Coefficient of variation of red cell distribution width has correlations to periodontal inflamed surface area in non obese hypertensive patients

Srirangarajan Sridharan, Paruchuri Sravani and Dr. Ravi J Rao

Department of Periodontics, Bangalore Institute of Dental Sciences and Post Graduate Research Centre, 5/3, Hosur road, Bangalore – 560029; Karnataka, India.

ABSTRACT

Aims: To test the hypothesis that higher periodontal inflammed surface area (PISA) has a positive correlation with the coefficient of variation of red cell distribution width (RDW-CV) in non-obese hypertensive patients.

Materials and Methods: Hypertensive subjects aged between 40-60 years with and without periodontitis were enrolled for the study. They completed a structured questionnaire which included gender, height, weight and other variables. Body mass index was calculated to exclude obese individuals. Clinical periodontal parameters were recorded. PISA was calculated and participants were divided into four groups: Group 1: Non-hypertension without periodontitis, Group 2: Non-hypertensive with periodontitis, Group 3: Hypertensive without periodontitis and Group 4: Hypertensive with periodontitis. Hematologic evaluation included red cell distribution width analysis.

Results: ANOVA showed age and diastolic blood pressure was significantly related to RDW-CV at $p < 0.001$. Periodontal parameters showed significant association with RDW-CV in both hypertensive and non hypertensive groups at $p < 0.001$. Pearson correlation test showed significant association between RDW-CV and PISA. Multivariate regression models showed PISA to be a significant predictor for RDW-CV in periodontitis group when compared to a non-periodontitis group.

Conclusions: The increase in the RDW-CV in periodontitis group in both hypertensive and non hypertensive indicates the independent role of increased inflammation on pathogenic alteration of red cell morphology.

Keywords: Periodontal inflamed surface area; Periodontitis; Red cell distribution width; Hypertension.

Introduction

Periodontitis has been shown to cause an increase (19%) in the risk of cardiovascular disease and this risk increases to 44% in people aged above 65 years (Janket *et al.,* 2003). Among cardiovascular diseases, hypertension and myocardial infarction are the foremost causes of death globally (Sundstrom *et al.,* 2011). Both periodontitis and hypertension are associated with increased inflammation. Blood count parameters are shown to predict the severity of both these diseases (Uysal *et al.,* 2016, Jain *et al.,* 2013). Smoking, diabetes mellitus, obesity and poor oral hygiene are common risk factors for both of the above mentioned diseases (Leong *et al.,* 2014; Modeer *et al.,* 2011; Teles and Wang, 2011). Interestingly, studies have suggested a specific association between systemic hypertension and pathogenic periodontal pockets (Angeli *et al.*, 2003; Franek *et al.*, 2005; Franek *et al.*, 2009). In periodontitis low-grade chronic inflammation has been associated with endothelial dysfunction (Fichtlscherer *et al.*, 2000), low bacteremia with low immune response (Paraskevas *et al.*, 2008) and oxidative stress (Touyz RM, 2004) resulting in hypertension (Tsakos *et al.,* 2010; Vidal *et al.,* 2011). On the other hand hypertension can affect

Correspondence to: Dr. Srirangarajan Sridharan MDS, Professor and Head, Department of Periodontics, Bangalore Institute of Dental Sciences and Postgraduate Research Centre. 5/3, Hosur Road, Bangalore- 560029. Email: docranga@yahoo.com; Fax: 080-41506025.

© International Academy of Periodontology

periodontal membrane vessels, inducing malfunction of small arterioles and gingival bleeding, causing alterations to the tooth-supporting alveolar bone (Peres *et al*., 2012). We propose that a reliable linking factor between these diseases which could help us to predict certain cardiovascular events is the estimation of the width of the red cell (RDW). Estimation of RDW-CV is relatively inexpensive and performed as part of a complete blood cell count and quantifies the variability in size of circulating red blood cells. An increase in RDW-CV reflects the presence of immature red blood cells in the peripheral circulation. This is caused by increased red blood cell destruction, pathologic iron homeostasis and ineffective erythropoiesis (Zalawadiya *et al.,* 2010). RDW-CV has been studied in coronary artery disease, stroke, hypertension, LVM, and is novel marker for stress erythropoiesis, and has a prognostic role in coronary heart disease (Bujak *et al.,* 2015). Currently, there are a number of classifications that can be used to describe periodontitis. However, none of these, quantify the inflammatory burden associated with this disease. Assessment of the periodontal inflammed surface area (PISA) reflects surface area of the bleeding pocket epithelium in square millimeters and is quantifiable, hence it may provide decisive conclusions on periodontitis as a risk factor for other diseases (Nesse, *et al*., 2008). To date, no studies have analyzed the changes in RDW-CV in periodontitis stage II and III individuals (Caton *et al*., 2018). Hence we investigated the changes associated with these two inflammatory diseases on RDW-CV. The aim of this study was to test the hypothesis that higher PISA values have positive correlation with RDW-CV in hypertensive patients.

Materials and methods

Power and sample size

The sample size was estimated using the software G Power v. 3.1.9.2. Assuming the size of difference to be larger, the effective size was fixed at 0.4 (40%), power of the study at 80% and the margin of the error at 5%, the mean difference of 1mm in CAL determines the group to which the subjects will be belonging (periodontitis or not). The effective sample size for each group was 16 in each group. (Bonato *et al.,* 2012)

Study population

Eighty gender-matched individuals aged between 40-60 years with a minimum of 20 teeth (excluding third molars) were enrolled in this study. The study protocol, was prepared in accordance with the Declaration of Helsinki of 1973 (as revised in 2013) and was approved by the Institutional Review Board and ethical committee (No:BIDS/786/2015) of Bangalore Institute of Dental Sciences and Post graduate research center. The study details were explained and written informed consent were obtained for all participants in this study. Individuals were excluded from the study if they had any of the following: obese individuals (BMI >30) according to the world health organization 1995; any history of blood dyscrasia such as anemia, thrombocytopenia; systemic diseases such as diabetes mellitus and thyroid disease; patients with renal disorders, cardiac disorders (valve problems congenital heart disease), patients taking any drug which could affect the blood pressure apart from the antihypertensive drugs; smokers both former and currently showing high dependency according to the Fagerstrom test score and pregnant women. None of the included individuals had received treatment for periodontal disease within six months before commencement of the study. Occasional smokers (showing low dependency on the Fagerstrom test score) were included although on minimal numbers (n= <10%).

Questionnaire

All the individuals filled in a questionnaire which included information concerning gender; height; weight; calculation for body mass index in accordance to WHO 1995 as follows ≤ 18.50kg/m^2- Underweight,18-24 kg/m^2- normal weight, 25-29 kg/m^2- pre obese and ≥ 30 kg/m^2 as obese. Detailed medical and dental history was obtained; in addition oral hygiene habits were recorded, medication used for dental and other systemic diseases if any were noted.

Blood pressure evaluation

A standard mercury sphygmomanometer (Diamond, Bangalore, India) was used to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP) on the participants' right arm in a seated position after at least 5 min of rest before the initial measurement. The blood pressure measurements were taken twice at a five minute interval and in all three postures standing, sleeping and sitting; the average values were recorded. For our analyses, pre-hypertension was defined as having DBP < 90 mmHg and hypertension was defined as having an average DBP ≥ 90 mmHg or medicated for hypertension (Rivas-Tumanyan *et al*., 2013).

Examiner calibration

Two examiners (SS and PS) were calibrated, using five subjects who were not included in the study, and were requested to volunteer for calibration exercises of the clinical parameters. The periodontal probing depth estimation was judged to be reproducible if the intra examiner agreement was within ± 1 mm between the repeated measurements was at least 80%. The kappa value for intra examiner variability agreement, between the two measurements was recorded to be 0.89.

Study groups

Plaque index (PI) (Turesky *et al.*,1970), gingival index (GI) (Löe and Silness 1963), Gingival recession, probing pocket depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) were measured at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual) of all the teeth using UNC 15 periodontal probe (HuFriedy Il,Chicago). Individuals were categorized according to the classification of periodontal diseases and conditions (American Academy of Periodontology and European Federation of Periodontology 2017) into four groups consisting of 20 subjects in each group as; Group 1 - non-hypertensive without periodontitis; Group 2 - non-hypertensive with periodontitis stage II and III; Group 3 - hypertensive without periodontitis and Group 4 - hypertensive with periodontitis stage II and III.

Periodontal inflamed surface area (PISA) calculation

The PISA scores reflecting the amount of inflammed periodontal tissue were calculated (Hujoel *et al.*, 2001). The CAL, recession, bleeding on probing and periodontal epithelial surface area (PESA) measurements were used and in a step by step approach the PISA values for each patient were calculated.

Analysis of red cell distribution width (RDW-CV)

5 ml of blood was drawn from the ante-cubital fossa by veni-puncture and transferred into EDTA anticoagulant containing vaccutainers. The samples were then analyzed using the Sysmex KX-21N automated hematology analyzer (Sysmex Corporation, Kobe, Japan). RDW-CV measures the deviation of the red cell width and not the actual width or size of the individual cell. The RDW- CV is a calculation based on the width of the distribution curve and the mean cell size. The formula as follows was used: RDW-CV= 1SD/ MCV × 100

Data analysis

All the data were compiled and subjected to statistical analysis using SPSS software 16.0. Age, socio-economic status, gender, body mass index, smoking status and diastolic blood pressure were taken as potential confounding factors; chi square test and ANOVA was performed to find the association between red cell distribution width, hypertension and periodontal disease. ANCOVA followed by Bonferroni's post hoc analysis was performed to compare the clinical parameters to RDW-CV after adjusting for possible covariates. Pearson correlation coefficient was calculated to study the relationship between RDW-CV and various parameters such as PI, GI, PD, CAL, PISA, BMI and diastolic blood pressure. Multivariate linear regression between dependent variable (RDW-CV) and independent variables at 95% confidence interval was calculated. Levels of significance were accepted at p values less than 0.05.

Results

Of the individuals included in the study (n = 80) 40 were systemically healthy and 40 were hypertensive patients. The demographic data are presented in Table 1 and there were no statistically significant differences between gender, socio-economical status, smoking and body mass index. Post hoc analysis showed age and diastolic blood pressure had significant associations to RDW at $p<0.001$ (Table 2). For the mean PI, GI, PPD, CAL, PISA (dependent variables) and RDW-CV there were statistically significant associations for both the hypertensive and non-hypertensive individuals at $p<0.001$ (Figures 1, 2 and 3). Pearson correlation coefficient tests were performed to check for the relationship between RDW-CV and clinical parameters in all of the study groups (Table 3). The results showed there was a strong positive correlation between RDW-CV and PISA in the periodontitis group of both non-hypertensive and hypertensive group than the non-periodontitis group ($p <0.001$). Diastolic blood pressure was significantly correlated to RDW-CV in the non-hypertensive group with periodontitis and hypertensive group without periodontitis ($p <0.01$ and $p <0.03$ respectively). Multivariate linear regression analysis with RDW-CV as a dependent variable showed a significant association with PISA and DBP in the non-hypertensive group with periodontitis. PI was found to be associated with RDW-CV in the non-hypertensive group without periodontitis ($p <0.004$). In the hypertensive and non-hypertensive groups with periodontitis, PISA was found to be a significant predictor for RDW-CV ($p<0.001$) (Table 4 and 5).

Discussion

This cross sectional study evaluated the effect of increased PISA scores (as in periodontitis stage II and III) on red cell distribution width in non-hypertensive and hypertensive individuals. Results from this study demonstrated PISA scores to be significantly associated with an increase in red cell width in the periodontitis group in non-obese individuals. Hypertension may also individually affect the red cell width. Together both hypertension and chronic periodontitis could increase the inflammatory burden and width of the red cell which we hypothesize to be a cause for unprecedented cardiac events.

For periodontitis to be a risk factor for cardiovascular disease in non-hypertensive and hypertensive individuals, studies have proposed that if a causative relationship exists between periodontitis and cardiovascular diseases it is more likely to be attributed to an incremental contribution of systemic inflammation rather than direct colonization of atherosclerotic plaques with

Table 1. Demographic data amongst the study groups

Variables		*NH+NP*		*NH+P*		*H+NP*		*H+P*		*P-Value*
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	
Sex	Males	7	35%	7	35%	6	30%	6	30%	0.97[a]
	Females	13	65%	13	65%	14	70%	14	70%	
SES	Lower	6	30%	7	35%	7	35%	8	40%	0.14[a]
	Middle	9	45%	2	10%	8	40%	8	40%	
	Upper	5	25%	11	55%	5	25%	4	20%	
SMOKING	No	18	90%	17	85%	16	80%	18	90%	0.86[a]
	Yes	1	5%	2	10%	2	10%	2	10%	
	Former	1	5%	1	5%	2	10%	0	0%	
BMI	Under wt.	1	5%	3	15%	0	0%	0	0%	0.13[a]
	Normal wt.	19	80%	12	60%	18	90%	14	100%	
Age	Mean ± SD	41.6	3.4	50.8	10.0	42.6	4.8	48.4	7.4	<0.001*[b]
DBP	Mean ± SD	72.2	5.6	76.3	6.1	96.0	3.4	95.5	4.2	<0.001*[b]

* - Statistically Significant ; [a] - p-value obtained by chi square test; [b] - p-value obtained by ANOVA test;
SES- socio economic status; DBP- Diastolic blood pressure; NH+NP - non hypertensive without periodontitis; NH+P- non hypertensive with periodontitis; H+NP - Hypertensive without periodontitis; H+P- Hypertensive with periodontitis;

Table 2. Bonferroni post hoc analysis depicting significance of age and diastolic blood pressure amongst groups.

		Age		*DBP*	
		Mean diff	*P value*	*Mean diff*	*P value*
NH+NP	NH+P	-9.20	.000*	-4.1	.065
	H+NP	-.95	1.000	-23.85	.000*
	H+P	-6.75	.016*	-23.35	.000*
H+P	H+NP	8.25	.002*	-19.75	.000*
	H+P	2.45	1.000	-19.25	.000*
H+NP	H+P	-5.8	.055	.50	1.00

NH+NP - non hypertensive without periodontitis; NH+P- non hypertensive with periodontitis; H+NP - Hypertensive without periodontitis; H+P- Hypertensive with periodontitis

periodontal pathogens (Dave and Van Dyke, 2008; Nazir, 2017; Beukers *et al*., 2017). A limited number of studies have evaluated the association between PISA and systemic diseases (Iwasaki *et al*., 2012; Susanto, *et al*., 2012; Yoshihara, *et al*., 2016; Tamelli *et al*., 2018). The present study is the first to measure PISA and correlate it to RDW-CV in periodontitis individuals. As expected, the total inflammatory burden, as measured by the PISA, was greater in the periodontitis (1099.11mm^2) group than in patients without periodontitis (262.26 mm^2). It is important to note from the results of this study that inflammatory conditions affected the diameter of the red cells. It has been suggested that low level inflammation may induce over production of cytokines and acute phase reactants which could interfere with erythropoiesis (Gemmell, *et al*., 1997; Tsioufis *et al.,* 2011) which is evident in the present study, as the PISA scores for the periodontitis group RDW-CV were higher than the control group (> by 0.45mm – 2.26mm). It has been also suggested that evaluating other blood parameters like mean platelet volume and platelet distribution width may be valuable for cardiac risk stratification (Temelli *et al*., 2018). However, for this study, we chose RDW-CV because it is accepted as a strong independent, diagnostic and prognostic marker for myocardial infarction, and is also thought to initiate atherosclerosis and platelet activation (Bujak *et al*., 2015; Zalawadiya *et al*., 2010; Güngör *et al*., 2014). RDW-CV has also been related to inflammatory markers such as erythrocyte sedimentation rate and high sensitivity C-Reactive protein (CRP) both of which have been associated frequently in periodontitis (Lappé *et al*., 2011). Obesity, diabetes mellitus and anemia

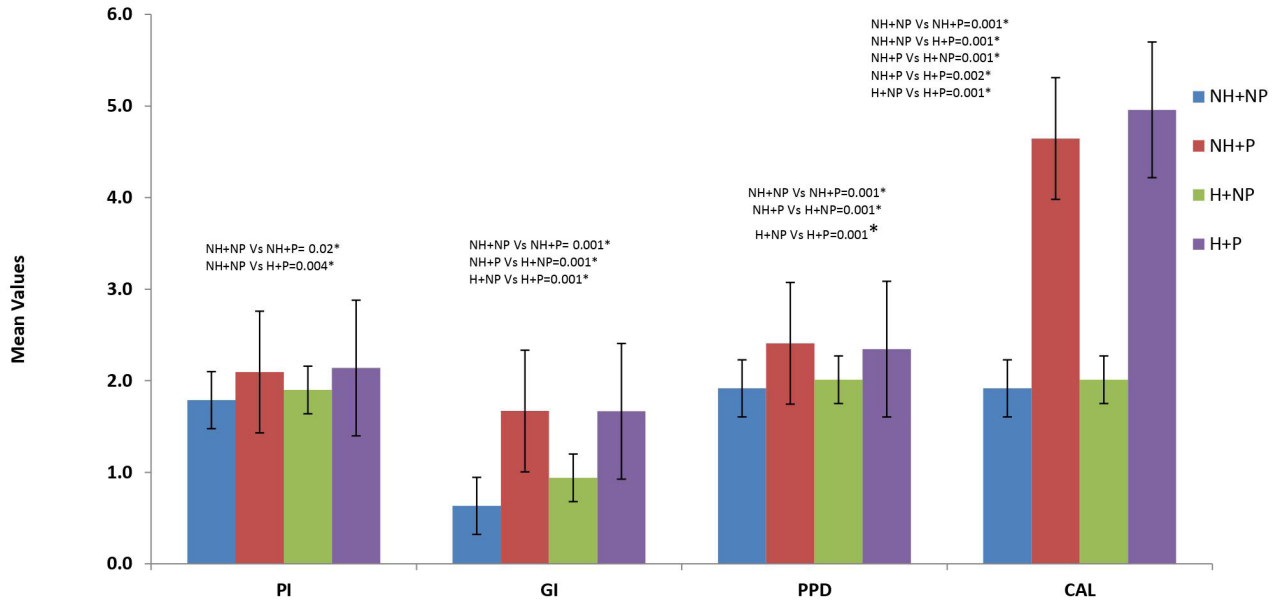

Figure 1. Comparison of PI, GI, PPD and CAL amongst the groups using ANCOVA followed by Bonferroni post hoc analysis.

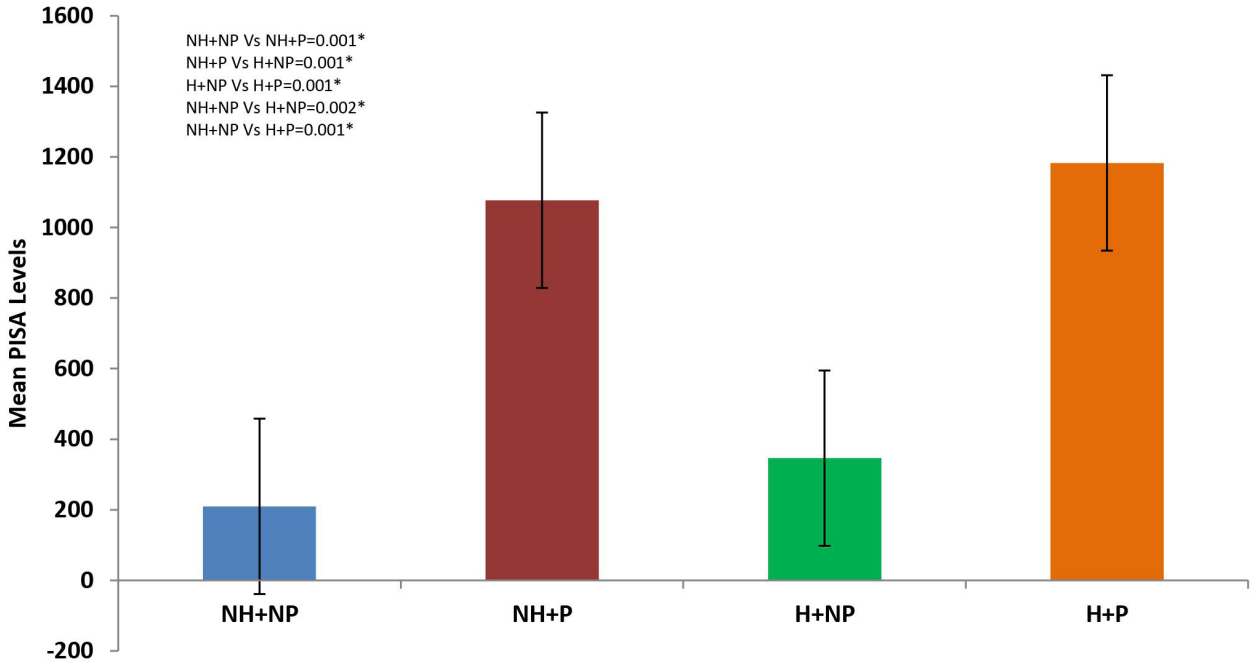

Figure 2. Comparison of PISA amongst the groups using ANCOVA followed by Bonferroni post hoc analysis.

have also been shown to be effective modifiers of cell morphology, vascular architecture and periodontitis (Tumanyana *et al.*, 2012; Lee *et al.*, 2015), hence in this study we excluded these potential confounding factors. Considering the lack of agreement as to which reading can be considered to reflect normal, hypertensive and pre-hypertensive states corresponding to age in non-obese middle aged individuals, we reported our findings on raised diastolic blood pressure, instead of pre-hypertension or pathological hypertension. We included individuals within the age group of 40-60 since both periodontitis and systemic hypertension is more prevalent in this age group. Multivariate regression analyses revealed that RDW-CV could be independently affected by PISA and DBP than other parameters evaluated in the study. The results also suggest a possible additive effect of periodontitis on RDW-CV in hypertensive individuals with periodontitis and warrants strict emphasis on meticulous control of periodontitis in hypertensive individuals which may help to prevent adverse cardiac outcomes (Beck and Offenbacher, 2001; Blaizot *et al.*, 2009).

In summary this study found both hypertension and periodontitis to independently influence changes in the red cell width. Using PISA to assess periodontal inflammation allows an objective score and allows regression analysis to be carried out, which helps us

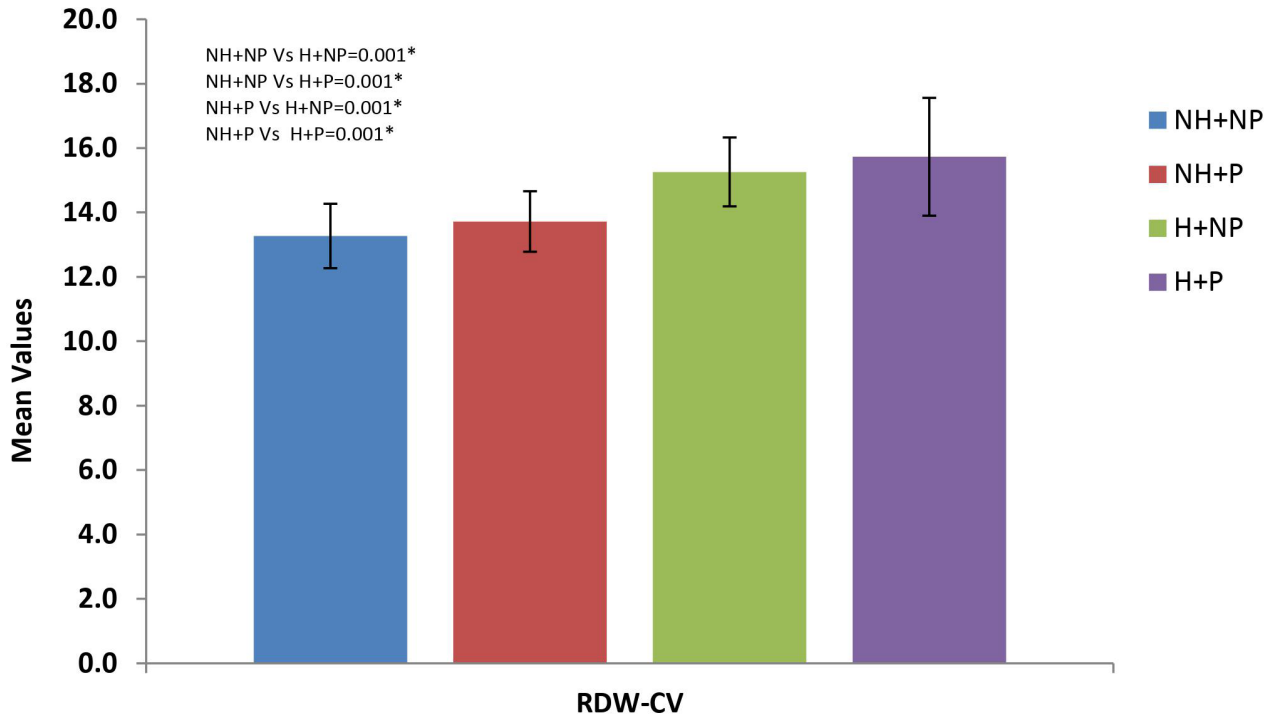

Figure 3. Comparison between RDW-CV amongst the groups ANCOVA followed by Bonferroni post hoc analysis.

Table 3. Pearson's Correlation test to compare RDW-CV, periodontal parameters and DBP.

Group	*Variable*	*Values*	*P I*	*G I*	*PPD*	*CAL*	*PISA*	*DBP*	*BMI*
		r	-0.62	-0.51	-0.36	-0.36	-0.15	0.48	0.40
NH+NP	RDW-CV	P	0.004*	0.02*	0.12	0.12	0.54	0.34	0.08
		N	20	20	20	20	20	20	20
		r	0.76	0.60	-0.24	0.34	0.80	0.55	0.10
NH+P	RDW-CV	P	<0.001*	0.005*	0.31	0.14	<0.001*	0.01*	0.66
		N	20	20	20	20	20	20	20
		r	0.46*	0.07	0.43	0.43	0.50	0.50	-0.04
H+NP	RDW-CV	P	0.04*	0.76	0.06	0.06	0.03*	0.03*	0.87
		N	20	20	20	20	20	20	20
		r	0.80	-0.20	0.05	-0.02	0.88	0.43	0.10
H+P	RDW-CV	P	<0.001*	0.39	0.84	0.95	<0.001*	0.06	0.68
		N	20	20	20	20	20	20	20

PI-Plaque index; GI-Gingival index; PPD- Probing depth; CAL-Clinical attachment level; PISA- Periodontal inflammed surface area; RDW-CV-Red cell distribution width; DBP- Diastolic blood pressure; BMI- Body mass index; NH+NP- Non hypertensive without periodontitis; NH+P- Non hypertensive with periodontitis; H+NP- Hypertensive without periodontitis; H+P- Hypertensive with periodontitis.

understand the periodontal–systemic relationship with more clarity than mere hypothetical associations. A possible correlation between the RDW-CV and the severity and characteristics of inflammation may exist which explains the elevated RDW-CV observed in the study group, This would have been more definitive had we considered inflammatory parameters like h-CRP (Bassuk *et al*., 2004; Lippi *et al*., 2009) and other pro-inflammatory cytokines which deserve further attention.

A strength of the present study is its detailed full mouth periodontal examination along with quantification of the inflammatory burden of the individual, selecting the study population within the critical age range of 40-60 with only hypertension and no other coexisting diseases apart from periodontitis. Recording the blood pressure in all three postures and taking average of the diastolic pressure for classifying between hypertensive and non hypertensive makes the results

Table 4. Coefficients for Dependent Variable – RDW-CV.

				95% CI for the Coefficient			
Group	*Model*		*Coefficients*	*Lower*	*Upper*	*Std. Error*	*P-Value*
NH+NP	1	Intercept	15.87	14.21	17.54	0.79	<0.001*
		P I	-1.46	-2.38	-0.54	0.44	0.004*
NH+P	1	Intercept	8.41	6.39	10.42	0.96	<0.001*
		PISA	0.01	0.00	0.01	0.00	<0.001*
	2	Intercept	3.22	0.69	5.76	1.20	0.02*
		PISA	0.01	0.00	0.01	0.00	<0.001*
		DBP	0.07	0.04	0.10	0.01	<0.001*
H+NP	1	Intercept	0.21	-12.74	13.15	6.16	0.97
		DBP	0.16	0.02	0.29	0.06	0.03*
H+P	1	Intercept	2.62	-0.95	6.19	1.70	0.14
		PISA	0.01	0.01	0.01	0.00	<0.001*

PI-Plaque index; PISA- Periodontal inflammed surface area; RDW-CV-Red cell distribution width; DBP- Diastolic blood pressure; NH+NP- Non hypertensive without periodontitis; NH+P- Non hypertensive with periodontitis; H+NP- Hypertensive without periodontitis; H+P- Hypertensive with periodontitis.

Table 5. Multivariate linear regression models evaluating each independent variable in relation to RDW-CV

		Model Summary			
Group	*Model*	*R*	*R^2*	*Adjusted R^2*	*S.E.E*
NH+NP	1	0.62[a]	0.38	0.35	0.58
NH+P	1	0.80[b]	0.63	0.61	0.58
	2	0.92[c]	0.85	0.84	0.38
H+NP	1	0.50[d]	0.25	0.21	0.95
H+P	1	0.88[b]	0.77	0.76	0.90

a. Predictors: (Constant), PI; b. Predictors: (Constant), PISA; c. Predictors: (Constant), PISA, DBP; d. Predictors: (Constant), DBP

PI-Plaque index; GI-Gingival index; PISA- Periodontal inflammed surface area; RDW-Red cell distribution width; DBP- Diastolic blood pressure; NH+NP- Non hypertensive without periodontitis; NH+P- Non hypertensive with periodontitis; H+NP- Hypertensive without periodontitis; H+P - Hypertensive with periodontitis.

more authentic. Eliminating or rejecting individuals with other co-morbidities such as obese individuals, smokers with high dependency for nicotine as determined from the Fagerstrom score and diabetes. A limitation of this study is its cross sectional design which did not allow us to explore the mechanisms involved in changes induced by the increased inflammatory state of periodontitis on the red cell width. Additional longitudinal studies with larger sample size equating severity of periodontitis along with inflammatory markers to RDW-CV are necessary to confirm its usage as a risk stratification tool.

Conclusion

Within the limitations of this preliminary study we propose that the extent of periodontal inflammation independently affects the coefficient of variation of the red cell width. Furthermore, in combination with systemic hypertension, there is a substantial increase in the red cell width. This suggests that RDW-CV could be used for relating periodontitis to cardiovascular disease.

Acknowledgements and Conflict of interest

The authors would like to thank Mr. Santosh, Department of Biostatistics, Rajarajeshwari College of Dental Sciences for the detailed statistical analysis. The authors state that the study is self funded and has no commercial relationship. There is no potential conflict of interest between the authors.

References

Angeli F, Verdecchia P and Pellegrino C *et al.* Association between periodontal disease and left ventricle mass in essential hypertension. *Hypertension* 2003; **41**:488-492.

Beck JD and Offenbacher S. The association between periodontal diseases and cardiovascular diseases: a state-of-the-science review. *Annals of Periodontology* 2001; **6**:9-15.

Bassuk SS, Rifai N and Ridker PM. High sensitivity C-reactive protein: clinical importance. *Current Problems in Cardiology 2004*; **29**:439-493.

Beukers NGFM, Van Der Heijden GJMG, Van Wijk AJ, *et al.* Periodontitis is an independent risk indicator for atherosclerotic cardiovascular diseases among 60 174 participants in a large dental school in the Netherlands. *Journal of Epidemiology and Community Health* 2017; **71**:37–42.

Blaizot A, Vergnes JN, Nuwwareh S, Amar J and Sixou M. Periodontal diseases and cardiovascular events: meta-analysis of observational studies. *International Dental Journal* 2009; **59:**197-209.

Bonato CF, doAmarai CCF, Belini L, Salzedas LMP and Oliveira SHP. Hypertension favors the inflammatory process in rats with experimentally induced periodontitis. *Journal of Periodontal Research* 2012; **47**:783-792.

Bujak K, Wasilewski J, Osadnik T *et al.* The Prognostic Role of Red Blood Cell Distribution Width in Coronary Artery Disease: A Review of the Pathophysiology. *Disease Markers* 2015 :**1**-12.

Caton JG, Armitage G, Berglundh T, *et al.* A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *Journal of Clinical Periodontology* 2018; **45(Suppl 20):**51-58.

Dave S, and TE Van Dyke TE. The link between periodontal disease and cardiovascular disease is probably inflammation. *Oral Diseases* 2008; **14**:95-101.

Franek E, Blach A, Witula A *et al.* Association between chronic periodontal disease and left ventricular hypertrophy in kidney transplant recipients. *Transplantation* 2005; **80**:3-5.

Franek E, Klamczynska E, Ganowicz E, Blach A, Budlewski T and Gorska R. Association of chronic periodontitis with left ventricular mass and central blood pressure in treated patients with essential hypertension. *American Journal of Hypertension* 2009; **22**:203-207.

Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S and Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. *Circulation* 2000; **102**:1000-1006.

Gemmell E, Marshall RI and Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontoogyl 2000* 1997; **14**:112–143.

Güngör B, Özcan KS, Erdinler İ, *et al.* Elevated levels of RDW is associated with nonvalvular atrial fibrillation. *Journal of Thrombosis and Thrombolysis* 2014; **37**:404– 10.

Hujoel, PP, White, BA, Garcia, RI and Listgarten, MA. The dentogingival epithelial surface area revisited. *Journal of Periodontal Research* 2001; **36**:48–55.

Iwasaki M, Taylor GW, Nesse W, Vissink A, Yoshihara A and Miyazaki H. Periodontal disease and decreased kidney function in Japanese elderly. *American Journal of Kidney Disease* 2012; **59**:202-209.

Janket SJ, Baird AE,Chuang SK and Jones JA. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics* 2003; **95**:559-569.

Jain K, Das SJ and Jain M. Comparison of red cell parameters in smokers and non smokers with chronic periodontitis. *Journal of Investigative and Clinical Dentistry* 2013; **4:**84-88.

Lappé JM, Horne BD, Shah SH, *et al.* Red cell distribution width, C-reactive protein, the complete blood count, and mortality in patients with coronary disease and a normal comparison population. *International Journal of Clinical Chemistry and Diagnostic Laboratory Medicine 2*011; **412**:2094–2099.

Leong XF, Ng CY, Badiah B and Das S. Association between Hypertension and Periodontitis: Possible Mechanisms. *The Scientific World Journal* 2014:768237.

Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G and Guidi GC: Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Archives of Pathology and Laboratory Medicine* 2009; **133**:628632.

Löe H and Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* 1963; **21**:533-551.

Lee KS, Lee SG, Kim EK *et al.*, Metabolic Syndrome Parameters in adolescents may be determinants for the future periodontal diseases. *Journal of Clinical Periodontology* 2015; **42**:105-112.

Modeer T, Blomberg C, Wondimu B, Lindberg TY and Marcus C. Association between obesity and periodontal risk indicators in adolescents. *International Journal of Pediatric Obesity* 2011; **6**:264-270.

Nesse W, Abbas F, van der Ploeg I, Spijkervet FKL, Dijkstra PU and Vissink A. Periodontal inflamed surface area: quantifying inflammatory burden. *Journal of Clinical Periodontology* 2008; **35**:668–673.

Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. *International Journal of Health Sciences* 2017; **11**:72–80.

Paraskevas S, Huizinga JD and Loos BG. A systematic review and meta analyses on C-reactive protein in relation to periodontitis *Journal of Clinical Periodontology* 2008; **35:**277-290.

Peres MA, Tsakos G, Barbat PR, Silva DA and Peres KG. Tooth loss is associated with increased blood pressure in adults - a multidisciplinary population based study. *Journal of Clinical Periodontology* 2012; **39**:824-33.

Rivas-Tumanyan S, Campos M, Zevallos JC, Joshipura KJ. Periodontal disease, hypertension and blood pressure among older adults in Puerto Rico. *Journal of Periodontology* 2013; **84**:203-211.

Sundstrom J, Neovius M, Tynelius P and Rasmussen F. Association of blood pressure in late adolescence with subsequent mortality: cohort study of Swedish male conscripts. *British Medical Journal* 2011; 342:643.

Susanto H, Nesse W, Dijkstra PU, *et al*. Periodontal inflamed surface area and C reactive protein as predictors of HbA1c: a study in Indonesia. *Clinical Oral Investigation* 2012; **16**:1237-1242.

Temelli B, Yetkin Ay Z, Aksoy F, *et al*. Platelet indices (mean platelet volume and platelet distribution width) have correlations with periodontal inflamed surface area in coronary artery disease patients: a pilot study. *Journal of Periodontology* 2018; **89**:1203-1212.

Turesky S, Gilmore ND and Glickman I. Reduced plaque formation by the chloromethyl analogue of vitamin C. *Journal of Periodontololgy* 1970; **41**:41-43.

Teles R and Wang CY. Mechanisms involved in the association between periodontal diseases and cardiovascular disease. *Oral Diseases* 2011; **17**:450-461.

Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? *Hypertension* 2004; **44**:248-252.

Tsakos G, Sabbah W, Hingorani AD *et al*. Is periodontal inflammation associated with raised blood pressure? Evidence from a National US Survey. *Journal of Hypertension* 2010; **28**:2386-2393.

Tsioufis C, Kasiakogias A, Thomopoulos C and Stefanadis C. Periodontitis and blood pressure: the concept of dental hypertension. *Atherosclerosis* 2011; **219**:1-9.

Tumanyana SR, Spiegelmana D, Curhana GC, Formand JP and Joshipura KJ. Periodontal Disease and Incidence of Hypertension in the Health Professionals Follow-Up Stud. *American Journal of Hypertension* 2012; **25**:770-776.

Uysal HB, Dağlı B, Akgüllü C, *et al*. Blood count parameters can predict the severity of coronary artery disease . *Korean Journal of Internal Medicine* 2016; **31**:1093-1100.

Vidal F, Figueredo CM, Cordovil I and Fischer RG. Higher prevalence of Periodontitis in patients with refractory arterial hypertension: a case-control study. *Oral Diseases* 2011; **17**:560-563.

WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series 854. Geneva: *World Health Organization*, 1995.

Yoshihara A, Iwasaki M, Miyazaki H and Nakamura K. Bidirectional relationship between renal function and periodontal disease in older Japanese women. *Journal of Clinical Periodontology* 2016; **43**:720-726.

Zalawadiya SK, Veeranna V, Niraj A, Pradhan J and Afonso L. Red cell distribution width and risk of coronary heart disease events. *American Journal of Cardiology* 2010; **106**:988993.