Original article:

Anisocytosis in peripheral smear as a tool for screening Dyslipidemia in the Community.

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Abstract:

Background: Dyslipidemia is one of the important modifiable risk factor for CVD. If this is screened at the earliest , the morbidity and mortality due to CVD can be reduced in the community. Anisocytosis is frequently reported in patients suffering from cardiovascular disorders. This study is aimed to find the correlation between anisocytosis and dyslipidemia, and whether anisocytosis can be used as a tool for screening dyslipidemia.

Methods : This study is done in 100 subjects , 50 with normal lipid profile and 50 with dyslipidemia. The following data of total cholesterol, triglycerides, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Very Low Density Lipoprotein (VLDL) were collected. Computer based image analysis, using UTHSCSA image tool software was used to measure anisocytsis .

Results: Pearson's coefficient correlation and Paired sample t-tests were used in this study to compare the relationship between anisocytosis and lipid profile. Anisocytosis was more in dyslipidemic group, when compared with control group. Total cholesterol, LDL, VLDL, triglyceride levels were positively correlated strongest being total cholesterol in female subject with r value 0.87 and least being VLDL in male subject with r value 0.58. HDL level was negatively correlated with anisocytosis with r value of – 0.84 and - 0.83 in male and female subject respectively. Using Paired sample t-test, Compared to control group , anisocytosis was increased significantly in the dyslipidemic group with the p value < 0.001.

Conclusion: Dyslipidemia leads to a decrease in deformability of erythrocytes by altering the RBC membrane structure, and hence cause anisocytosis. Study of anisocytosis may serve as a simple, reliable and inexpensive tool for risk assessment and screening of dyslipidemia .

Key words: CardioVascular Disease , Dyslipidemia, Anisocytosis , Image tool.

Introduction:

Incidence and prevalence of cardiovascular disease is increasing at an alarming rate , thanks to urbanization, stressful and sedentary life style. Important modifiable risk factor for cardiovascular disease is dyslipidemia. Cardiovascular diseases are responsible for one in four deaths at global level[1]. Burden of cardiovascular disease is increasing alarmingly in developing countries like India. Creating awareness among masses through health education will help in controlling cardiovascular diseases by early screening, diagnosis, initiation of effective treatment and modifying risk factors. This will help in reducing cardiovascular diseases related mortality and morbidity. Dyslipidemia is manifested as elevation or attenuation of plasma concentration of

lipoproteins. In dyslipidemia, cholesterol accumulates in RBC membrane. The accumulated cholesterol alters the deformability of RBC membrane, affects the normal size of RBC [2]. Dyslipidemia causes atherosclerosis[3].The pathophysiology behind the emergence of arterial hypertension is decreased elasticity of large arteries[4].The gradual change in erythrocyte size, as observed microscopically, may reflect the effect of cholesterol in the arterial vessels[5].

Anisocytosis is variation in the size of the red blood cells. Anisocytosis is used in the differential diagnosis of anemia, but otherwise has received little attention. Increased values has been recently reported in several cardiovascular disorders such as ischaemic heart disease, acute and chronic heart failure [6]. Limited information is available, on the association between anisocytosis and plasma lipids in the general population.

Aim and objective:

The aim of this study is to screen dyslipidemia at the earliest by presence of anisocytosis in peripheral smear, and to prevent the morbidity and mortality due to CVD. The objective of this study is to assess the existence of potential association between anisocytosis and total cholesterol, low density lipoprotein [LDL], high density lipoprotein [HDL], Very Low Density Lipoprotein [VLDL] and triglycerides.

Materials and Methods :

This study was conducted in 100 subjects between the age group 25 -50 years who attended the Annapoorana Medical College Hospital(VMU). This study was started after getting Ethical clearance from the Ethical committee of Annapoorana Medical College and Hospitals. Fifty (25 male and 25 female) subjects with normal lipid profile were included in the control group. Fifty (25 male and 25 female) subjects with dyslipidemia were included in the test group. Patients with anaemia, malnutrition, and other chronic diseases whose RBCs may show anisocytosis were excluded. Written consent was got from all the subjects who participated in the study. Venous blood samples were routinely drawn in the morning after an overnight fast. Plasma lipid profile was assayed in semi auto analyser. The smears were stained with leishman's stain, and focused under oil immersion objective, and the image was captured using a digital camera fitted to the microscope with a help of suitable adapter. Images were transferred to the computer system and diameter of RBCs were measured using the software, S. Brent Dov, UTHSCSA Dental Diagnostic Science. The calibration image was taken from the smallest square of RBC in Neubauer's counting chamber ,which is 50 micron. Magnification factor was kept constant for all the images. Anisocytosis was measured manually using computer based image analysis method .

Since the details of morphology of RBCs cannot be obtained by automated analysers , manual method of measuring anisocytosis is preferred in this study. Best diagnostic support is given by Systematic examination of blood film and all the other test are either complimenting or confirming it [7]. Computer based image analysis method to determine red cell size, provides an accurate and reliable measurement, which is simple and cost effective [8]. The diameter of 25 RBCs were measured from each image. The variation in the size of RBCs (anisocytosis) (largest diameter_ smallest diameter) , between the normolipidemic control and dyslipidemic subjects were compared.

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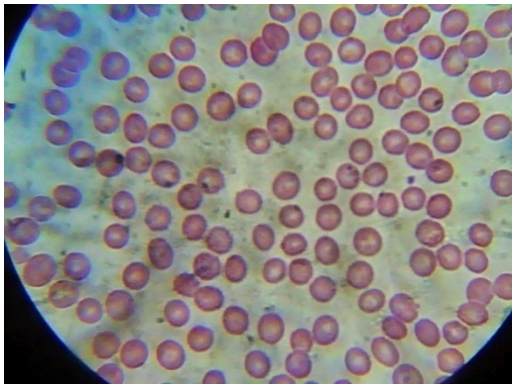

Image of peripheral smear from Normolipidemic control.
Largest –smallest RBC =7.9-6.2=1.7 microns.

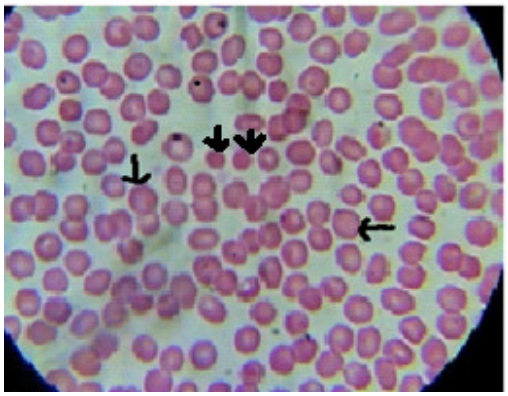

Image of peripheral smear from dyslipidemic Subject showing anisocytosis. (arrow)
Largest –smallest RBC = 9.7-5.9= 3.8 microns.

Normal range of rbc size is 6-8 microns[2]. Normal subjects the variation between the RBC diameter is 1-2 microns (ie) the difference between the largest RBC and smallest RBC is 1- 2 microns. Grading of anisocytosis is done as follows. Mild :- Difference between largest and smallest RBC is 1-2microns. Moderate:- Difference between largest and smallest RBC is 2-3microns. Severe:- Difference between largest and smallest RBC is more than 3 microns. In this study, severe anisocytosis is present in subjects with increased cholesterol, triglycerides, LDL, VLDL levels and decreased HDL levels.

Statistical analysis :

Pearson's coefficient correlation and Paired sample t test were used in this study to compare the relationship between anisocytosis and lipid profile parameters in normolipidemic controls and dyslipidemic subjects. Anisocytosis was compared with each lipid profile parameter, individually. If the r value is near 1 the relation between anisocytosis and lipid parameter is highly significant. If the r value is positive , there is positive correlation and if r value is negative , there is negative correlation between anisocytosis and lipid profile parameter. The statistical software SPSS (version 17) was used for data analysis. The mean values of all the parameters of lipid profile tests and anisocytosis were analyzed. Data were expressed as mean ± SD. Paired sample t-test was used for group wise comparisons (anisocytosis and every lipid profile parameter individually) and p-value of < 0.001 is considered to be significant. Anisocytosis is significantly increased in dyslipidemic subjects with p value < 0.001.

Observation and Results:

The variation in the size of RBCs (anisocytosis) is more in subjects with dyslipidemia. Total cholesterol, LDL,VLDL,triglyceride levels are positively correlated with anisocytosis and HDL level is negatively correlated with anisocytosis. The normal variation in the rbc size is 1-2 microns. But, in dyslipidemia , the variation in the rbc size (anisocytosis) is 3.8 microns, which gives the significant r values. By comparing the anisocytosis with individual dyslipidemic parameters using paired sample t-test , the significant p value of < 0.001 is got .

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Variation in RBC size between control and dyslipidemic subjects

Study subjects.	Range of RBC size (microns)
Male controls (n= 25)	6.2 to 7.9
Male subjects (n= 25)	5.8 to 10.2
Female controls (n= 25)	6.1 to 7.8
Female subjects (n= 25)	5.7 to 9.1.

Correlation of various lipid parameters with anisocytosis.

Variable	Male control r value	Male subject r value	Female control r value	Female subject r value
Total cholesterol	0.06	0.75	0.01	0.87
triglycerides	0.16	0.64	0.15	0.60
LDL	0.28	0.79	0.16	0.73
VLDL	0.20	0.58	0.14	0.85
HDL	-0.24	– 0.84	-0.38	-0.83

Paired sample t –test.			
Subjects.	variable	Mean ± sd	P value.
Control: female	anisocytosis	1.7 ± .2	
	Total cholesterol	193 ± 19.8	0.949
	LDL	96 ± 8.9	0.450
	VLDL	31.6 ± 4.1	0.476
	Triglycerides	140.4 ± 38.1	0.477
	HDL	59 ± 7.9	0.051
MALE	anisocytosis	1.7 ± .2	
	Total cholesterol	180.6 ± 29.8	0.764
	LDL	95.6 ± 11.7	0.173
	VLDL	33 ± 9	0.328
	Triglycerides	168.6 ± 39.1	0.444
	HDL	52.4 ± 11.5	0.260
Dyslipidemic female	anisocytosis	3.4 ± .2	
	Total cholesterol	290.6 ± 34.1	< 0.001
	LDL	156.4 ± 13.9	< 0.001

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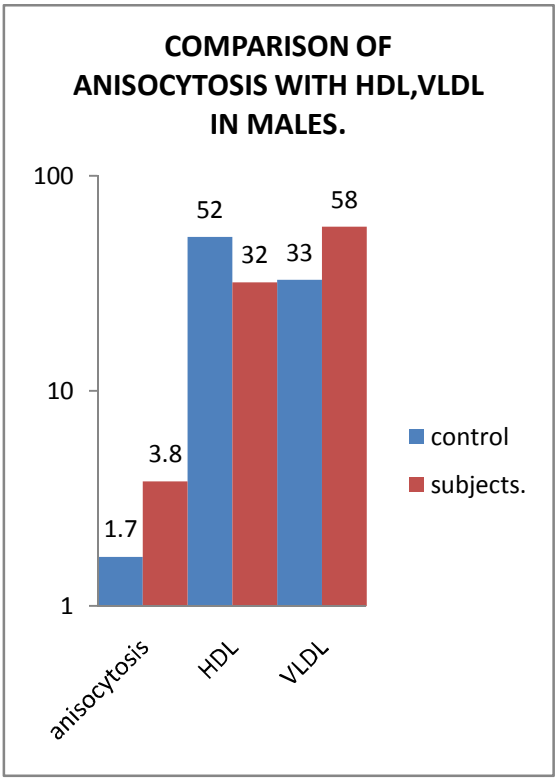
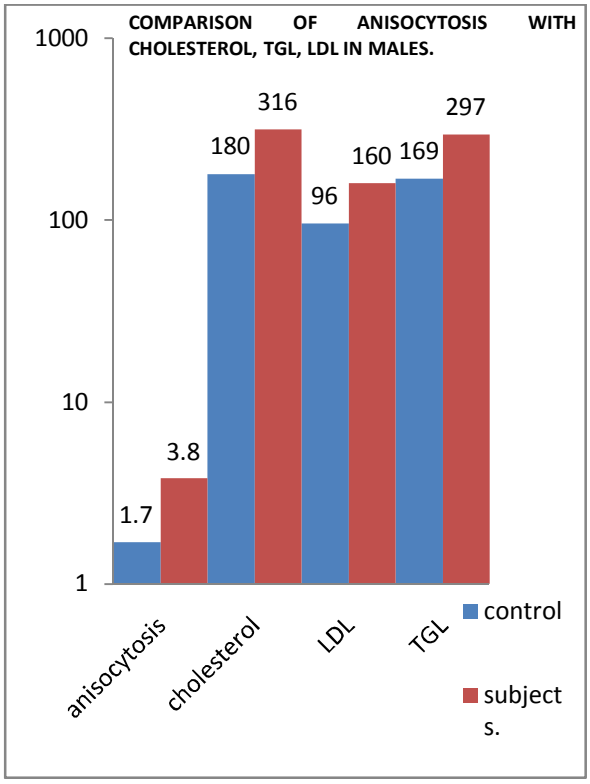
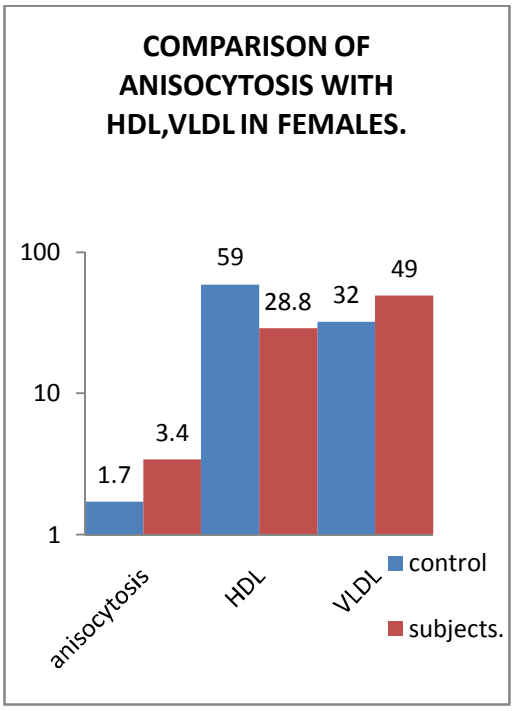
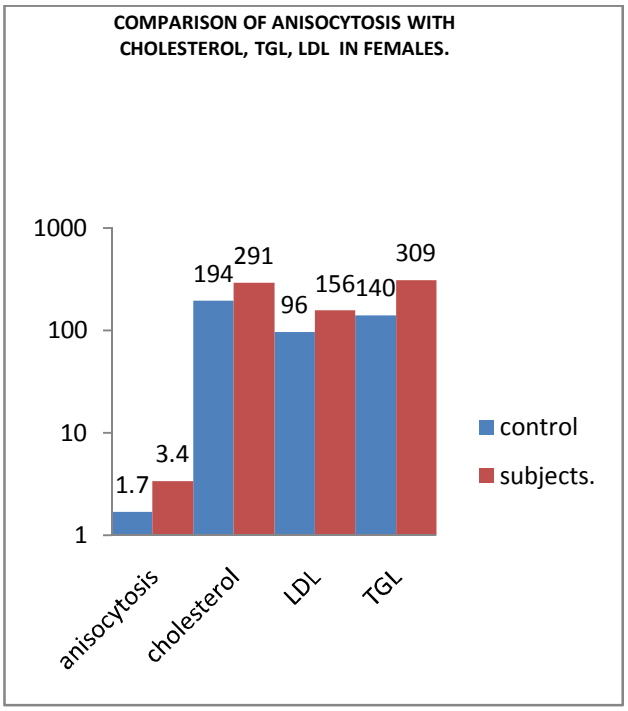
	VLDL	49.4 ± 4	< 0.001
	Triglycerides	309.3 ± 40.2	< 0.001
	HDL	28.8 ± 1.7	< 0.001
Male	anisocytosis	3.78 ± .4	
	Total cholesterol	315 . 48 ± 34.4	< 0.001
	LDL	160.0 ± 14.8	< 0.001
	VLDL	58.3 ± 7.2	< 0.001
	Triglycerides	297 ± 39.4	< 0.001
	HDL	32.1 ± 2.7	< 0.001









Discussion:

In cardiovascular system the erythrocytes deform in large vessels as well as small vessels to carry out its functions. Acquired changes like dyslipidemia, hyperglycemia etc affect the deformability of RBCs adversely. This study shows that dyslipidemia affects the size of RBCs (anisocytosis) by altering the deformability of RBC membrane .Before going into the discussion about how anisocytosis is brought about by dyslipidemia, we should know the rheological characteristics of RBC membrane in brief. The RBC membrane consists of two domains namely, lipid bilayer and cytoskeleton[9]. The lipid bilayer is composed of cholesterol and phospholipids. Cholesterol is evenly distributed and phospholipids are asymmetrically dispersed in the lipid bilayer. Important function of this cholesterol is to alter the flexibility (deformability) and providing stability to RBC membrane [10]. Phospholipid is essential for functioning of Na^+ K^+ ATP ase activity[11], which is needed for control of hydration, nutrient uptake and fluidity of RBC membrane[12].

Any variation in the structural constituents of lipid bilayer leads to impairment of deformability[13]. In dyslipidemia, hypercholesterolemia decreases deformability of RBC membrane, and increased lipoproteins [14] decrease the Na^+ K^+ ATP ase activity. In dyslipidemic subjects there is negative correlation between cholesterol, triglyceride, LDL and VLDL levels and positive correlation between HDL and Na^+ K^+ ATP ase activity [15]. In the study done by Vaya et al[3], they found out, In patients with hypercholesterolemia, the cholesterol content in RBC membrane is increased, and there is borderline correlation between LDL levels and RBC membrane cholesterol.[16] In the study conducted by Koter M et al [17] they have concluded that hypercholesterolemia causes changes in the structure and fluidity of erythrocyte plasma membrane. In the study done by Broncel M et al [18], concluded that

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mixed hyperlipidemia may have influence on the erythrocyte membrane structure caused significant decrease of membrane fluidity. From our study we come to know that anisocytosis is proportional to dyslipidemia.

Conclusion:

To conclude, the possible mechanisms by which dyslipidemia produces anisocytosis, are 1) increase in RBC membrane cholesterol [19], leading to decreased deformabiity, 2) dyslipidemia causing decreased Na^+ K^+ ATP ase activity, leading to decreased deformability and increased rigidity[20]. The reason why we are bothered about screening dyslipidemia at the earliest is,dyslipidemia is the etiological factor behind the pathophysiology of atherosclerosis and hypertension and CVD. Dyslipidemia leads to hyperaggregability of RBCs,which leads to microvascular complications. Cholesterol is a marker or an underlying sign of atheroma plaque instability. The gradual change in erythrocyte size in this study, reflects the effect of increased cholesterol in the arterial vessels[21]. This study indicates that anisocytosis reflects the dyslipidemic status. If dyslipidemia, is screened early, the future complications like CVD, endothelial damage, CAD and stroke ctc. can be prevented. This study concludes that anisocytosis in peripheral smear can be used as a simple, reliable and inexpensive tool for screening dyslipidemia in the community.

References:

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regiona mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–128.
2. Vaya A, Martinez M, Sloves P, Barbera JL, Aznar J. Red blood cell deformability determined by the rheodyn SSD in familial hypercholesterolemia. Clin Hemorheol 1996;16:515-22.
3. Oparil S, Zaman MA, Calhoun DA. Pathogenesis of hypertension. Ann Intern Med 2003; 139:761-76.
4. Chobanian AV. Clinical practice. Isolated systolic hypertension in the elderly. N Engl J Med 2007; 357:789-96.
5. Giuseppe Lippi, Fabian Sanchis-Gomar, Elisa Danese,, Martina Montagnana. Association of red blood cell distribution width with plasma lipids in a general population of unselected outpatients. Kardiologia Polska 2013; 71, 9: 931–936.
6. Montagnana M, Cervellin G, Meschi T et al. The role of red blood cell distribution width in cardiovascular and thrombotic disorders. Clin Chem Lab Med, 2012; 50: 635–641.
7. Dr. Prasanna N Kumar, peripheral blood examination , Laboratory haematology 11:2005, 83-90.
8. Satish Kumar NS , Aswini Dutt R , Maruthy KN , Dinakar Nadig ,Neevan D R Dsouza A Simple imaging method for demonstrating red cell sizes to life sciences students. National Journal of Basic Medical Sciences. Volume - I, Issue – 3 2011 :133-36.
9. Çetin M, Kocaman SA, Bostan M et al. Red blood cell distribution width (RDW) and its association with coronary atherosclerotic burden in patients with stable angina pectoris. Eur J Gen Med, 2012; 9: 7–13.
10. Smith J E. Erythrocyte membrane: Structure, function and pathophysiology.Vet Pathol . 24 (1987) 471.

11. Bikesh K Nirala, Shelza Saluja1, Anoop Mishra2, Nivedita K Gohil1 Erythrocyte ATPase activity in relation to hyperglycemia and hyperlipidemia in diabetic urbanIndian population int J Diabetes & Metab (2012) 20 :75 -79.
12. Prasad SK.1,Bhattacharjee A.1, Bandyopadhyay P.2, Pal S.3, Syamal AK.4, Mukherjee S1 RBC functional status and lipid profile of coal miners . Erythrocyte functional status and lipid profile of coal mine workers of West Bengal, India. Prog Health Sci 2014, Vol 4, No2.
13. Glader B & Lukens J, Hereditary spherocytosis and other anemias due to abnormality of red cell membrane, in Wintrobe's Clinical Hematology , Vol 1, edited by G R Lee, J Forester, J Lukens, F Paraskevas, J Greer & G Roger (Lippincott Williams and Wilkins, Baltimore) 1999, 1132, 1150.
14. Sehyun Shin ,Yunhee Ku., Narayanan Babu,& Megha Singh1, Erythrocyte deformability and its variation in diabetes mellitus,Indian Journal of Experimental Biology ,Vol. 45, January 2007, p. 121-128
15. Kumar R, Kumar AN, Ahmed S (2011) Changes in Erythrocyte Membrane in Type-2 Diabetes Mellitus with and without Dyslipidemia. J Diabetes Metab 2:141. doi:10.4172/2155-6156.1000141.
16. A. Vayá a, M. Martínez Triguero a,,E.Réganon b,V.Vila b,V. Martínez Sales b,,E.Solá c,A. Hernández Mijares c,and A. Ricart d. Erythrocyte membrane composition in patients with primary hypercholesterolemia .Clinical Hemorheology and Microcirculation 41 (2009) 67–72 .
17. Koter M, Franiak I, Strychalska K, Broncel M, Chojnowska-Jezierska J. Damage to the structure of erythrocyte plasma membranes in patients with type-2 hypercholesterolemia Int J Biochem Cell Biol. 2004 Feb;36(2):205-15.
18. Broncel M, Balcerak M, Bała AKoter-Michalak MDuchnowicz PChojnowska-Jezierska J, The erythrocyte membrane structure in patients with mixed hyperlipidemia, Clin Hemorheol Microcirc. 2009;41(3):169-77.
19. Kanakaraj P, Singh M. Influence of hypercholesterolemia on morphological and rheological characteristics of erythrocytes. Atherosclerosis 1989;9:209-17.
20. Caimi G, Serra A, Lo Presti R, Montana M, Catania M,& Samo A, Erythrocyte membrane fluorescence parameters in diabetes mellitus, Diabetes Res,21 (1992) 19.
21. F.D. Kolodgie, A.P. Burke, G. Nakazawa, Q. Cheng, X. Xu and R. Virmani, Free cholesterol in atherosclerotic plaques: where does it come from?, Curr. Opin. Lipidol. 18 (2007), 500–50.