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RED BLOOD CELL DISTRIBUTION WIDTH: AN EMERGING DIAGNOSTIC MARKER FOR CHRONIC PERIODONTITIS.

Dental Science

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ABSTRACT

INTRODUCTION: Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms inducing a major vascular response which may impair erythropoiesis and degradation of erythrocytes. Red Blood Cell Distribution Width(RDW)is measurement of variation in size of the circulating erythrocytes(anisocytosis).

AIM: To evaluate RDW in chronic periodontitis(CP)and periodontally healthy subjects.

MATERIALS AND METHODS: 90 participants,45 CP and 45 healthy subjects were randomly selected from department of PMNM Dental College and Hospital. 4.5ml of blood was collected from all the subjects after obtaining informed consent and analysed for RDW. The obtained data were statistically analysed.

RESULTS:A statistically significant(p=0.006)and positive correlation was found between RDW of CP than the control group.

CONCLUSION: Increased RDW reflects chronic inflammation and increased oxidative stress,telltale signs of CP. RDW,a simple and inexpensive chair side diagnostic test can be used as reliable biomarker for early detection and therapeutic intervention in CP.

KEYWORDS

Anisocytosis,Chronic inflammation,Chronic periodontitis,Oxidative stress.

INTRODUCTION

Periodontitis is a slowly proceeding annihilation of infrastructure of periodontium such as periodontal ligament, alveolar bone by specific bacteria leading to formation of pocket and apical migration of gingiva resulting in recession or both.[1] In periodontitis, pathogens provoke a host mediated immune response by fibroblasts and macrophages thereby producing several cytokines as mediators of inflammatory response and immune reaction.[2] However, abnormal host responses to bacterial pathogens also play a pivotal role in the development of periodontitis.[3] Polymorphonuclear leukocytes (PMNs) are recruited by the cytokines towards the site of infection[4] and the bacterial antigens (e.g., lipopolysaccharide), also stimulate polymorphonuclear leukocytes to produce proteolytic enzymes such as elastase and Reactive Oxygen Species (ROS) via the oxidative burst, catalyzed by NADPH oxidase.[5]

Cell death occurs as a result of tissue destruction which releases various molecules into the blood stream thus analysis of the blood components can enable us in diagnosis of periodontitis.[6] One of these parameters is Red blood cell distribution width (RDW). The RDW, a widely available and economical test accomplished by automated haematology analysers and reported as an index of the complete blood cell count, a quantitative measure of anisocytosis.[7] It is calculated as follows:

RDW-CV (coefficient of variance) = (Standard deviation of red blood cell volume ÷ mean cell volume) ×100.[7]

The standard range for the RDW-CV is 11.5%–14.5%, and higher values specify greater variations in cell sizes.[7] High RDW designates a high degree of anisocytosis[8] reflecting chronic inflammation and an increased level of oxidative stress.[9] Thus, RDW is documented as global marker of chronic inflammation and oxidative stress.[10]

Quite a few recent studies have reported positive correlation of RDW with the Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) levels and RDW value is increased in inflammatory condition analogous to other inflammatory parameters.[11] Few studies discovered the changes in RDW link with cardiac and non-cardiac related deaths.[12-15] Malandrino et al., reported a significant positive correlation of high RDW and incidence of macro and microvascular complications without marked vascular complications in Diabetes Mellitus (DM) patients.[16]

Although there is a strong precedent for correspondence between RDW, chronic inflammation and increased oxidative stress, their relationship has not been meticulously examined in the case of chronic periodontitis. So, this is the first study of its kind that aims to evaluate the relationship between RDW and chronic periodontitis.

MATERIALS AND METHODS

The present cross-sectional study was initiated after the protocol was reviewed and approved by the Ethical Committee of PMNM Dental College& hospital, Bagalkot. The duration of the study was six months. The power of study was fixed at 91%, error fixed at 5%, as per calculation the sample size was estimated to be 78. To reduce bias and increase power of study, 90 subjects were considered for the study of which 45 subjects with chronic periodontitis were selected from patients referred to Department of Periodontics at PMNM dental college and hospital, Bagalkot. Subjects having more than 30% of their teeth with ≥4mm periodontal probing depth and ≥2mm attachment loss were included in the chronic periodontitis group.[17] A total of 45 subjects with a clinically healthy periodontium were included in the control group.

All subjects in the study were systemically healthy. Pregnant or lactating females, Subjects using Non Steroidal Anti-Inflammatory

Drugs (NSAIDS), antibiotics, contraceptives and drugs which were known to alter haematological parameters, subjects having or who had underwent scaling & root planing in past six months, smokers and alcoholics were excluded from the study.

A written informed consent was obtained before commencement of the current study from all the subjects who have fulfilled the above criteria. Full mouth clinical periodontal examination was performed by single examiner to reduce bias. The following clinical parameters- Gingival Index (GI, loe and silness, 1963), Periodontal Probing Depth (PPD), Clinical Attachment Loss (CAL) were measured at the mesial, distal, buccal, lingual aspects of each tooth.[18]

Sample Collection:

A 4.5 ml of venous blood was withdrawn with negligible stasis from the antecubital vein using a dry sterile disposable syringe and needle and was dispensed into Ethylene Diamine Tetraacetic Acid (EDTA, Anticoagulant) tubes. The samples were labelled with subject's age, sex, and identification number and were stored at room temperature till processed, within 4 hours of collection.[19]

Laboratory Analysis:

A complete blood count was performed using the YUMIZEN HORIBA 500, a six-part auto analyser able to run 27 parameters per sample, including haemoglobin concentration, packed cell volume, red blood cell concentration, red blood cell distribution width, mean corpuscular haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration, white blood cell and platelet count and mean platelet volume. Standardization, calibration of the instrument and processing of the samples were carried out as per the manufacturer's instructions. At the time of processing, approximately 20 µL of blood was aspirated by the equipment sampling probe of auto analyser from the well-mixed blood samples. The results of the analysis were revealed within 30 seconds.[19]

STATISTICALANALYSIS

The data acquired was statistically analysed by using computer software, IBM Statistical Package for Social Sciences Version 20.0. Unpaired t-test, Mann Whitney U-test and Spearmans correlation tests were used for the analysis. Data were expressed as mean and standard deviation. A p-value less than 0.05 were considered as significant.

RESULTS

The mean age of chronic periodontitis subjects is higher than the healthy group [Table*1*]. RDW value was found to be highest in the chronic periodontitis group than the healthy group. The mean gingival index, pocket probing depth and clinical attachment loss was found to be highest in the chronic periodontitis group [Table *2*]. These parameters showed a positive correlation with RDW values in the study groups [Table*3*].

Table 1:- comparison of the age between the healthy and chronic periodontitis (CP) subjects using Independent sample t test.

	Group	N	Mean (SD)	Mean difference (95% CI)	t	df	p-value
AGE	Healthy	45	27.56 (4.91)	-14.84 (-17.83, -11.86)	-9.93	69.27	<0.001 *
	CP	45	42.40 (8.74)				

*p<0.05 statistically significant. SD:Standard Deviation

Table 2:- comparison of the study parameters between the healthy and CP subjects using Mann whitney U test.

Group		N	Mean (SD)	Range	Median (Q1-Q3)	U statistic	p-value
Gingival index(GI)	Healthy	45	0.52(0.15)	0.2 - 0.8	0.5 (0.4 - 0.6)	0.00	<0.001*
	CP	45	2.26 (0.24)	1.7 - 2.8	2.3 (2 - 2.4)		
Pocket probing depth(PPD)	Healthy	45	2.42(0.50)	2 - 3	2(2 - 3)	0.00	<0.001*
	CP	45	5.89 (0.80)	5 - 7	6 (5 - 7)		
Clinical attachment level(CAL)	Healthy	45	0(0)	0 - 0	0(0 - 0)	0.00	<0.001*
	CP	45	6.38 (0.94)	5 - 8	6 (6 - 7)		
Red blood cell distribution width(RDW) value	Healthy	45	12.10 (0.98)	10.5- 14.3	12 (11.45 -	672.50	0.006*
	CP	45	13.14 (1.87)	10.4 - 18.2	12.8 (11.8 -		

*p<0.05 statistically significant. SD:Standard Deviation

Table 3:- correlation between study parameters in healthy and CP subjects using Spearmans corelation test.

		RDW VALUE	
		Healthy	CP
Age	Correlation Coefficient	-0.08	-0.11
	p-value	0.62	0.48#
Gingival index	Correlation Coefficient	0.78	0.80
	p-value	<0.001*	<0.001*
Pocket probing depth	Correlation Coefficient	0.52	0.74
	p-value	<0.001*	<0.001*
Clinical attachment level	Correlation Coefficient	-	0.80
	p-value	-	<0.001*

p<0.05 statistically significant # P>0.05 Non significant

DISCUSSION

Periodontitis is highly prevalent, but largely hidden, chronic inflammatory disease. Moreover, it has negative and profound influences on many aspects of daily living and excellence of life, distressing confidence, social interactions and food choices.[20] There are evidences signifying that periodontitis induces excessive Reactive Oxygen Species (ROS) production in periodontal tissue. [21] However, proteins, lipids and DNA are oxidized by overproduced ROS that contribute to tissue destruction.[22]

ROS, comprising superoxide, hydrogen peroxide and hydroxyl anions, are products of normal cellular metabolism. The host defense system against pathogens, leads to production of ROS which is the most secured protective barrier in contrast to diseases associated with phagocytic infiltration. [23,24] As the ROS release is not target-specific, generalized destruction to host tissue correspondingly occurs.[21]

Even though the RDW values were within the normal range, there is significant increase of RDW in CP (mean =13.14±1.87) as compared to healthy subjects (mean=12.10±0.98). This can be attributed to the fact that with the advancement of periodontitis, ROS produced by periodontal inflammation diffuses into the blood stream [25,26] resulting in increased oxidative stress causing the oxidation of various molecules in blood.[27] Inflammatory cytokines such as Interleukin (IL)-1 b, IL-6, Tumour Necrosis Factor (TNF)-α produced due to inflammation, desensitize the bone marrow erythroid progenitors affecting erythropoiesis due to an increase in the quantity of circulating premature erythrocytes, in turn inhibiting red blood cell maturation.[28] Increased RDW is seen in high inflammatory condition and in oxidative stress, which reduce the red blood cell survival therefore, resulting in anisocytosis and increased blood oxidative stress which is closely related to the clinical periodontal status.[29,30]

Various studies have established RDW as a marker of inflammation in various systemic conditions like diabetes, inflammatory bowel disease, cardiovascular diseases. Heba Sherif et al., in 2013 investigated RDW as a marker of inflammation in type 2 diabetes (T2DM) and it showed high levels of RDW to be associated with macrovascular complications.[31] Yeşil A et al., conducted a study on RDW in Inflammatory Bowel Disease (IBD) concluded that there is an increase in RDW in active IBD.[11] Giuseppe Lippi et al., studied the relation between RDW and inflammatory biomarkers. They analysed the RDW, Hb%, MCV (mean corpuscular volume), ferritin, hs-CRP(high sensitivity C-reactive protein), ESR and concluded that there is strong, graded association of RDW with hs-CRP and ESR. Hence, RDW can be used as an algorithm for cardiovascular risk prediction.[14] Chronic inflammation could cause RDW level elevation and predict a higher risk of cardiovascular diseases.[15]

In this study, RDW has shown a significant positive correlation with all the clinical parameters like gingival index, probing pocket depth, clinical attachment loss where the production of inflammatory cytokines and increased oxidative stress and also known to reduce

lifespan of red blood cells representing anisocytosis.

RDW is characteristically elevated in conditions of increased red cell destruction (such as haemolysis), impaired red cell production (such as B12 or folate deficiency, iron deficiency, and haemoglobinopathies), or after blood transfusion.[7] Conceivably, RDW may represent an integrative measure of multiple pathologic processes in heart failure (e.g., nutritional deficiencies, renal dysfunction, hepatic congestion, inflammatory stress), liver disease, malnutrition, occult colon cancer and neoplastic metastases to marrow.[8,32] However, the influence of such confounders are insignificant as all the subjects selected for the study were healthy individuals (control group) or subjects with chronic periodontitis without systemic complications or apparent comorbidities at the time of the study.

Thus the increase in RDW values in chronic periodontitis in the current study shows that there exists a positive association between chronic periodontitis and RDW. Therefore, RDW can be used as a diagnostic marker for chronic periodontitis. Monitoring of RDW in chronic periodontitis is the need of the hour in order to prevent its complications.

Limitations:

Standardization of age and sex should be determined for better correspondence, which is lacking in the present study. Further studies with larger sample size and longitudinal studies are required to establish a strong correlation between RDW and chronic periodontitis.

CONCLUSION

Red cell distribution width, a largely overlooked variable, available to clinicians providing further evaluation of its association with chronic periodontitis for better understanding of pathophysiology and to identify risk-stratify individuals which can be promoted for use in estimating treatment prognosis.

In this booming era of ever changing trends and technologies, where prevention is better than cure, efficient use of RDW as a diagnostic marker in CP can prevent generalized systemic complications.

REFERENCES:

[1] Sharma M, Sharma V, Gupta MK, Mand T, Pathak AK. Quantitative changes of immune complexes and leukocyte count due to periodontitis and their effect on diabetes mellitus. Recent Research in Science and Technology 2011;3:44-46.
[2] Bharath N, Sowmya NK, Mehta DS. Determination of antibacterial activity of green coffee bean extract on periodontogenic bacteria like Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans: An in vitro study. Contemp Clin Dent 2015;6:166-69.
[3] Page RC, Kornman K. The pathogenesis of human periodontitis: an introduction. Periodontol 2000 1997;14:9-11.
[4] Lamont RJ, Jenkinson LRJ. Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis. Microbiol Mol Biol Rev 1998;62:1244-63.
[5] Sculley DV, Langley-Evans SC. Salivary antioxidants and periodontal disease status. Proc Nutr Soc 2002;61:137-43.
[6] Rock KL, Kono H. The inflammatory response to cell death. Annu Rev Pathol 2008;3:99-126.
[7] Evans T, Jehle D. The red blood cell distribution width. J Emmerg Med 1991;9:71–74.
[8] Spell DW, Jones J, Harper WF, Bessman DJ. The value of a complete blood count in predicting cancer of the colon. Cancer Detect Prevent 2004;28:37-42.
[9] Ferrucci L, Guralnik JM, Woodman RC, Bandinelli S. Proinflammatory State and circulating erythropoiesis in persons with or without anaemia. Am J Med 2005;118:1288.
[10] Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: Association of acute phase reac¬tants and IL-6 with Metabolic Syndrome. Diabetologia 1997;40:1286–92.
[11] Yeşil A, Şenateş E, Bayoğlu IV, Erdem ED, Demirtunç RK, Övünç AOK. Red Cell Distribution width: a novel marker of activity in inflammatory bowel disease. Gut and Liver 2011;5:460-67.
[12] Tonelli M, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M. Relation between red cell distribution width and cardiovascular event rate in people with coronary disease. Circulation 2008;117:163–65.
[13] Perlstein TS, Weuve J, Pfeffer MA, Beckman JA. Red blood cell distribution width and mortality risk in a community based prospective cohort. Arch Intern Med 2009;169:588–90.
[14] Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. Arch Pathol Lab Med 2009;133:628–32.
[15] Felker GM, Allen LA, Pocock SJ, Shaw LK, McMurray JJV, Pfeffer MA, et al. Red cell distribution width as a novel prognostic marker in heart failure: Data from the charm program and the duke databank. J Am Coll Cardiol 2007;50:40–47.
[16] Malandrino N, Wu WC, Taveira TH, Whtlach HB, Smith RJ. Association between red blood cell distribution width and macrovascular complications in diabetes. Diabetologica 2012;55:226–35.
[17] American Academy of Periodontology Task Force Report on the update to the 1999 Classification of Periodontal Diseases and Conditions*. J Periodontol 2015;86:835-38.
[18] Mistry S, Kundu D, Bharati P. Epidemiology: it's application in periodontics. Periodontal Diseases – A Clinician's Guide 2012;253-278.
[19] Dada OA, Oshinaike O, Ogbera AO, Uche E, Akinbami A, Arogundade O, et al. The relationship between red blood cell distribution width and blood pressure in patients with type 2 diabetes mellitus in Lagos, Nigeria. J Blood Med 2014;5:185–89.
[20] O'Dowd LK, Durham J, McCracken GI, Preshaw PM. Patients' experiences of the impact of periodontal disease. J Clin Periodontol 2010;37:334–39.
[21] Tomofuji T, Irie K, Sanbe T, Azuma T, Ekuni D, Tamaki N, et al. Periodontitis and increase in circulating oxidative stress. Jpn Dent Sci Rev 2009;45:46-51.
[22] Valko M, Leibffritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
[23] Hyslop PA, Hinshaw DB, Scraufstatter IU, Cochrane CG, Kunz S, Vosbeck K. Hydrogen peroxide as a potent bacteriostatic antibiotic: implications for host defense. Free Radic Biol Med 2007;19:31-37.
[24] Fialkow L, Wang Y, Downey GP. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radic Biol Med 2007;42:153-64.
[25] Tomofuji T, Ekuni D, Yamanaka R, Kusano H, Azuma T, Sanbe T, et al. Chronic administration of lipopolysaccharide and proteases induces periodontal inflammation and hepatic steatosis in rats. J Periodontol 2007;78:1999-2006.
[26] Sobaniec H, Sobaniec-Lotowska ME. Morphological examinations of hard tissues of periodontium and evaluation of selected processes of lipid peroxidation in blood serum of rats in the course of experimental periodontitis. Med Sci Monit 2000;6:875-81.
[27] Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids 1987;45:337- 51.
[28] Macdougall IC, Cooper A. The inflammatory response and erythropoiesis sensitivity. Nephrol Dial Transplant 2002;17:48–52.
[29] Ruggiero C, Cherubini A, Ble A, Bos A JG, Maggio M, Dixit VD, et al. Uric acid and inflammatory markers. Eur Heart J 2006;27:1174–81.
[30] Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cell Mol Biol Lett 2005;10:255-64.
[31] Sherif H, Ramadan N, Radwan M, Hamdy E, Reda R. Red cell distribution width as a marker of inflammation in type 2 diabetes mellitus. Life Sci J 2013;10(4):32-39.
[32] Ozkalemkas F, Ali R, Ozkocaman V, Ozcelik T, Ozan U, Ozturk H, et al. The bone marrow aspirate and biopsy in the diagnosis of unsuspected non hematologic malignancy: a clinical study of 19 cases. BMC Cancer 2005;5:144.