Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(7A):2322-2327 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2016.v04i07.003

Original Research Article

A Simple Slide Test to Assess Grades of Erythrocyte Aggregation in Acute ST-Elevated Myocardial Infarction and Its Prognostic Significance

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Abstract: Erythrocytes are involved in a developing thrombus or clot formation. Increased endothelial adherence of red blood cells with endothelium by increase adhesive proteins has been observed. The causes that effect EAAT include proteins of acute-phase response and changes in the phospholipid composition of erythrocyte membrane especially in a case of hyperlipidemia. The aim is to study erythrocyte aggregation and adhesiveness by a simple slide test in subjects with acute ST-elevated myocardial infarction (STEMI) in predicting the outcome within 1week**.** Fifty patients of acute STEMI who came to the ICCU of Government Royapettah Hospital were included in the study. Subjects with onset of chest pain within 6hrs, with retrosternal chest pain persisting for more than 30 min and with ST segment elevation more than 1mm in limb leads and more than 2mm in chest leads in at least two contiguous ECG leads were included in the study. Citrated blood was subjected to simple slide test and Stained smears were examined under 400X and graded into four grades. 'P' value between the Grades of RBC aggregation and the prognosis of the patients with STEMI was < 0.05. EAAT is a simple bedside slide test for erythrocyte aggregation, which indirectly finds out the presence and proportion of inflammation. This test also has the potential to assess the prognosis of STEMI patients. It can also be used as a screening test for patients with STEMI to find out high-risk individuals so that necessary interventions (like PCI) could be adopted.
Keywords: Erythrocytes, myocardial infarction, EAAT

INTRODUCTION

Myocardial infarction (MI) is the irreversible necrosis of heart muscle due to prolonged ischemia and occurs from an imbalance in oxygen supply and demand, most often caused by atherosclerotic plaque rupture with thrombus formation in a coronary vessel [1]. Erythrocyte acts like as a scavenger toward reactive oxygen species and nitrogen species under the normal physiologic conditions. If the amount of reactive oxygen and nitrogen species production is
beyond the antioxidant capacity of the RBCs, it consequently loses its morphological (structural) features [2]. This results in increase adhesiveness and aggregation of erythrocytes to the vascular endothelium, contributing to vascular damage. The presence of erythrocyte aggregation is an independent risk factor for thrombosis or occlusion both in arterial and venous systems. With these properties of erythrocyte aggregation would also be identifiable with poor circulation in the small capillaries [3]. These studies show that erythocyte aggregation is also related to occlusion in the vascular network, caused by the formation of atheromatous plaques. Erythrocyte aggregation is also available indirectly through Erythrocyte Sedimentation Rate (ESR) [4]. But ESR correlates poorly with erythrocyte aggregation. Erythrocyte aggregation can be demonstrated more accurately by Myrene Rheometer, Cell flow analyzer [CFA], Laser- assisted optical rotation cell analyzer [LORCA] and INFLAMET as designed in Israel. The main disadvantage is that all of these require expensive equipments. EAAT (erythrocyte aggregation and adhesiveness test) has been put up as a simple bedside slide test which is a very feasible, cost effective and fast method of directly visualize and assessing the erythrocyte-aggregation status to detect internal inflammation [5].

Erythrocyte sedimentation rate

The erythrocyte sedimentation rate is done by Westergren method or Wintrobes method. ESR, is the commonest test and it is used for measuring the inflammation rate at which Erythrocyte sedimentation rate done in a 1 hour. When an inflammation occurs,

leads to increase fibrinogen level in the serum that causes RBCs to stick to each other. The erythrocytes form stacks called rouleaux formation. which settle faster [6].

Table-1: Normal Range of ESR

Sex	Male	Female
50 yrs. old or less	15mm/h	20mm/hr
Over 50 yrs old	20mm/hr	30mm/hr

Erythrocyte aggregation and adhesiveness test (EAAT)

Erythrocyte aggregation and adhesiveness test (EAAT) is a simple bedside slide test. It is a very feasible, cost effective, fast method of directly visualizing the erythrocyte aggregation status. It is a useful biomarker to detect internal inflammation in individuals with atherothrombotic risk factors.1. The test has the potential to assess the risk of acute MI 2. It can be used to assess the prognosis in patient with STEMI [7].

MATERIALS AND METHODS

50 Patients admitted in ICCU with STEMI at Government Royapettah Hospital during study period.

Inclusion criteria

Patients admitted in ICCU with acute STEMI Retrosternal chest pain >30 min, within <6 hrs of onset and 1 mm ST elevation in atleast 2 contiguous ECG leads.

Exclusion criteria

1. Recent myocardial infarction, 2. Sepsis, 3. Infections-any bacterial infection 4. Neoplasms 5. Liver failure, ESRD 6. DVT, 7. Patient on antiplatelet drugs and statins. Detailed history of the patient was collected and routine examination like both general and systemic examination was done. complete history including risk factors of the cardio vascular diseases were recorded. Blood sample was collected from anticubital vein immediately after admission. Subsequently, the subjects were treated according to the standard treatment protocol of our hospital. The blood samples were and collected from the subjects and immediately send to the laboratory and ESR (erythrocyte sedimentation rate) by Wintergreen's method and slide test (EAAT) were performed along with other investigations. Citrated blood was subjected to simple slide test and stained smears were examined under 400X and graded into four grades.

Slide Preparation

A single drop of anticoagulant 0.8% sodium citrate solution and 3 drops of blood was taken in a watch glass and mixed. This anticoagulated blood was placed on a slide, which was inclined at 45°. The slide was left in that position for 10 seconds during which the blood ran down by gravity leaving a fine film. An adsorbing paper was used to wipe blood from the lowest part of the slide. The slides were dried at room temperature in a completely horizontal position.The drop size and also the angle at which the slides were placed was maintained constant for all the slides. The slides were then stained with 5-6 drops of Leishman stain and left for 2 minutes. Distilled water poured over the slide gently double the amount of Leishman stain. We waited for 8 minutes and, the slides were washed under the tap water slowly, dried at the room temperature and then the slides were examined. First the slides were subjectively assessed without diagnosis [7, 8].

Analysis of The Slides

First the slides were subjectively assessed without the diagnosis. The slides were studied under the microscope at 40× (400×) magnification and searched all areas of the slide and grades were subjectively assigned grades of erythrocytes aggregation by the investigator.

The grading criteria

Figure 1, grade A, RBCs are discrete with uniform distribution throughout with no clear areas in between.

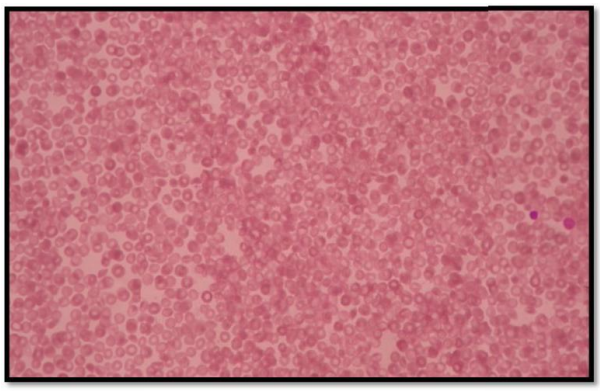

Fig-1: Grade A

Figure 2, Grade B, RBCs are aggregated in some areas of the slide with areas of small clear spaces.

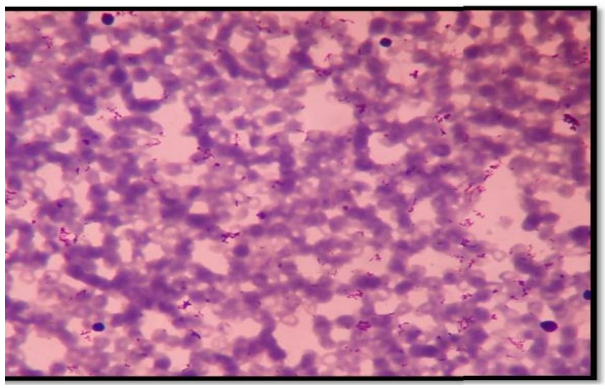

Fig-2: Grade B

Figure 3, Grade C, Variable sizes of erythrocytes aggregates in all the areas of slide with small clear spaces.

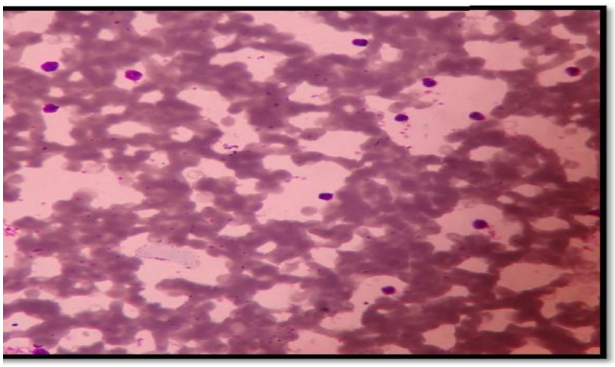

Fig-3: Grade C

Figure 4, Grade D, Large thick RBC aggregates with rounded/clear borders and large clear spaces.

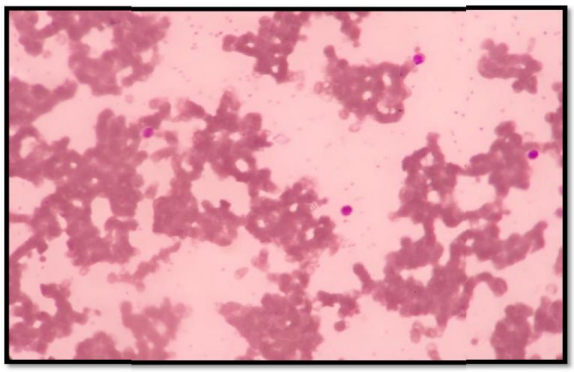

Fig-4: Grade D

Interpretations were done in the microscope in oil immersion field of peripheral blood. Standard criteria were used in this method for diagnosing erythrocyte grades. One week follow-up was done for the prognosis of the patient and was observed in 1 week follow up period. Prognosis: Good prognosis was documented in patients with complete recovery. Bad prognosis was documented in patients with recurrent chest pain or angina reinfarction, LV failure, Cardiogenic shock, Cardiac arrhythmias, VT, VF, AF, mortality from cardiac causes and mmortality from non-cardiac causes [8].

OBSERVATION AND RESULTS

Table 1 Out of 50 patients males were 38 females were 12. In out of 100 percentage males were 76% and females were 24%. Age group more commonly came with acute myocardial infarction are 51-60 years, next age group are 41-50 years then 61-70 years.

Out of fifty patients mild aggregation Grade A are observed in 5(10%) patients, Grade B erythrocyte aggregation observed in 19(38%) patients. Grade C aggregation observed in 21(42%) patients. Grade D aggregation observed in 5(10%) patients.

Out of fifty patients 13 patients STEMI had Bad prognosis within one week period of followup. 37 patients had Good prognosis. Out of fifty patients 12 patients were female and 7(58.3%) patients had good prognosis and 5(41.7%) patients had bad prognosis. 38(78.9%) patients were male among this 8(21.1%) patients had bad prognosis. Out of fifty patients with STEMI 5 showed Grade A RBCs aggregation, all of them had Good prognosis (100%).

In patients Grade B RBCs aggregation were observed, out of this 17(89.5%) had good prognosis and 2(10.5%) patients had bad prognosis. Grade C RBCs aggregation observed in 21 patients, out of this 14 patients (66.7%) had good prognosis and 7 patients (33.3%) had bad prognosis. Grade D RBCs aggregation observed in 5 patients, out of this 4(80%) had good prognosis and 1(20%) had bad prognosis.

Table 2 Out of this fifty patients 37(74%) patients had good prognosis, 4(8%) patients had arrhythmia, 2 patients (4%) had LVF, one patient went to cardiogenic shock. Six (12%) patients died from STEMI.

Table 1: Distribution and frequency

		Frequency	%	Valid %	Cumulative %
Valid	21-30 yrs	2	4.0	4.0	4.0
	31-40 yrs	5	10.0	10.0	14.0
	41-50 yrs	15	30.0	30.0	44.0
	51-60 yrs	16	32.0	32.0	76.0
	61-70 yrs	8	16.0	16.0	92.0
	71-80	4	8.0	8.0	100.0
	Total	50	100.0	100.0	

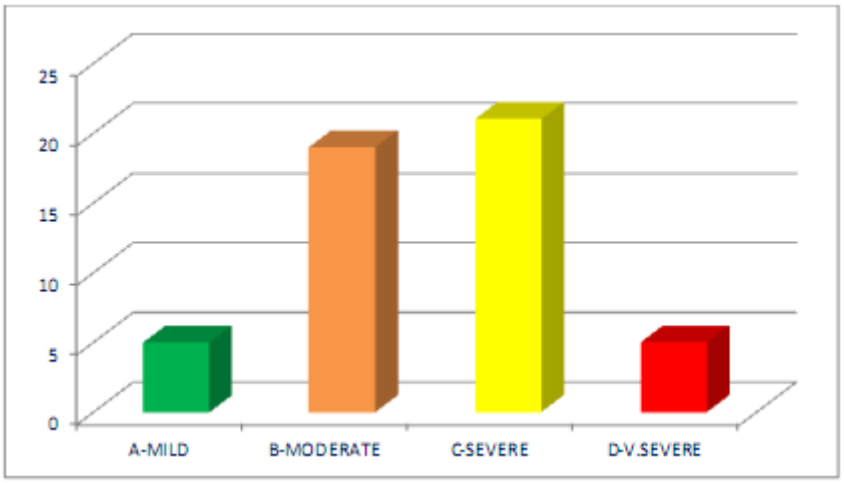

Fig-5: Frequency of Erythrocyte aggregates in STEMI patients

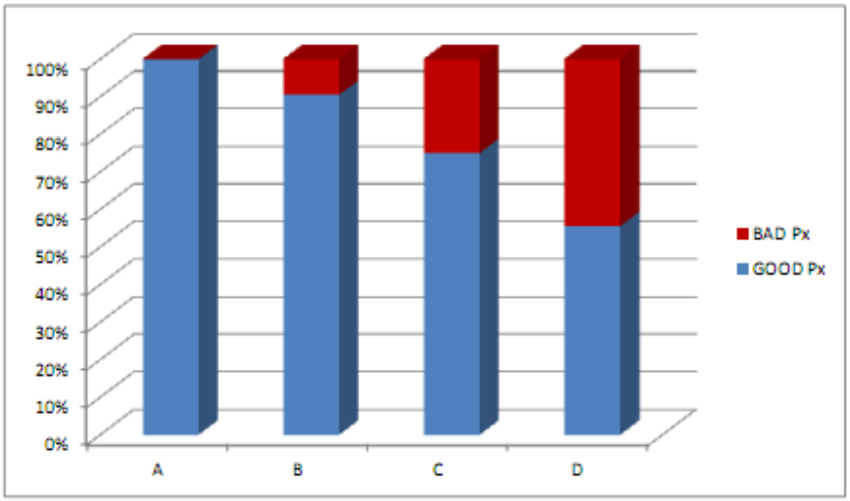

Fig-6: Erythrocyte aggregation grades Vs prognosis within 1 week

Table 2:

		Frequency	%	Valid %	Cumulative %
Valid	Nil	37	74.0	74.0	74.0
	Died	6	12.0	12.0	86.0
	Arrythmia	4	8.0	8.0	94.0
	LVF	2	4.0	4.0	98.0
	Cardiogenic Shock	1	2.0	2.0	100.0
	Total	**50**	**100.0**	**100.0**	

DISCUSSION

In acute myocardial infarction, many inflammatory markers have been studied and have been linked to bad prognosis. Neumann et al did a study to determine the prognostic significance of altered plasma viscosity and erythrocyte aggregation in unstable angina[9]. It was a study of 96 patients with unstable angina up for six months or until surgical intervention, results showed that plasma viscosity and erythrocyte aggregation were better predictors of AMI than age, gender, fibrinogen levels, ST segment changes. Also, Holter monitoring with ST segment analysis showed that ischemic episodes were more common in cases with high erythrocyte aggregation [10]. Erythrocyte aggregation value was studied in groups of patients with Metricell apparatus. The results showed that erythrocyte aggregation strongly associated with Myocardial Infarction and arteriosclerosis of peripheral arteries [11]. A study showing erythrocyte aggregation as a cause of slow blood flow in acute coronary syndrome. In 1988, the International Committee for Standardization in Hematology recommended that ESR can be an important test for monitoring chronic inflammatory processes Various studies were done and ESR was found to have potential predictive importance

in Coronary artery disease (CAD) [12]. In view of various such studies done, of all the parameters available, ESR came out as a cheap, fast and widely applicable inflammatory marker to assess inflammation and erythrocyte aggregation in patients with CAD. But ESR correlates poorly with erythrocyte aggregation due to the confounding effects of temperature, PCV, albumin, hemodilution by anticoagulants. ESR also does not differentiate whether erythrocyte aggregation is because of cellular factors or of plasma factors [13]. The ESR is a useful test in clinical practice also as an indicator of other inflammatory processes, infection, trauma or malignant disorders, stroke etc. As the age of patients prone to coronary artery diseases and other inflammatory processes like arthritis, vasculitis etc. is common; specificity of the test becomes low. And thus arises the need of a test that correlates better to assess erythrocyte aggregation in CHD patients [14]. Berliner for the first time did a study known as slide test showed that erythrocyte aggregation may be a useful biomarker showing superiority over other commonly used markers to detect inflammation in subjects with atherosclerotic heart disease. Erythrocyte aggregation can be determined accurately by commercially available systems, the Myrene Rheometer and the LORCA (Laser-assisted optical rotation cell analyzer), which employs light transmission or light scattering to obtain indices of erythrocyte aggregation [15].

A cell flow analyzer (CFA) has been developed in the laboratory of Yedgar for monitoring erythrocyte aggregation by direct visualization of the aggregation. It analyzes the aggregate size distribution expressed as the number of cells per aggregate, as a function of shear stress. However, they are very expensive and need a well-established laboratory with expensive equipment [16]. In A study 50% (15 of 30) of patients of AMI belonged to C and D grades. Among the controls, only 2 of the 30 subjects belonged to grades C and D. In our study, 8 patients showed Grade A, 53 patients show Grade B, 34 patients showed Grade C and 5 patients showed Grade D RBC aggregation [17].

CONCLUSION

Erythrocyte adhesiveness/aggregation is a useful biomarker to detect internal inflammation in individuals with atherothrombotic risk factors. In the present study, greater erythrocyte aggregation was seen in subjects of acute ST elevation myocardial infarction and significant correlation was found between grades of erythrocyte aggregation and prognosis of the patient. Patients with severe grades of erythrocyte aggregation C&D are high risk individuals for complications. These high risk individuals are candidates for PCI (delayed PCI) and hence, referred to higher centre for the same. However, this needs further studies. In the present study, stained slides were assessed and graded and were found to be good prognostic indicators in patients with MI. It is a simple bed side procedure and is cost-effective. Thus, it can be used as a screening test for high-risk individuals. In this study, it is concluded that slide test showed better correlation with the prognosis and aided well to detect the presence of internal inflammation in STEMI.

REFERENCES

1. Jayavanth S, Singh M; Computerized analysis of erythrocyte aggregation from sequential video-microscopic image sunder gravitational sedimentation. Techno Med Bio., 2004; 25:67-74.
2. Lakshmi A B, Uma P, Venkatachalam C, Nageswar Rao S; A simple slide test to assess erythrocyte aggregation in acute ST-elevated myocardial infarction and acute is chemic stroke: Its prognostic significance. Indian J Pathol Microbiol., 2011; 54:63-9.
3. Ozlu MF, Sen, Karakas MF, Turak O, Ozcan F, Kanat S; Erythrocyte sedimentation rate in acute myocardial in farction as a predictor of poor prognosis and impaired reperfusion Med Glas Ljekkomore- Zenicko-dobojkantona, 2012; 9(2):189-97.
4. Rampling MW, Meiselman HJ, Neub B, Baskurt OK; Influence of cell specific factors on red blood cell aggregation. Biorheology, 2004; 41:91-112.
5. Bamuer GR, PorzP, Boss, L Ehmache, Stam D; The Erythrocyte Aggregation Value as a measure of the risk of Myocardial Infarction and Arteriosclerosis of Peripheral rteries Clin. Cardiol., 1985; 8: 529-534.
6. Arbel Y, Banai S, Benhorin J, Finkelstein A, HerzI, HalkinA; Erythrocyte aggregation as a cause of slow flow in patients of acute coronary syndromes, International Journal of Cardiology, 2012; 154: 322–27.
7. Danesh J, Collins R, Peto R, Lowe GD; Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies ofcoronary heart disease. Eur Heart J., 2000; 21: 515-20.
8. NataliA, L'Abbate A,Ferrannini E; Erythrocyte sedimentation rate, coronary atherosclerosis, and cardiac mortality. EurHeartJ., 2003; 24:639-48.130
9. Fusman R, Zeltser D, Rotstein R, Chapman J, Avitzour D, Shapira II, Eldor A, Elkayam O, Caspi D, Arber N, Berliner S; INFLAMET: An image analyzer to display erythrocyte adhesiveness/aggregation. Eur J Intern Med., 2000;11:271-6.
10. Hysi E, Ratan K, Kolios MC; Characterization of Red BloodCell Aggregation with Photoacoustics: A Theoretical and Experimental Study IEEE International Ultrasonics Symposium Proceeding, 2011; 1187-90.
11. Berliner S, Rogowski O, Aharonov S, Mardi T, Tolshinsky T; Erythrocyte adhesiveness/

aggregation: A novel biomarker for the detection of low-grade internal inflammation in individuals with athero thrombotic risk factors and proven vascular disease. American Heart Journal, 2005; 260-5.

12. Zeltser D, Rotstein R, Rogowski O, Fusman R, Shapira I, Prochorov V; The erythrocyte adhesiveness/aggregation test (eaat) in the peripheralblood of patients with ischemic heart and brain disease with normal fibrinogen concentrations. Applied Rheology, 2000: 10(5):231-7.131.
13. Weng X, Roederer GO, Beaulieu R, Cloutier G; Contributionofacutephase proteins and cardiovascular risk factors to erythrocyte aggregation in normolipidemic and hyperlipidemic individuals. Thromb Haemost., 1998; 80:903-8.
14. Sharshun Y, Brill S, Mardi T, Justo D, Rozenblat M, GoldinY, Serov J, Berliner S, Shapira I; Inflammation at a glance, erythrocyte adhesiveness/aggregation test to reveal the presence of inflammation in people with atherothrombotic. Heart Dis., 2003; 5:182-3.
15. Stolez JF, Donner M; The Importance of erythrocyte aggregation inblood rheology: Considerations on the pathophysiology of thrombotic disorders. Blood, 1997; 89:4236.
16. Assayag E, Bornstein N, Shapira I, Mardi T, Goldin Y, Tolshinski T, Vered Y, Zakuth V, Burke M, Berliner S, Bonet DS; Inflammation sensitive proteins and erythrocyte aggregation in atherothrombosis.Int J Cardiol., 2005; 98:271-6.132
17. Danesh J, Collins R, Peto R, Lowe GD; Haematocrit, viscosity,erythrocyte sedimentation rate::meta-analyses of prospective studiesof coronary heart disease. Eur Heart J., 2000; 2:121-50.