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# Relationship Between Red Blood Cell Indices and Myxomatous Mitral Valve Disease in Small-Breed Dogs: A Retrospective Study

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## ABSTRACT

**Background:** Red blood cell (RBC) indices provide information on the size and haemoglobin content of erythrocytes. The RBC distribution width (RDW) is an index of size variability of the circulating RBC population. The correlation between various diseases and RDW in dogs has been demonstrated. Some studies have evaluated RDW in dogs with myxomatous mitral valve disease (MMVD), and conflicting results have been reported.

**Objectives:** We aimed to evaluate the association between RBC indices, complete blood cell counts (CBC), and serum biochemical and echocardiographic variables in small-breed dogs with MMVD.

**Methods:** RBC indices, CBC, and serum biochemical and echocardiographic variables were retrospectively investigated in 102 client-owned dogs with MMVD at various disease stages.

**Results:** RBC indices were not statistically significant among groups (control group, compensated group, decompensated group). RDW had a significant positive correlation with haematocrit (Hct) (correlation coefficient, 0.452) and a negative correlation with MCH (correlation coefficient, −0.498) and MCV (correlation coefficient, −0.357). The end-diastolic volume index, fractional shortening (%), and left atrial-anteroposterior diameter normalised for body weight were echocardiographic variables that affected MMVD severity.

**Conclusions:** We observed no correlation between RBC indices and MMVD. However, conflicting results have been reported in several other studies; thus, further studies should be considered.

## 1 | Introduction

Red blood cell (RBC) indices, including the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW), provide information regarding the size and haemoglobin content of erythrocytes (Kumiega et al. 2020). RBC indices are used for differential anaemia diagnosis (Harvey et al. 2012). Using these indicators determines whether an anaemia is haemorrhagic, haemolytic, or hyporegenerative.

RDW is an index of size variability of the circulating red blood cell population, easily measured by modern haematology analysers (Guglielmini et al. 2021). The extent of the RDW change varies depending on the degree of anisocytosis. Since reticulocytes are larger than mature RBCs and spherocytes are smaller than mature RBCs, RDW increases as these RBC types are mixed (Harvey et al. 2012). Generally, increased RDW indicates ineffective red cell production (such as iron and vitamin B12 deficiency), haemolysis, or can occur after blood transfusion. However, many other pathological causes can increase RDW values, including cardiorespiratory, vascular, neoplastic, endocrine, renal, hepatic, chronic, and acute systemic inflammation in humans (Felker et al. 2007; Salvagno et al. 2015). In dogs, high RDW values may be associated with hypothyroidism, hyperadrenocorticism, hepatic disease, neoplasia, immune-mediated haemolytic anaemia, chronic kidney disease, and pneumonia (Martinez et al. 2019).

In humans, chronic heart failure (CHF) patients are often known to have anaemia, and anaemia increases mortality in CHF patients (Anand et al. 2004). RDW is a factor used to evaluate the prognosis of patients with heart failure and prior myocardial infarction without heart failure in humans (Felker et al. 2007; Oh et al. 2009). Some studies have evaluated RDW in dogs with myxomatous mitral valve disease (MMVD), the most common cardiac disease in small-breed dogs, and conflicting results have been reported (Guglielmini et al. 2013, 2021; Martinez et al. 2019).

We aimed to evaluate the association between RBC indices, especially RDW in MMVD, and compare it with other laboratory variables and echocardiographic prognostic indices of MMVD in small-breed dogs. We also identified the variables affecting MMVD severity.

## 2 | Materials and Methods

### 2.1 | Selection of Cases and Controls

This study was a retrospective study conducted on 102 dogs that visited Kangwon National University Veterinary Teaching Hospital between 2018 and 2022. This study was limited to small-breed dogs; therefore, dogs that weighed 10 kg or more were excluded. The included dogs were classified into three groups: control group, compensated group, and decompensated group by MMVD staging. MMVD was diagnosed through a classification based on 2019 guidelines of the American College of Veterinary Internal Medicine consensus in this study. The stages included stage B1 (asymptomatic dogs with valvular disease but no cardiac remodelling), stage B2 (asymptomatic dogs with cardiac remodelling), stage C (symptomatic dogs with evidence of heart failure), and stage D (symptomatic dogs with heart failure refractory to standard treatment) (Keene et al. 2019). Twenty-five healthy client-owned dogs who visited our hospital for various purposes were enrolled as the control group. Of the 77 dogs diagnosed with MMVD, 49 dogs diagnosed with MMVD stage B1 and B2 were named the 'compensated group', and the other 28 dogs diagnosed with MMVD stage C and D were named the 'decompensated group'. Enrolment was based on the results of physical examination, echocardiographic and Doppler echocardiographic examination, and complete blood cell count (CBC), including RBC indices and serum biochemical profile.

Dogs with underlying diseases known to increase RDW and affect the erythrocyte indices, such as hypothyroidism, hyperadrenocorticism, hepatic disease, neoplasia, immune-mediated haemolytic anaemia, chronic kidney disease, pneumonia, and systemic inflammatory diseases were excluded from this study (Martinez et al. 2019). Dogs with concomitant congenital or acquired heart disease were also excluded. To limit the impact of anaemia, cases with haematocrit values below the lower reference limit were excluded.

### 2.2 | Blood Analysis

CBC and serum biochemical analyses were performed within 72 h of their echocardiographic examination for the stability of the test results. CBCs were performed using a commercial automated haematology analyser (IDEXX procyte), and biochemistry was measured using a commercial automated biochemistry analyser (IDEXX catalyst one, mindray BS-240Pro).

### 2.3 | Echocardiographic Examination

Echocardiographic and echo-Doppler examinations were performed in the unsedated dogs. Standard echocardiographic scan planes were used for each dog. The left ventricular internal diastolic diameter normalised for body weight (LVIDDn), end-diastolic volume index (EDVI), end-systolic volume index (ESVI), and fractional shortening (FS) were obtained from two-dimensional (2D)-guided M-mode images from the right parasternal short-axis view. Normalization for the effect of body weight (BW) of the measured left ventricular diameters was obtained by applying previously published equations (LVIDd (cm)/BW (kg)$^{0.294}$) (Cornell et al. 2004). Left ventricular systolic and diastolic diameters were used to calculate end systolic volume and end diastolic volume, respectively, using the Teichholz formula (De Madron et al. 2015). The diameter of the left atrium (LA) and aortic root (Ao) was measured at early diastole from the 2D method derived from the right parasternal short axis view. The LA-to-Ao ratio (LA/Ao) was calculated. The left atrial anteroposterior diameter (LAD) was obtained using a 2D method using the right parasternal long-axis four-chamber view. The effect of BW on the measured LA diameter was normalised by applying the previously described method: [LAD (cm)/BW(kg)$^{0.324}$] (Marchesotti et al. 2019). Trans-mitral blood flow was recorded from the left parasternal apical four-chamber view, and the measurement of the peak velocity of early diastolic blood flow (E-max) was obtained. Early diastolic mitral annular velocity (E') was measured from the left apical four-chamber view using tissue Doppler imaging. The early mitral inflow velocity to early diastolic mitral annular velocity ratio (E/E') was then calculated.

### 2.4 | Statistical Analysis

SPSS version 26.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The normal distribution of all data was assessed using the Shapiro–Wilk test. Normally and non-normally distributed data were reported as the mean ± standard deviation (SD) values and median and range, respectively. Categorical variables (sex) were presented as frequency counts. Analysis of variance (ANOVA) and Kruskal–Wallis tests were

used to compare normally distributed continuous variables (RBC, MCH, MCHC, white blood cell, haemoglobin, haematocrit (Hct), RDW, MCV, total protein, albumin, LA/Ao, LVIDDn, EDVI, FS, E-max, LADn, and E/E') and non-normally distributed variables (weight, platelet, triglyceride, cholesterol, blood urea nitrogen [BUN], creatinine, and ESVI) in dogs divided into three groups (control, compensated, and decompensated groups). The Mann–Whitney U test was used when the null hypothesis was rejected. The chi-squared test was used to evaluate categorical variables (sex).

Pearson's correlation analysis was used to assess the correlation between the RDW and all variables included in the study (demographic, haematologic, serum biochemical variables, and echocardiographic indices). Multiple linear regression analysis was used to determine which factors were associated with RDW. A logistic regression model was used to examine the relationship between MMVD severity and the variables.

The significance level was set at $p$-values $< 0.05$.

## 3 | Results

### 3.1 | Study Population

The inclusion criteria were met by 102 dogs (51% females and 49% males) of different breeds. Their mean age was $12.57 \pm 0.346$ years, and the median BW was 4.15 kg (1.7–9.3). Of these, 25 dogs were healthy, 49 dogs diagnosed with MMVD stages B1 or B2 were in the compensated group, and 28 dogs diagnosed with MMVD stage C or D were in the decompensated group. The most represented breeds of dogs with control group were Maltese ($n = 11$), Yorkshire Terrier ($n = 3$), Pomeranian ($n = 3$), Poodle ($n = 2$), Mix ($n = 2$), and others (Chihuahua, Pekinese, Dachshund, Bichon Frise, $n = 4$). The most represented breeds of dogs with MMVD were Maltese ($n = 37$), Shih Tzu ($n = 16$), Mix ($n = 7$), Pomeranian ($n = 5$), Yorkshire Terrier ($n = 4$), Poodle ($n = 2$), Spitz ($n = 2$), Chihauhau ($n = 2$), and others (Italian greyhound, Toy fox terrier, $n = 2$). Twenty dogs (20%) had concomitant non-cardiac diseases, including neurological ($n = 7$), respiratory ($n = 6$), urinary ($n = 3$), genital ($n = 3$), and dermatological ($n = 1$) diseases. Dogs in the control group and dogs diagnosed with MMVD stage B1 were not prescribed any drugs for MMVD. Dogs diagnosed with MMVD stage B2 were prescribed only cardiotonic vasodilators, while dogs diagnosed with MMVD stage C and D were prescribed phosphodiesterase 3 inhibitors, diuretics, angiotensin-converting enzyme inhibitors, and vasodilators. Dogs with MMVD were older than those in the control group.

### 3.2 | Laboratory and Echocardiographic Variables

A summary of the characteristics of the 102 dogs with various variables, including their demographic, laboratory, and echocardiographic data, divided into three groups according to MMVD severity, is presented in Table 1. No differences in RBC indices were observed among the control, compensated, and decompensated groups. The median serum BUN concentration in the decompensated group was significantly ($p < 0.05$) higher than in the control and compensated groups. The mean ± SD albumin concentration in the decompensated group was significantly ($p < 0.05$) lower than that in the control and compensated groups. The median serum creatinine concentrations of the compensated and decompensated groups were significantly ($p < 0.05$) higher than that of the control group.

The compensated and decompensated groups had significantly ($p < 0.05$) higher values of all echocardiographic variables (LA:Ao, LVIDDn, ESVI, EDVI, FS, E-max, LADn, E/E') than the control group. Among them, the decompensated group had higher ESVI, EDVI, FS, LADn, and E/E' than the control and compensated groups.

### 3.3 | Correlation and Odds Ratio

Pearson's correlation analysis was conducted to understand the correlation between RDW and the laboratory and echocardiographic variables. The results of Spearman's correlation between the RDW and various variables are presented in Table 2. Hct, MCH, and MCV were correlated with RDW, and Hct had the highest positive correlation, with a correlation coefficient of 0.452. Conversely, MCH had the highest negative correlation, with a correlation coefficient of −0.498. A graph explaining the linear correlation of the factors with RDW is presented in Figure 1.

Based on the correlation identified through Pearson's correlation analysis, a multiple linear regression analysis was conducted to examine the influence of each factor on RDW. This analysis method selected the "enter" method. This regression model was considered suitable based on the $F$ value and Durbin–Watson analysis results, and 43.1% of the explanatory power was confirmed through the adjusted $R^2$ value. This result is presented in Table 3. MCV, MCH, and Hct significantly affected RDW at $p < 0.05$. MCV and MCH had a negative effect on RDW, whereas Hct had a positive effect on RDW.

Logistic regression analysis was performed to identify the factors that significantly affected MMVD severity among the various variables. This result is presented in Table 4. Analysis revealed that three variables—EDVI, FS, and LADn—had statistically significant effects on MMVD severity at $p < 0.05$. For LA/Ao, the 95% confidence intervals were not statistically significant and excluded. MCV was also excluded because it was not statistically significant via ANOVA.

## 4 | Discussion

We focused on whether there was a significant association between laboratory variables, especially RBC indices, echocardiographic indices, and MMVD severity in small-breed dogs. The findings of this study revealed no difference in any RBC indices values, including RDW, among the control, compensated, and decompensated groups. Age, laboratory variables, including BUN, creatinine, and albumin, and all echocardiographic variables revealed significant differences between the three groups. Hct, MCH, and MCV were correlated with RDW, and all three variables had significant effects on RDW. Logistic regression analysis revealed that EDVI, FS, and LADn had statistically significant effects on MMVD severity.

**TABLE 1** | Demographic, laboratory, and echocardiographic data comparisons between three groups of dogs with MMVD (control, compensated, decompensated).

| Variables | Control ($n = 25$) | MMVD | | Overall $p$ value |
|---|---|---|---|---|
| | | Compensated ($n = 49$) | Decompensated ($n = 28$) | |
| Age (year) | 9.88 ± 3.26 | 13.06 ± 3.31 | 14.11 ± 2.67 | <0.05 |
| Weight (kg) | 4.2 (1.8–7.2) | 4.25 (1.7–9.3) | 3.89 (2–8.1) | 0.637 |
| Sex (F/M) | 14/11 | 21/28 | 17/11 | 0.273 |
| Haematocrit (%) | 46.09 ± 5.37 | 45.037 ± 6.56 | 47.589 ± 7.37 | 0.261 |
| MCH (pg) | 22.09 ± 1.15 | 22.2 ± 1.15 | 22.30 ± 1.26 | 0.804 |
| MCHC (g/dL) | 34.56 ± 1.18 | 34.95 ± 1.15 | 35.05 ± 1.71 | 0.444 |
| MCV (fL) | 63.9 ± 2.53 | 63.6 ± 3.24 | 63.7 ± 3.47 | 0.93 |
| RDW (%) | 18.080 ± 1.9155 | 18.253 ± 1.5969 | 18.3 ± 1.7751 | 0.821 |
| TG (mg/dL) | 71 (28–249) | 73 (24–207) | 92.89 (26–178) | 0.835 |
| Cholesterol (mg/dL) | 167.49 (58–317) | 203 (80–336) | 180 (81–321) | 0.221 |
| BUN (mg/dL) | 19.12 (4.93–48) | 17 (4–39) | 26 (7–72.3) | <0.05 |
| Creatinine (mg/dL) | 0.7 (0.3–1.3) | 0.8 (0.3–1.6) | 0.9 (0.5–1.9) | <0.05 |
| TP (g/dL) | 6.32 ± 0.76 | 6.692 ± 0.6 | 6.504 ± 0.77 | 0.08 |
| Albumin (g/dL) | 3.041 ± 0.3708 | 2.967 ± 0.3772 | 2.742 ± 0.527 | <0.05 |
| LA/AO | 1.34 ± 0.146 | 1.626 ± 0.267 | 2.41 ± 0.59 | <0.05 |
| LVIDDn | 1.31 ± 0.24 | 1.53 ± 0.252 | 1.97 ± 0.24 | <0.05 |
| ESVI (mL/m$^2$) | 4.12 (1.17–13.8) | 5.35 (1.16–12.98) | 7.8 (2.98–18.3) | <0.05 |
| EDVI (mL/m$^2$) | 23.36 ± 7.95 | 31.0.7 ± 12.64 | 61.48 ± 20.85 | <0.05 |
| FS (%) | 47.75 ± 12.72 | 50.46 ± 8.45 | 58.25 ± 9.03 | <0.05 |
| E-max (cm/s) | 72 ± 14.57 | 90.09 ± 23.36 | 132.67 ± 34.31 | <0.05 |
| LADn | 11.88 ± 2.59 | 13.04 ± 2.77 | 20.78 ± 4.75 | <0.05 |
| E/E’ | 11.31 ± 3.04 | 12.10 ± 3.07 | 14.63 ± 4.7 | <0.05 |

*Note*: Age, BUN, creatinine, albumin, and all echocardiographic data showed significant differences between the three groups. Normally distributed data are expressed as mean ± SD; nonnormally distributed data are expressed as median (range).

Abbreviations: BUN, blood urea nitrogen; EDVI, end diastolic volume index; E/E’, early mitral inflow velocity to early diastolic mitral annular velocity ratio; E-max, peak velocity of early diastolic blood flow; ESVI, end systolic volume index; FS, fractional shortening; LA/AO, left atrium to aorta ratio; LAD, left atrial anteroposterior diameter normalised for body weight; LVIDDn, left ventricular internal diastolic diameter normalised for body weight; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RDW, red blood cell distribution width; TG, triglyceride; TP, total protein.

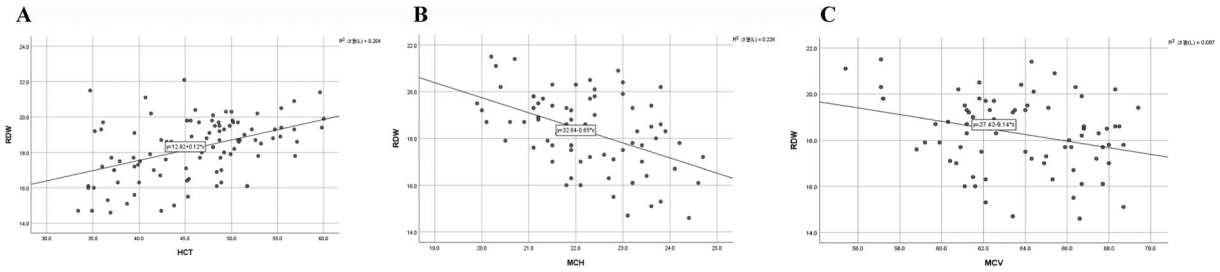


**FIGURE 1** | Correlation between (A) Hct, (B) MCH, (C) MCV, and RDW. Hct was positively correlated with RDW and MCH, and MCV was negatively correlated with RDW.

Our study results indicated no association between RBC indices and the three groups. RBC indices, including MCV, MCH, MCHC, and RDW, provide information on haemoglobin concentration and erythrocyte size. Abnormal values usually mean that anaemia exists and are used to distinguish the type of anaemia. Anaemia commonly occurs in human patients with chronic heart failure (Tang and Katz 2006). However, a study has revealed a low anaemia prevalence in dogs with MMVD (Guglielmini et al. 2013).

**TABLE 2** | Correlations between demographic, laboratory, and echocardiographic variables and RDW in 102 dogs with MMVD by Pearson's correlation analysis.

| Variable | Correlation coefficient | *p* value |
|---|---|---|
| Age (year) | −0.36 | 0.72 |
| Weight (kg) | 0.104 | 0.297 |
| Haematocrit (%) | 0.452 | <0.05 |
| MCH (pg) | −0.498 | <0.05 |
| MCHC (g/dL) | −0.18 | 0.07 |
| MCV (fL) | −0.357 | <0.05 |
| TG (mg/dL) | −0.29 | 0.775 |
| Cholesterol (mg/dL) | −0.121 | 0.225 |
| BUN (mg/dL) | 0.007 | 0.946 |
| TP (g/dL) | 0.053 | 0.594 |
| Albumin (g/dL) | −0.005 | 0.959 |
| Creatinine (mg/dL) | −0.075 | 0.452 |
| LA/AO | 0.001 | 0.992 |
| LVIDDn | 0.004 | 0.971 |
| ESVI (mL/m$^2$) | 0.006 | 0.952 |
| EDVI (mL/m$^2$) | −0.006 | 0.952 |
| FS (%) | 0.049 | 0.622 |
| E-max (cm/s) | 0.024 | 0.812 |
| LADn | −0.087 | 0.383 |
| E/E' | −0.046 | 0.647 |

*Note*: Hct showed a positive correlation with RDW, and MCH and MCV showed negative correlation with RDW.

Abbreviations: BUN, blood urea nitrogen; EDVI, end diastolic volume index; E/E', early mitral inflow velocity to early diastolic mitral annular velocity ratio; E-max, peak velocity of early diastolic blood flow; ESVI, end systolic volume index; FS, fractional shortening; LA/AO, left atrium to aorta ratio; LAD, left atrial anteroposterior diameter normalised for body weight; LVIDDn, left ventricular internal diastolic diameter normalised for body weight; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RDW, red blood cell distribution width; TG, triglyceride; TP, total protein.

Combined with the results of this study, it can be inferred that anaemia prevalence does not significantly increase as MMVD worsens.

The most common causes of heart failure in humans are coronary artery disease, high blood pressure, diabetes, obesity, and smoking. However, MMVD is the most common cause of heart failure in dogs. The cause of valvular degeneration in dogs is uncertain, but it has been presumed that genetic factors play an important role. The pathophysiological mechanism of heart failure is similar in humans and dogs, but there are differences in epidemiological aspects (Johnson 2014). Since heart failure usually occurs as another underlying disease in humans, the pathophysiological mechanism of the underlying disease may have affected the RBC indices. RDW increases in an obesity state in humans (Fujita, Strodthoff et al. 2013). Unlike humans, dogs have a high concentration of HDL cholesterol and lack the enzyme cholesterol ester transfer protein, which makes it less likely to develop coronary artery disease (Ettinger et al. 2016).

In this study, kidney values, creatinine and BUN, were significantly higher in the MMVD group than in the control group, although creatinine shows no clinical difference when evaluated by International Renal Interest Society CKD staging guideline. The heart and kidneys are pathophysiologically related. Cardiorenal syndrome is a disorder of the heart and kidneys, resulting in a cascade of different feedback mechanisms, whereby acute or chronic dysfunction of one organ may induce acute or chronic dysfunction of the other (Ronco et al. 2008). When heart failure progresses, prerenal hypoperfusion occurs, resulting in renal ischemia. Due to the activation of the renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system, preglomerular vasoconstriction occurs, which worsens renal ischemia and further lowers GFR (Rangaswami et al. 2019). Furosemide administration may play a role in cardiovascular and renal disorders (Martinelli et al. 2016). Several studies have also revealed that heart failure affects renal impairment in dogs, similar to this study's results (Guglielmini et al. 2013, 2021; Pfeifer et al. 2022).

In humans, hypoalbuminaemia is associated with increased mortality risk in patients with systolic heart failure (Horwich et al. 2008). Hypoalbuminaemia in patients with heart failure may result from haemodilution, malnutrition, chronic inflammation, proteinuria, or other mechanisms (Horwich et al. 2008). Our study's result indicated that albumin concentration decreases as MMVD progresses. Therefore, hypoalbuminaemia may adversely affect the prognosis of dogs with MMVD.

This study reveals that Hct, MCH, and MCV are linearly correlated and significantly affect RDW. Hct, MCH, and MCV were correlated with RDW, and Hct had the highest positive correlation, with a correlation coefficient of 0.452. Conversely, MCH had the highest negative correlation coefficient (−0.498). Regarding MCV, when RDW increased by 1, MCV decreased by 0.155 times, and MCH decreased by 0.37 times. Conversely, Hct increased by 0.123 times when the RDW increased by 1.

An increased RDW indicates that a variation in RBC size exists. The decrease in MCV and MCH as RDW increases indicates hypochromic and microcytic RBC increase, which can appear in portosystemic shunts, malnutrition, and iron deficiency (Valenciano and Cowell 2019). Iron deficiency is common in human patients with systolic heart failure, even if patients are not anaemic (Jankowska et al. 2010). Iron deficiency is associated with decreased aerobic performance and exercise intolerance, as observed in chronic heart failure (Jankowska et al. 2011). Similar to humans, iron deficiency is frequently associated with MMVD in dogs. Almost 20% of patients with MMVD are iron-deficient (Savarese et al. 2018). Similarly, it assumes that there is an association between iron deficiency and MMVD in our results. However, since research on iron deficiency was not conducted in this study, additional research is needed to prove this.

Echocardiographic variables were selected based on the values used for left cardiac pressure evaluation, cardiac remodelling evaluation, and systolic function evaluation.

**TABLE 3** | Multiple linear regression analysis for examining the influence of each factor on RDW.

| Variables | *B* | S.E | *β* | *t* | *p* value | TOL | VIF |
|---|---|---|---|---|---|---|---|
| Constant | 30.676 | 2.841 | | 10.797 | <0.01 | | |
| MCV | −0.155 | 0.056 | −0.288 | −2.755 | 0.007 | 0.516 | 1.939 |
| MCH | −0.37 | 0.147 | −0.259 | −2.512 | 0.014 | 0.53 | 1.886 |
| Hct | 0.123 | 0.021 | 0.479 | 5.923 | <0.01 | 0.859 | 1.164 |
| $F(p)$ | | | | 26.553 (<0.01) | | | |
| Adj. $R^2$ | | | | 0.431 | | | |
| Durbin-Watson | | | | 1.822 | | | |

MCV, MCH, and Hct had a significant effect on RDW at $p < 0.05$. MCV and MCH had a negative effect on RDW, and Hct had a positive effect on RDW.
Abbreviations: MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; Hct, haematocrit; TOL, tolerance; VIF, variance inflation factors.

**TABLE 4** | Logistic regression analysis between demographic, laboratory, and echocardiographic variables and MMVD severity.

| | | | | | | 95% CI | |
|---|---|---|---|---|---|---|---|
| Variables | *B* | SE | Wald | *p* value | OR | LLCI | ULCI |
| EDVI Tei | 0.111 | 0.049 | 5.196 | 0.023 | 1.118 | 1.016 | 1.23 |
| FS (%) (Tei) | 0.264 | 0.109 | 5.933 | 0.015 | 1.303 | 1.053 | 1.612 |
| LADn | 0.621 | 0.265 | 5.513 | 0.019 | 1.862 | 1.108 | 3.128 |

*Note*: EDVI, FS, and LADn have statistically significant effects on MMVD severity at $p < 0.05$.
Abbreviations: EDVI, end diastolic volume index; FS, fractional shortening; LAD, left atrial anteroposterior diameter; LLCI, lower limit confidence interval; ULCI, upper limit confidence interval; B, unstandardised coefficient; Tei, Teichholz formula.

All echocardiographic variables revealed differences between the three groups. When logistic regression analysis was performed to identify the factors that significantly affected MMVD severity, only three echocardiographic variables were identified as factors. An increase in EDVI by 1 increases the probability of belonging to the decompensated group 1.118 times. An increase in FS by 1 increases the probability of belonging to the MMVD decompensated group 1.303 times. In addition, if LADn is increased by 1, the probability of belonging to the MMVD decompensated group increases 1.862 times.

In MMVD, regurgitation occurs due to valve incompetence, resulting in cardiac remodelling with an increased activation of RAAS and increased preload and afterload. As MMVD progresses, it deteriorates into ventricular dysfunction (Keene et al. 2019), associated to EDVI, FS, and LADn increase (Nyland and Mattoon 2002; O'Gara et al. 2008).

This study had some limitations because of its retrospective design. First, since there was no age limit for choosing samples, the fact that the control group is much younger can be a bias given that the incidence of MMVD increases with age. Second, the small sample size may have affected our results. Finally, nutritional status and body condition score evaluations that may affect haematological values were not always documented.

## 5 | Conclusion

We evaluated variables correlated with RBC indices and risk factors for MMVD deterioration. No difference was observed in any RBC indices value, including RDW, between the control, compensated, and decompensated groups. This is not the first study published on a lack of association between RBC indices and MMVD, but this correlation remains controversial given the recently published study. For this reason, the correlation between RBC indices and MMVD remains controversial, so further studies including a larger animal population should be needed.

### Author Contributions

EJH and JOA conceived and designed the study and drafted the manuscript. EJH, YJ and JHA performed the treatments and analysed the data. SC and JYC interpreted data. All the authors interpreted the data, revised the manuscript, and approved the final version.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Ethics Statement

Since the study was based on archived clinical case records, ethical review and approval were not required.

### Data Availability Statement

The datasets generated for this study are available on request from the corresponding author.

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