum chemistry. The EDTA whole blood samples were immediately subjected to complete blood count using hematology analyzer (Celltac α, Nihon Kohden, Japan). The RDW were obtained as part of hematology analysis and expressed as percentage mean (standard deviation) of the measurement of size variability of the red blood cells.

Screening for hepatic and renal disease

The collected serum samples were analyzed for performing the serum chemistry. The serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), creatinine, total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), total protein (TP) and albumin (Alb) were estimated using commercially available kits (Tulip Diagnostic Pvt Ltd).

Ultrasonography (USG)

The patients showing alteration in serum biochemical parameters were subjected to ultrasonographic imaging (Acuson X 300, Siemens, USA) using 5 MHz curvilinear probe for confirmation of hepatic and renal pathologies.

Data analysis

The findings of study were analyzed by Pearson correlation coefficient and one way ANNOVA using statistical analysis package IBM SPSS 20 (SPSS, Science, Chicago, USA). The multiple comparisons

between the groups was done by using Tukey test at 5% level of significance.

Results and discussion

RDW is inexpensive and readily available information as part of a complete cell count investigations. The RDW has been proved to be a quantitative marker of anisocytosis in RBCs. Besides, its role as supplemental diagnostic marker in anaemia, the prognostic potential of RDW in range of diseases is well appreciated in human patients. However, the studies on canine patients are scarcely available in literature. The alteration in RDW may have a stronger prognostic value than other costly and clinically inaccessible indicators. Therefore, the present study was hypothesized to study the prognostic significance of RDW for diagnosis of hepatic and renal diseases of canines.

In the present study, the renal ($n = 11$), hepatic disease ($n = 11$) and 6 healthy canines were enrolled for comparison. The hepatic group consists of Labrador Retriever ($n = 5$), German Shepherd ($n = 2$) and Naplean Mastiff, Pitbull, Pomeranian and Non-discript ($n = 1$ each). The renal diseases were confirmed in 8 males and 3 females of the study. Breed distribution showed highest number of cases in Labrador Retriever ($n = 5$) followed by German Shepherd ($n = 2$) and Naplean Mastiff, Pitbull, Pomeranian and Non-discript ($n = 1$ each). Similarly, the hepatic disease group breed distribution showed highest number of cases in Labrador Retriever ($n = 6$) followed by Pomeranian and Non-discript ($n = 2$ each) and 1 case in German Shepherd. A total of 6 males and 5 females were found affected with hepatic disease out of 11 canines enrolled in the study. In the healthy control group all animals were male German Shepherd breed with median age of 3.75 years (range 3-4.5 years). The median age in hepatic disease group was 6.4 years (range: 3-10 years) and renal disease group was 6.8 years (range: 2.5-12 years). The marked ultrasonographic features in hepatic diseases group were hepatic enlargements, chronic hepatic fibrosis with and without ascites, and cholecystohepatitis with splenitis. In the renal diseases group, the prominent ultrasonographic findings included markedly reduced cortico-medullary differentiation, markedly thickened, granular and hyperechoic renal cortex and dilation in renal pelvis. The five canines were diagnosed with chronic kidney disease and six were having glomerulonephritis, ureteral obstruction and pyelonephritis and acute kidney injury.

The findings of hemato-biochemical changes in hepatic, renal and healthy groups are summarized in table-1. The mean RDW value in healthy, hepatic and kidney diseases groups were found as $12.37 \pm 0.41\%$ (range-11.4 to 13.4%), $17.40 \pm 0.94\%$ (range-13.1 to 23.6%) and $14.24 \pm 0.56\%$ (range-12.5 to 17.8%), respectively. There was a statistically significant ($P \leq 0.0021$) difference in RDW values between hepatic and renal disease group. When compared to the control group, RDW value was significantly increased ($p < 0.05$) in the dogs diagnosed with hepatic disease. In present study, a significant increase in RDW value was observed in hepatic disease group compared to healthy control, which is in consistent with the findings of Hu *et al.* (2013) [12] and Martinez *et al.* (2019) [3]. The exact underlying mechanism of the increased RDW values in hepatic disease is still unclear, however, several possible explanations are documented such as inflammation of hepatic parenchyma leading to release of the inflammatory cytokines adversely affecting the erythrocyte maturation and promoting the release of immature reticulocytes into the peripheral circulation (Pierce *et al.*, 2005; Cengiz *et al.*, 2013) [19, 18]. Similar association between RDW and inflammatory response has been reported by Lippi *et al.* (2009) [20] and Patel *et al.* (2010) [21]. Zhao *et al.* (2014) [22] also reported the elevated RDW levels due to oxidative stress and inflammation in canine. The increased RDW was also found associated with lower concentration of folic acid in hepatic disease patients (Hu *et al.*, 2013) [12]. Folic acid deficiency may result in impaired erythropoiesis and erythrocyte maturation leading to increased heterogeneity of erythrocytes. Further, alteration in RDW is also associated with portal hypertension commonly reported in hepatic disease could cause hypersplenism resulting in accelerated erythrocytes destruction and release of immature, larger RBCs from bone

marrow into the circulation (Evans, 1991; Cardenas and Gines, 2009) [24, 23].

In hepatic diseases, compared to conventional prognostic indicators such as albumin, A/G ratio, bilirubin (TB, DB and IB) and platelets, the RDW could serve as more reliable indicator possibly due to limitation of short half-lives for serum bilirubin, platelet and albumin as 10 h, 4 days and 10 – 20 days, respectively (Hu *et al.*, 2013) [12]. Whereas the lifespan of RBCs are 110-115 days. Therefore, RDW may reflects the hepatic disease status for longer period whereas the conventional parameters reflects current status (Adili *et al.*, 2014) [25].

The RDW value in renal disease group was also found to be increased as compared to the healthy control group but this increase was statistically insignificant (Table-1). RDW value in renal disease group of present study was found non significantly increased as compared to hepatic and healthy groups. Contrary to findings of the present study Martinez *et al.* (2019) [3] has reported significantly higher RDW values in the dogs with renal diseases. The possible reason of non-significant elevation of RDW in present study could be inclusion of canines in early stages of renal diseases along with chronic kidney disease. In present study, only 5 canines were having significantly elevated BUN and creatinine whereas, the remaining 6 canines were having mild to moderate elevation of serum BUN and creatinine concentration.

Despite increased RDW values in both the diseased groups, the MCV values of all the groups were within the reference range (Table-1). The previous studies on RDW have suggested that the RDW values should not be interpreted separately. The alternation in RDW with MCV can provide more useful prognostic information than the RDW alone. In the present study, regardless of significantly increased RDW values, MCV values were found within the reference range. Martinez *et al.* (2019) [3] also reported the normal MCV values with higher RDW in early cobalamin, iron, or folate deficiency and chronic hepatobiliary diseases. Further, the increased RDW and decreased MCV are also reported in hemorrhagic diseases (Arslan *et al.*, 2017) [26].

The overall correlation of RDW and hemato-biochemical parameters of this study is presented in correlation matrix (Table 2). The correlation between Hb and PCV was 0.606 ($P \leq 0.01$) and Hb and TEC was 0.53 ($P \leq 0.01$). The association between Hb and various parameters such as MCV, MCH, platelets, IB, TP and Alb was positive but non-significant. The non significant correlation was also found between Hb and MCHC, RDW, TLC, SGPT, TB, DB, Globulin, creatinine and BUN.

The association between PCV and TEC was significant ($r = 0.89$, $P \leq 0.01$) however the association between PCV and other hemato-biochemical parameters of study remain non-significant. The associations of TEC with other hemato-biochemical parameters of study also remain non-significant. The correlation between MCV and MCH was positive and significant ($r = 0.594$, $P \leq 0.01$) and with MCHC was negative but significant ($r = -0.586$, $P \leq 0.05$).

The correlation between RDW and SGPT was significant ($r = 0.394$, $P \leq 0.05$). The correlation of TB with DB was positive and significant ($r = 0.783$, $P \leq 0.01$) while the correlation of DB with IB was also positive and significant ($r = 0.500$, $P \leq 0.05$). The correlation of IB and BUN was 0.480 ($P \leq 0.05$). The significant positive correlation ($r = 0.52$, $P \leq 0.01$) was observed between Alb and TP. The correlation between BUN and TB ($r = 0.491$, $P \leq 0.05$), BUN and IB ($r = 0.480$, $P \leq 0.05$) and BUN and creatinine ($r = 0.727$, $P \leq 0.01$) was positive and significant. There was moderate but significant positive correlation between RDW and SGPT ($r = 0.394$) and a moderate negative correlation was found between RDW and PCV ($r = -0.309$) and TP ($r = -0.323$). There were no correlation between RDW and other

hematobiochemical parameters (Table 3 and Table 4). In the hepatic diseases group, the RDW has significant but negatively correlated ($r = 0.610$, $P \leq 0.05$) with Hb. However, no significant correlation was found with RDW and other parameters in hepatic and renal disease groups (Table 3 and Table 4).

The correlation data of the present study showed moderate but significant positive correlation between RDW and SGPT and a moderate negative correlation between RDW and PCV and TP. The RDW was reported to be a good predictor of hepatic fibrosis and showed significant association between RDW with SGPT and serum proteins (Xu *et al.*, 2015) [27]. Further, in hepatic diseases group of this study, the RDW has significant but innegative correlation with Hb. Similar findings were also reported by Chen *et al.*, (2013) [28]. The other hemato-biochemical parameters of the study did not show correlation with RDW.

Conclusion

The findings of the present study suggested that the increased RDW could be used as useful supplemental information along with standard biochemical markers of hepatic and renal diseases. The author concludes that RDW may also be proved useful prognostic marker in monitoring the therapeutic outcomes after suitable studies in canine patients.

Table 1. Mean±SE of hemato-biochemical parameters in hepato-renal disease

Parameters	Liver disease	Kidney disease	Healthy Control
Hb (g/dL)	9.81 ± 1.00 ^B	9.38 ± 0.76 ^B	13.10 ± 0.85 ^A
PCV (%)	28.24 ± 4.05	26.40 ± 2.54	38.67 ± 2.56
TEC (10 ⁶ /μL)	4.56 ± 0.67	4.44 ± 0.49	5.80 ± 0.42
MCV (fL)	62.64 ± 2.55	64.28 ± 1.88	66.70 ± 0.42
MCH (pg)	22.35 ± 0.90	22.61 ± 0.60	22.62 ± 0.28
MCHC (g/dL)	36.09 ± 1.17	35.42 ± 1.00	33.87 ± 0.27
RDW (%)	17.40 ± 0.94 ^A	14.24 ± 0.56 ^B	12.37 ± 0.41 ^B
Platelet (10 ³ /μL)	139.20 ± 32.88	171.64 ± 39.02	192.75 ± 17.72
TLC (10 ³ /μL)	11.70 ± 1.84	17.56 ± 3.13	9.48 ± 0.91
SGPT (U/L)	158.34 ± 21.61 ^A	47.21 ± 6.86 ^B	54.30 ± 2.65 ^B
Total bilirubin (mg/dL)	0.79 ± 0.09	0.80 ± 0.27	0.35 ± 0.03
Direct bilirubin (mg/dL)	0.39 ± 0.05	0.47 ± 0.16	0.20 ± 0.04
Indirect bilirubin (mg/dL)	0.33 ± 0.08	0.33 ± 0.14	0.15 ± 0.03
Total Protein (g/dL)	4.85 ± 0.35 ^B	5.06 ± 0.35 ^B	6.85 ± 0.37 ^A
Albumin (g/dL)	2.58 ± 0.23	2.48 ± 0.32	2.90 ± 0.27
Globulin (g/dL)	2.32 ± 0.35	2.58 ± 0.22	1.97 ± 0.12
BUN (mg/dL)	36.66 ± 10.36 ^B	100.31 ± 21.82 ^A	10.53 ± 1.25 ^B
Creatinine (mg/dL)	1.25 ± 0.09 ^B	7.26 ± 1.62 ^A	1.12 ± 0.08 ^B

A,B differs significantly ($P \leq 0.05$)

Table 2: Over all correlation matrix of various hemato-biochemical parameters with RDW in hepato-renal diseases

Parameters	HB	PCV	TEC	MCV	MCH	MCHC	RDW	Platelet	TLC	SGPT	TB	DB	IB	TP	Alb	Glob	Creatinin	BUN
HB	1																	
PCV	.606**	1																
TEC	.530**	.890**	1															
MCV	.278	.213	-.031	1														
MCH	.017	-.011	-.071	.594**	1													
MCHC	-.321	-.265	-.030	-.586**	.293	1												
RDW	-.029	-.309	-.239	-.199	-.279	.002	1											
Platelet	.083	.164	.113	.024	-.239	-.295	-.270	1										
TLC	-.044	.115	.092	.019	-.126	-.097	-.119	-.107	1									
SGPT	-.054	-.178	-.133	-.091	.246	.356	.394*	-.328	-.235	1								
TB	-.167	-.262	-.153	-.310	-.152	.166	.247	.218	.157	.192	1							
DB	-.303	-.356	-.318	-.091	.108	.188	.198	.132	.105	.020	.763**	1						
IB	.023	-.104	.005	-.325	-.273	.082	.299	.323	-.065	.075	.783**	.500*	1					
TP	.221	.293	.135	.267	.393	.086	-.323	-.149	-.008	-.286	-.275	.005	-.209	1				
Alb	.040	-.045	-.040	.124	.276	.207	-.043	-.316	.127	-.161	-.101	.062	-.117	.521**	1			
Glob	-.213	.151	.007	.126	.325	.146	-.088	-.072	.073	-.046	.080	.379	-.006	.393	-.196	1		
Creatinin	-.228	-.215	-.099	.079	.244	.131	-.225	-.046	.062	-.353	.233	.341	.121	.037	.185	.084	1	
BUN	-.198	-.288	-.295	-.026	-.076	-.077	.050	.198	-.077	-.270	.491*	.440*	.480*	-.265	-.172	.050	.727**	1

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Table 3. Univariate correlations between RDW and other parameters of hepatic disease group

Parameters	r value	P
Hb	-0.610	0.046
PCV	-0.492	0.124
TEC	-0.425	0.193
MCV	0.011	0.974
MCH	-0.343	0.303
MCHC	-0.315	0.345
Platelet	-0.120	0.725
TLC	-0.261	0.438
SGPT	-0.210	0.535
TB	0.185	0.586
DB	0.499	0.118
IB	0.283	0.399
TP	-0.022	0.949
Alb	0.405	0.217
Glob	-0.367	0.267
Creatinin	0.134	0.694
BUN	0.445	0.170

Table 4. Univariate correlations between RDW and other parameters of renal disease group

Parameters	r value	P
Hb	0.483	0.132
PCV	0.373	0.259
TEC	0.361	0.275
MCV	-0.349	0.293
MCH	-0.298	0.373
MCHC	0.066	0.847
Platelet	-0.376	0.254
TLC	0.103	0.763
SGPT	0.361	0.375
TB	-0.037	0.913
DB	-0.173	0.611
IB	0.126	0.712
TP	-0.275	0.413
Alb	-0.572	0.065
Glob	0.447	0.168
Creatinin	-0.110	0.747
BUN	0.135	0.692

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Conflict of interest

The authors have no conflict of interest.

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