RED CELL DISTRIBUTION WIDTH AND NEUTROPHIL LYMPHOCYTE RATIO (NLR) AS NOVEL MARKER IN CORONARY ARTERY DISEASE

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ABSTRACT

BACKGROUND

Ischaemic Heart Disease (IHD) is one of the most common causes of death worldwide. There has been an increase in incidence of ischaemic heart disease in recent times particularly in the younger age groups with or without risk factors. Various parameters are used in different scoring systems to assess the risk of developing ischaemic heart disease. RDW and NLR are the two emerging parameters from the time Price-Jones C first described variation in RBC size, RDW has evolved as an easily available parameter after the invention of impedance haematology auto analysers. NLR is an inflammatory marker, which is superior to ESR and its level rises in ischaemic heart disease. RDW and NLR can be used as an independent parameter to assess risk of developing CAD. Many studies in the past have worked on either RDW or NLR alone; hence, in the present study, we have considered both RDW and NLR as an attempt to assess their correlation and also the utility of these parameters in risk scoring systems.

MATERIALS AND METHODS

This is a prospective study where blood from the patients diagnosed with ischaemic heart disease sent for haematological analysis was studied. A complete haemogram, ESR, and haematological analysis using a pure impedance fully automated 3-part differential haematology analyser was done. 100 patients of ischaemic heart disease in whom haemoglobin levels were >11 gm/dL were included in the study. 50 non-ischaemic heart disease subjects with haemoglobin >11 gm/dL were selected as control group.

RESULTS

RDW was found to be high in patients with IHD (47.9±11.02) as compared to control group (41.3±6) with p=0.0001. NLR was also found to be high in patients with IHD (5.09±2.5) as compared to the control group (2.8±1.5) with p<0.0001. ESR was also high in patients (26±6.7) compared to control group (18±7) with p<0.0001. RDW was independent of ESR or NLR or any other risk factor of IHD.

CONCLUSION

RDW is independent of inflammatory markers and other risk factors in IHD. All the three can be included in the screening programs for IHD, if standardised. NLR appears to be superior to ESR as plasma factors don't play a role in their ratio.

KEY WORDS

Red cell distribution width, Neutrophil lymphocyte ratio, Ischaemic heart disease, ESR.

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INTRODUCTION: Ischaemic Heart Disease (IHD)/Coronary Artery Disease (CAD) is one of the most common causes of death worldwide. WHO NMH (Non-communicable diseases and mental health) India has estimated that the projected rise in disease burden due to cardiovascular disease is expected to make it the prime contributor of total mortality and morbidity among general population.

Additionally, cardiovascular disease in Indians has been shown to occur prematurely at least a decade or two earlier than their counterparts in developed countries.[1] There has been an increase in incidence of ischaemic heart disease in young patients with or without any risk factors leading to significant burden on the country.[2] Framingham assessment recommended by the American Heart Association (AHA) is a risk-scoring system developed from the Framingham Heart Study. It estimates the risk of MI or coronary death within 10 years independently for men and women.[3] PROCAM scoring system is recommended by the international task

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force for prevention of coronary heart disease is based on the German PROCAM Munster Heart Study.[4]

The system estimates the risk of developing a fatal or non-fatal myocardial infarction or sudden death due to CHD in the next 10 years. RDW and NRL have been studies separately as risk assessment markers of cardiovascular disease in different studies. Current CAD risk assessment scoring systems (Framingham, PROCAM) doesn't include these two parameters. Martin H et al (2001) showed that older people have higher RDW values and it increases with age irrespective of haemoglobin status.[5] Patel K V, et.al showed that higher RDW are associated with high mortality in general population.[6] In one study, patients admitted with advanced heart failure, high NLR was reported with higher inpatient mortality.[7] In our study, we have included RDW and NLR together as an attempt to find novel and cheap screening tests for early risk assessment of CVD.

Red Cell Distribution Width (RDW) and Neutrophil Lymphocyte Ratio (NLR) have evolved as novel marker in predicting the risk of developing CVD.[8] Red Cell Distribution Width (RDW) is an electronic parameter of complete blood count, which measures the heterogeneity in the size of circulating erythrocytes. RDW-CV (RBC Distribution Width-Coefficient Variation) is RDW (%) calculated from the points defining 68.26% of the entire area spreading from the peak of the RBC particle distribution curve. RDW-SD (RBC Distribution Width-Standard Deviation) is the distribution width (fL) at the height of 20% from the bottom when the peak RBC particle distribution curve is taken as 100%.

AIMS AND OBJECTIVES: To study the pattern of Red Cell Distribution Width (RDW), Neutrophil Lymphocyte Ratio (NLR) in relation to the other risk factors in patients suffering from Coronary Vascular Disease (CVD)/Ischaemic Heart Disease (IHD) to determine the utility of these markers in the risk assessment and their inclusion into the risk assessment scoring systems of CVD/IHD.

MATERIALS AND METHODS: This is a study undertaken at Department of Pathology, Osmania General Hospital, Hyderabad, a tertiary care government institution. Cases were identified prospectively in this study spanning over a period of two years starting from October 2011 to October 2013.

Selection Criteria Were: Patients with CAD and haemoglobin >11 gm/dL were included in the study, patients with history of renal failure, chronic respiratory diseases, liver diseases, malabsorption syndromes, malignancies, and haematological disorders, which could affect the RDW or NLR were excluded from the study. 100 consecutive patients with CAD were selected and 50 non-CAD subjects with normal haematologic results were taken as control group. N/L ratio is calculated by the formula=absolute neutrophil count/total leukocyte count-absolute neutrophil count.[9] Questionnaire based on Framingham's risk scoring system i.e. age, sex, total cholesterol and HDL levels, hypertension, diabetes, smoking/tobacco use was used for data collection. The additional data included in our study were NLR, BMI (Body Mass Index), ESR, RDW, MCV, HCT, HB, and MCHC.

Risk Factors in Framingham's and PROCAM Scoring System:

Risk Factors	PROCAM	Framingham
Age and Gender	✓	✓
Total Cholesterol		✓
HDL-C	✓	
LDL-C	✓	
Triglycerides	✓	
Smoking	✓	✓
Systolic Blood Pressure	✓	✓
Diabetes	✓	✓
Family History of Coronary Heart Disease	✓	

RDW-SD was used for comparative analysis and RDW-CV for comparison with other studies. Data obtained was subjected to statistical analysis using statistical software MedCalc Version 12.7.2. Youden plots were used to compare two parameters. Each dot in a Youden plot represents a patient.

RESULTS: The results obtained from the study group (100 patients) are depicted in the tables and graphs below.

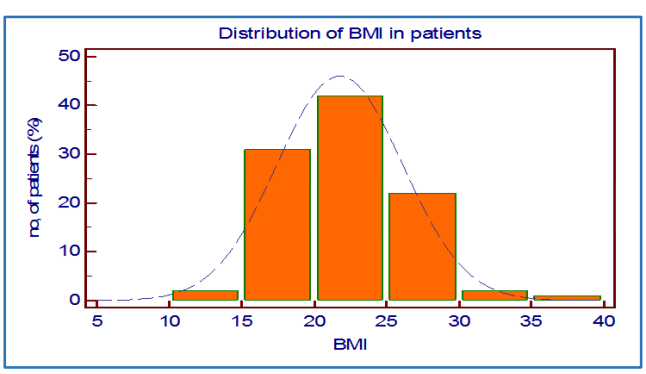
Age Group	No. of Patients	Percentage
30-40	15	15%
41-50	15	15%
51-60	38	38%
61-70	25	25%
>70	7	7%
Total	**100**	**100%**

Table 1: Age-Wise Distribution of the Patients

15% individuals were less than 40 years age and 63% were between 50 to 70 years.

Males	72%
Females	28%

Table 2: Sex Distribution of Study Group

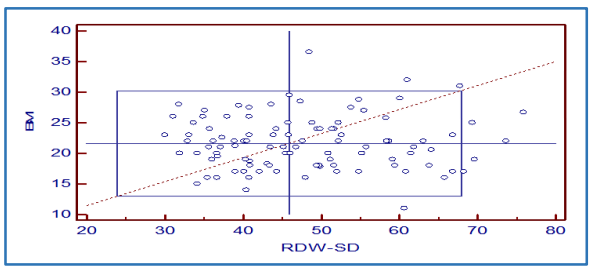


Graph 1: BMI Distribution of Study Group

BMI	No. of Patients	Percentage
<27	87	87%
27-30	10	10%
>30	03	3%

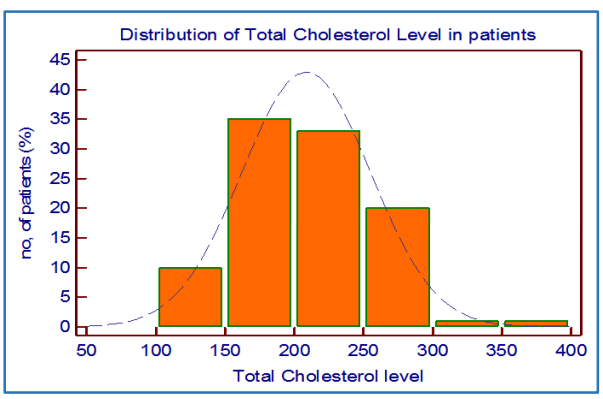
Table 3: Percentage of People with Normal/Overweight/Obesity

13% were overweight and 3% obese.



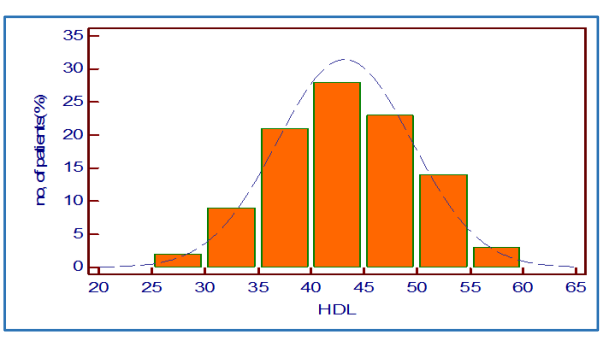
Graph 2: Correlation between RDW and BMI (Youden Plot)

Each dot represent a patient in the above graph, Dots are evenly distributed in all the four quadrants of the graph. BMI doesn't correlate with RDW.



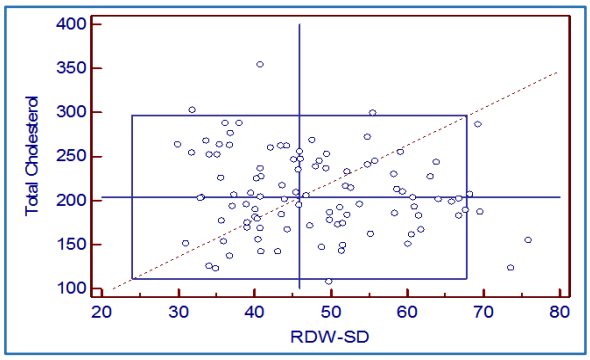
Graph 3: Total Cholesterol and HDL Levels in Study Group

65% of patients had total cholesterol levels between 150 and 250 mg/dL, total cholesterol levels detected in patients was 208±46 mg/dL.



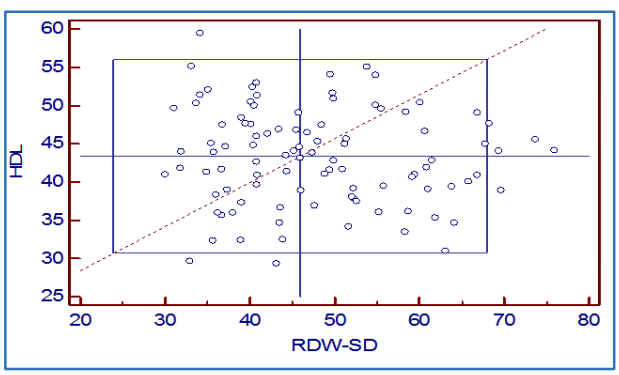
Graph 4: HDL Levels in Study Group

About 60% of patients had their HDL levels between 35 and 50 mg/dL. HDL levels detected in patients was 43.1±6.3 mg/dL.



Graph 5: Total Cholesterol vs. RDW

Dots are distributed in all the four quadrants of the graph. Total cholesterol levels do not correlate with RDW.



Graph 6: Correlation Between RDW and HDL

Dots are distributed in all the four quadrants of the graph. HDL levels do not correlate with RDW.

	Males (%)	Females (%)	Total	
Cases	38(54.1)	5(17.8)	44(44%)	N=100
Control	7(19.4%)	1(7.14%)	8(16%)	N=50

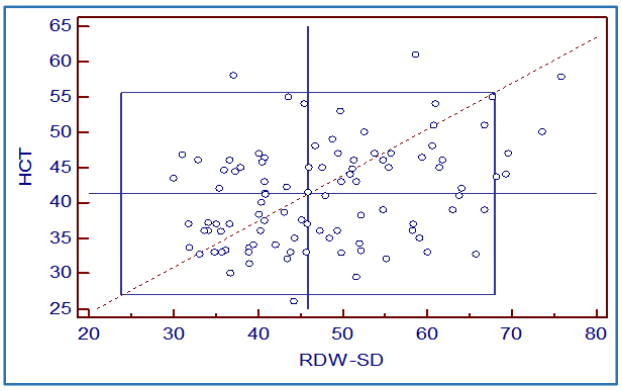
Table 4: Sex Distribution of Tobacco Smokers-Cases vs. Controls

44% of the patients showed history of smoking or use of smokeless tobacco. 16% of the subjects in the control group showed history of smoking or use of smokeless tobacco.

	Diabetes (%)	Hypertension (%)	Total
Cases	29(29%)	32(32%)	N=100
Control	6(12%)	9(18%)	N=50

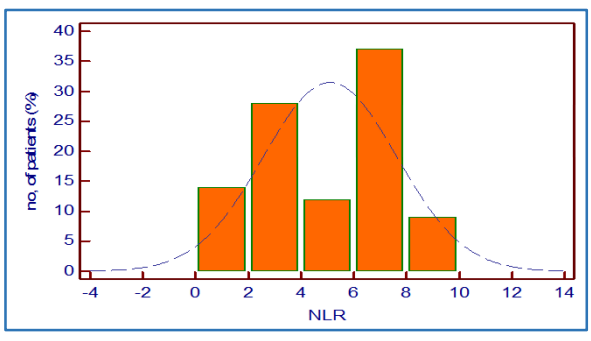
Table 5: Diabetes and Hypertension Among Cases vs. Control Group

29% of the patients of CAD also suffered from diabetes while 32% had hypertension compared to 12% of control group had diabetes and 18% had hypertension. The presence of these risk factors in CAD is statistically significant. Haemoglobin >11 gms were included in the study. 50% of the patients had haemoglobin level between 12 and 16. HB levels in the patients were 14.1±2.4 g/dL. 80% of patients had HCT between 30% and 50% HCT recorded in the patients 41±7.2%.



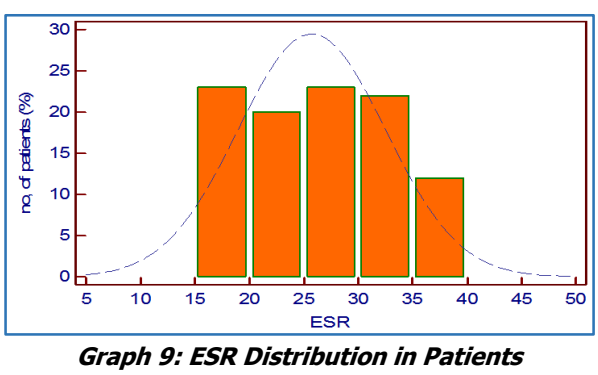
Graph 7: Correlation Between HCT vs. RDW

Dots are distributed in all the four quadrants of the graph. There is no relation exists between RDW and/or HCT.



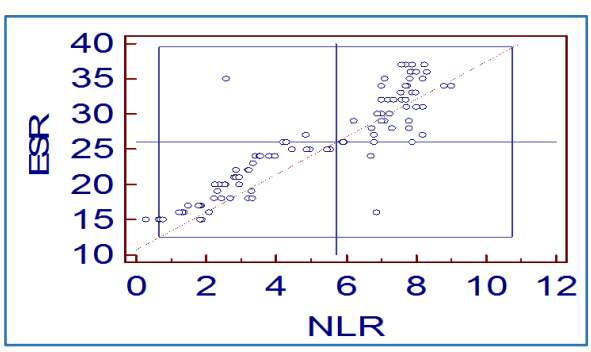
Graph 8: NLR Distribution in Study Group

35% of patients had NLR between 6 and 8. NLR recorded in the patients was 5.0±2.5.



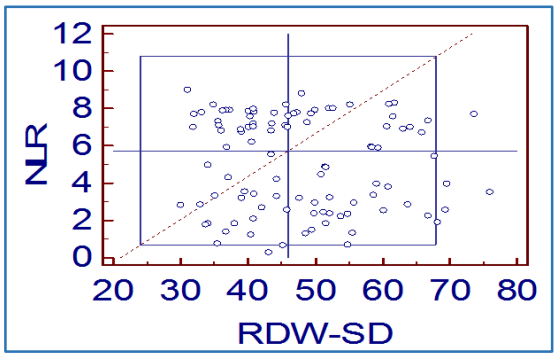
Graph 9: ESR Distribution in Patients

About 80% of patients had ESR between 15 and 35/1st hr. ESR recorded in patents was 25.7±6.7.



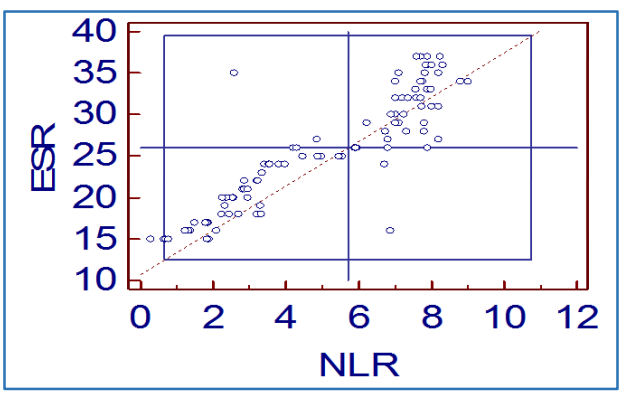
Graph 10: Comparative Analysis of ESR and NLR

Dots are arranged in a linear fashion along the diagonal line. NLR and ESR correlate well.



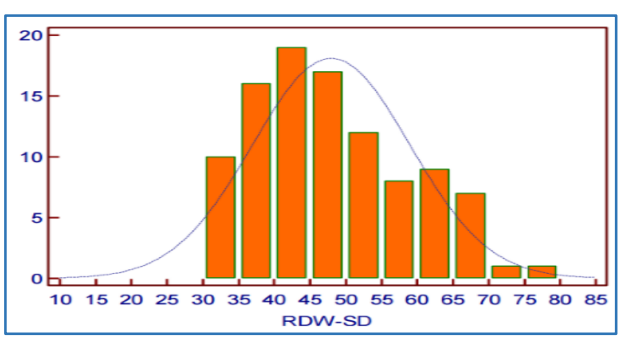
Graph 11: Comparison of NLR and RDW

Dots are arranged in all the four quadrants of the graph. NLR and RDW do not correlate.



Graph 12: Comparative Analysis of ESR and NLR

Distribution of NLR in Indian population (2.8±1.5) appears to be higher compared to others. The mean NLR and ESR is significantly higher in CAD patients compared to control group. RDW recorded in the patients was 47.9±11.02 femtolitres. RDW in patients is significantly higher than control group, which is 41.3 with a standard deviation of 6. (p=0.0001). RDW-SD was considered in the study as it is a better measure of anisocytosis. RDW-CV is corrected for HCT. RDW-SD was used for comparison with other parameter. RDW-CV mean and standard deviation was only enumerated for comparison with other studies, which is 14.206±1.98.



Graph 13: Distribution of RDW-SD in Study Group

>80% of the patients RDW was between 35 and 65 femtolitres. RDW-SD recorded among the patients 47.9±11.02 fL. The normal distribution of RDW in CAD patients is an inverted bell shaped curve with a positive skew.

	RDW-SD	N
Cases	47.9±11.02*	N=100
Control	41.3±6	N=50
P value	The two-tailed P Value equals 0.0001	

Table 6: RDW in Cases vs. Controls

*expressed as mean±SD

Mean RDW in the patients was 47.9 femtolitres with a standard deviation of 11.02. RDW in patients is significantly higher than control group, which is 41.3 with a standard deviation of 6. (p=0.0001).

Other Parameters Studied: The mean MCV is 84.6 femtolitres with standard deviation of 9.5. MCV less than 76 femtolitres is considered to be due to microcytosis. RDW in patients with CAD appears to be due to variation in size of normocytes and macrocytes rather than microcytes. MCHC in patients was variable ranging from 31 to 39 g/dL. MCHC in the patients was 34±2.27 g/dL.

DISCUSSION: The age and sex distribution of the patients studied correlates well with other studies (Nita A Tanna et al).[10] The important fact noted was that there were almost 15% of individuals <40 years of age. The number of young patients suffering from CAD seems to be increasing especially in Indian population.[11] About 87% of patients were with optimal BMI (<27 kg/m2). 13% were found to be overweight (>27 kg/m2) of which 3% were obese. CAD occurred even with BMI as low as 14 kg/m2. BMI in patients with risk factors such as smoking, diabetes, and hypertension was not different when compared with patients without those risk factors. We did not find any correlation between BMI and RDW. New studies show that central obesity correlates well with risk of developing CAD more than BMI.

Independent study of Thais Coutinho et al[12] showed similar results. CAD is associated with high cholesterol levels and low HDL levels and the correlation is statistically significant with p value <0.0001.[13] Total cholesterol and HDL levels have not correlated to any other known risk factors in our study not even with BMI or RDW. 44% of the patients showed history of smoking or use of smokeless tobacco compared to 16% in the control group. No correlation between tobacco usage to any other risk factor and RDW could be established in our study. 29% of the patients of CAD also suffered from diabetes while 32% had hypertension. The presence of these risk factors in CAD is statistically significant compared to 12% and 18% respectively in the control subject.

Only patients with haemoglobin >11 gms were included in the study in order to exclude high RDW recordings due to microcytosis (Iron-deficiency anaemia). No relation exists between RDW and haemoglobin status or HCT. Distribution of NLR in Indian population appears to be higher compared to others (2.8±1.5) as it is higher even in the control group.[14,15] The mean NLR and ESR is significantly higher in CAD patients compared to control group. There is a perfect linear correlation with ESR and NLR. There is no correlation between ESR/NLR with RDW. In the present study, the mean MCV is 84.6 with standard deviation of 9.5. (MCV less than 76 femtolitres is considered to be due to microcytosis.) RDW in patients with CAD appears to be due to variation in size of normocytes and macrocytes rather than microcytes. Mean RDW-SD in the patients was 47.9 with a standard deviation of 11.02, which is significantly higher than control group, which is 41.3 with a standard deviation of 6. (p=0.0001).[16] The distribution graph of RDW is inverted bell shaped with a positive skew.

CONCLUSION: Mean RDW in coronary artery disease patients are higher compared to normal subjects. Raised RDW is found independent of haemoglobin status.

Anisocytosis in coronary artery disease is due to normocytes and macrocytes. Relation between RDW and morbidity and mortality was thought to be mediated via immune-inflammatory response, but recent studies and our study shows that RDW behaves independent to inflammatory markers. However, the fact that RDW varies with race and the analyser used limits its inclusion into the prognostic scoring systems of coronary heart disease at present. NLR and ESR are potential and reliable inflammatory biomarkers for risk assessment for CAD. NLR appears superior to ESR because it is independent of plasma factors. Based on the previous literature review and our present study, RDW appears promising as simple, but cost-effective test for screening and prognostication of coronary heart disease when combined with other markers especially in developing country like India. A standardised method adopted worldwide for RDW could possibly include RDW into the risk assessment scoring systems for CAD.

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