Clinical Lipidology

ISSN: 1758-4299 (Print) 1758-4302 (Online) Journal homepage: https://www.tandfonline.com/loi/tlip20

The association of dyslipidemia with erythrocyte aggregation

Prajwal Gyawali, Ross S Richards, Phillip T Bwititi & Ezekiel Uba Nwose

Research Article

Clinical Lipidology

The association of dyslipidemia with erythrocyte aggregation

Aim: The present study aims to associate erythrocyte aggregation with plasma lipid concentration. **Materials & Methods**: Ninety-nine participants were divided into two groups: normolipidemic and dyslipidemic. Lipid parameters were measured from serum and erythrocyte aggregation parameters: critical stress and critical time were measured from whole blood. Data were analyzed using IBS SPSS statistics software. **Results:** Erythrocyte aggregation was higher in the dyslipidemic group when compared with the normolipidemic group. Erythrocyte aggregation was associated with the abnormalities in lipid parameters, with the association of HDL-cholesterol and triglyceride being stronger than that of LDL-cholesterol and total cholesterol. **Conclusion**: The strong association of triglyceride and HDL-cholesterol with erythrocyte aggregation emphasizes the role of these lipids in the cardiovascular system.

Prajwal Gyawali*[,1],
Ross S Richards[1],
Phillip T Bwititi[2]
& Ezekiel Uba Nwose[3]
[1]Biomedical Sciences, School of Community Health, Charles Sturt University, NSW 2640, Australia
[2]School of Biomedical Sciences, Charles Sturt University, NSW 2640, Australia
[3]School of Community Health, Charles Sturt University, NSW 2640, Australia
*Author for correspondence:
clbioprajwal@gmail.com

Keywords: dyslipidemia • erythrocyte aggregation • HDL-C • triglyceride

Background

Dyslipidemia is a modifiable risk factor for cardiovascular diseases (CVDs). Elevated LDL-cholesterol (LDL-C) levels in blood are associated with coronary heart diseases; hence, the National Cholesterol Education Programme, Adult Treatment Panel III (NCEP ATP III) report mentions LDL-C as a primary target of therapy [1]. The type of dietary lipids and the plasma lipid concentrations have an effect on the lipid composition of the erythrocytes [2]. An increased concentration of cholesterol in plasma implies its increased accumulation in the erythrocyte membrane, which leads to altered shape and stiffness [3]. It has been shown that increased erythrocyte aggregation is associated with adverse effects in the cardiovascular system [4]. A long-standing postulation is that erythrocyte aggregates could form a thrombus in the circulation at low shear stress, which may lead to ischemia and infarction [5]. However, this has yet to be considered in clinical applications. It has been reported that the odds of having myocardial infarction in patients with unstable angina was 5.7-times higher among groups with elevated erythrocyte aggregation when compared with groups with normal erythrocyte aggregation [6]. Thus, it is important to study the effect of dyslipidemia on erythrocyte aggregation and in this context, this study investigated the association of dyslipidemia with erythrocyte aggregation and compared the strengths of associations with the serum lipid measurements: LDL-C, HDL-cholesterol (HDL-C), triglyceride (TG) and total cholesterol (TC).

Materials & methods

Participants

Participants were recruited from two rural cities of Australia from June to December 2013 as part of studies into the association of hemorheological parameters with metabolic syndrome [7]. Pregnant women, nonambulatory patients and children under 18 years of age were excluded from the study. Ninety-nine participants who qualified for this study were divided into two groups on the basis of the absence or presence of dyslipidemia. An NCEP-ATPIII guideline was referred to in order to

Future Medicine part of fsg

classify dyslipidemia [1]. According to NCEP-ATPIII guidelines, hypercholesterolemia is defined as TC >200 mg/dl, LDL-C as >100 mg/dl, hypertriglyceridemia as TG >150 mg/dl and low HDL-C as <40 mg/dl. Dyslipidemia was defined by the presence of one or more abnormal serum lipid concentration. The study was approved by the University Human Research Ethics Committee (2012/131).

Laboratory analysis

Approximately 20 ml of blood was taken from the participants into ethylenediaminetetraacetic acid and plain vials. Erythrocyte aggregation measurement was carried out using a RheoScan-And 300 system (Rheo-Meditech, Inc., Seoul, Korea). All measurements were performed in duplicate according to the manufacturers' instructions within 1.5 h of blood collection and the principle involved was light transmission. The test kit was filled with 500 μl of 40% hematocrit adjusted whole blood and loaded in the instrument. While the blood was flowing through the microchannel, a laser beam emitted from the laser diode traversed the diluted erythrocyte suspension and was scattered by the erythrocytes. The backscattered light was captured by two photodiodes, linked to a computer. When a high pressure differential was initially applied, a strong shear flow occurred through the microchannel and the erythrocyte aggregates started to disaggregate and this was indicated by the corresponding increase in light intensity. As the pressure differential decreased exponentially, the shear flow also decreased and the disaggregated erythrocytes re-aggregated, resulting in a decrease in the corresponding light intensity. The initial increase of backscattered light was caused by the disaggregation of erythrocytes due to high-shear flow, and the decrease of backscattered light was a result of the re-aggregation of erythrocytes that were associated with low-shear flow [8]. Two measurement indices were used to define erythrocyte aggregation; critical time and critical stress. Critical time is the maximum point of backscattered light and indicates the starting point of the erythrocyte re-aggregation, which is dependent on shear stress and time. The time and shear stress values corresponding to the maximum point of backscattered light are defined as the critical time and the critical shear stress. Before and after critical time and critical shear stress, there is a critical transition period from disaggregation to re-aggregation of the erythrocytes that is associated with a decreasing shear flow. The lower the critical time, the faster the erythrocyte aggregation process and the higher the critical stress, the faster the erythrocyte aggregation [9]. Lipid profile was measured in a fully automated clinical chemistry analyzer in a commercial pathology laboratory.

Data analysis

Data were analyzed using IBM SPSS Statistics version 20. The normality of the data was tested using a Shapiro–Wilk test. Age and sex adjusted regression analysis was performed to find out whether erythrocyte aggregation could be predicted from the lipid parameters. Erythrocyte aggregation parameter (critical stress) was divided into quartiles and the value at highest quartile was considered an abnormal value (hyperaggregation). Ordinal regression analyses were used through generalized linear model to find out the odds of having dyslipidemia on the highest or lowest quartile of erythrocyte aggregation. A receiver operating characteristics (ROC) curve was constructed to find out the association of lipids parameters with hyperaggregation.

Results

The present study is a part of a larger study that was undertaken to investigate the association of hemorheology with oxidative stress and systemic inflammation among participants with and without metabolic syndrome (MetS). The detailed demographic and baseline characteristics of the participants with and without MetS have been published in our recent studies [7,10]. Among the 99 participants, 29 did not have dyslipidemia (controls), 21 had one, 36 had two, 11 had three and two had all four abnormal lipid parameters. The anthropometric measurements and clinical characteristics of participants between normolipidemic and dyslipidemic group are shown in Table 1.

Erythrocyte aggregation parameters (critical time and critical stress) were not normally distributed in either group ($p < 0.05$) as assessed by Shapiro–Wilk tests. Therefore, a Mann–Whitney U test was used to determine if there were differences in the median levels of erythrocyte aggregation parameters between normolipidemic and hyperlipidemic groups. The distributions of the parameters (critical time and critical stress) for both groups were similar, as assessed by visual inspection. Erythrocyte aggregation was found to be significantly higher in the dyslipidemic group when compared with normolipidemic group. Median score of the parameters in the two groups along with U and Z values is given in Table 2. Correlation of erythrocyte aggregation parameters with lipids parameters is shown in Table 3. Figure 1 shows the scatter plot of critical stress against HDL-C and TG.

The critical stress was divided into four groups (quartiles) using 25th, 50th and 75th percentiles. The values at 25th, 50th, and 75th percentile were 259.85, 329.25 and 476.9 Pa. Ordinal logistic regression (generalized linear model) showed that the dyslipidemia occurred 2.825 (95% CI: 1.267–6.299) times

Table 1. Clinical characteristics and anthropometric measurements among participants with and without dyslipidemia.

Demographic characteristics	Normolipidemic (n = 29)	Dyslipidemic (n = 70)	p-value
Mean age (±SD)	57.66 ±15.39	54.43 ±12.75	0.284
Male/female	11/18	42/28	–
Diagnosed diabetes mellitus	14	19	0.042
Diagnosed hypertension	12	29	0.996
Mean body mass index (±SD)	26.31 ±4.40	28.15 ±5.38	0.107

more frequently than normolipidemia, on the highest quartile of erythrocyte aggregation (critical stress), a significant effect, χ^2 (1) = 6.445, p = 0.011.

The lipid risk factors most strongly associated with hyperaggregation (critical stress >476.9 Pa) were decreased levels of HDL-C (odds ratio [OR]: 10.833; 95% CI: 3.749–31.307; p-value <0.0005) followed by increased concentration of TG (OR: 9.714; 95% CI: 3.412–27.659; p-value <0.0005) and LDL-C level (OR: 4.031; 95% CI: 1.438–11.304; p-value = 0.008). The association of TC level with critical stress did not reach to the significance level (p >0.05). ROC curve analysis showed that the area under the curve (AUC) was highest for TG followed by HDL-C for correctly classifying hyperaggregation (critical stress >476.9 Pa) (Table 4 & Figure 2).

Discussion

The present study investigated the effect of dyslipidemia on erythrocyte aggregation and demonstrated significant differences in erythrocyte aggregation parameters between normolipidemic and dyslipidemic group (Table 2). There was no difference in the mean age and BMI level among normolipidemic and dyslipidemic participants. Similarly, there was no difference in the distribution of hypertensive participants between groups; however, for some unknown reason, the proportion of diabetic participants was found to be higher in the normolipidemic group (Table 1). The higher value of critical stress in the dyslipidemic group indicated that higher initial pressure is required to disaggregate the erythrocyte suggesting increased erythrocyte aggregation or decreased erythrocyte disaggregation. Erythrocyte aggregation was associated with the abnormalities in lipids parameters with the association of HDL-C and TG being stronger than for LDL-C and TC. Similarly, erythrocyte aggregation parameters correlated negatively with HDL-C and positively with TG and LDL-C in the present study (Table 3). These data support the idea that there are some underlying processes related to dyslipidemia that are relevant to erythrocyte aggregation. LDL-C enhances erythrocyte interaction and increases erythrocyte aggregation [11,12] and the interaction of LDL-C with erythrocyte membrane is competitively reduced by HDL-C, thus decreasing erythrocyte aggregation [13]. This plausible mechanism reinforces the negative effect of LDL-C and positive effect of HDL-C in the cardiovascular system. It has also been hypothesized that changes in lipid composition of plasma and erythrocyte membrane may enhance the interaction between erythrocytes and plasma proteins that could lead to hyperaggregation [14,15]. Several studies have associated dyslipidemia with erythrocyte aggregation in the past supporting our findings [16,17].

ROC curve analysis (Table 4) and regression analysis showed that among the lipids parameters, levels of plasma TG and HDL-C were most strongly associated with erythrocyte aggregation. Studies have demonstrated increased level of erythrocyte aggregation due to elevated TG concentration [15,18]. It has been shown that among plasma lipids, TG was predominantly associated with increased erythrocyte aggregation in survivors of acute myocardial infarction [19] augmenting the findings of the present study. It is not clear whether TG is directly related to atherosclerosis and/or CVD [20]. The stronger association of TG among lipid parameters with erythrocyte aggregation

Table 2. Median, Mann–Whitney U, Z and p-values of erythrocyte aggregation parameters: critical time and critical stress.

Parameters	Median normolipidemic (n = 29)	Median dyslipidemic (n = 70)	U	Z	p-value
Critical time	8.10	7.5	707.5	-2.3	0.018
Critical stress	289.70	355.45	660.0	-2.7	0.006

Table 3. Correlation between lipids and hemorheological parameters.					
Erythrocyte aggregation parameters		**HDL-C**	**TG**	**LDL-C**	**TC**
Critical time	R	.280	-.438	.097	.034
	p-value	.005	<0.0005	.340	.737
Critical stress	R	-.459	.591	.204	-.117
	p-value	<0.0005	<0.0005	.043	.248

HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TC: Total cholesterol; TG: Triglyceride.

in the present study suggests that the possible effect of TG in the cardiovascular system should not be ignored. The American Heart Association considers TG as an independent cardiovascular risk factor [21] and, similarly, HDL-C has been considered as an independent CVD risk factor [22]. Patients who have achieved LDL-C target goal could still remain at risk for cardiovascular events if they have elevated TG and low HDL-C concentrations [23,24]. Hence, the European Atherosclerosis Society consensus panel recommends elevated TG and low HDL-C as targets for treatment [25]. The association of HDL-C with myocardial infarction was found to be stronger than LDL-C in the study of Sigdel *et al.* [26], possibly indicating that HDL-C could be the major determinant of clinical outcomes. The findings from the present study emphasize that HDL-C and TG should be given equal, if not more, importance than other routinely measured lipid parameters in the context of blood rheology and CVD.

It has been shown that increased erythrocyte aggregation leads to slow coronary blood f ow resulting in altered microcirculation [27]. Aggregates could form a thrombus at low shear stress [5]. Yedgar *et al.* [28] suggested that erythrocyte f ow could be altered due to abnormal rheological properties that may lead to vascular occlusion. Similarly, it has been demonstrated that increased erythrocyte aggregation increases the plasma layer formed along the wall of blood vessels which decreases oxygen diffusion from the vessels [29,30]. Moreover, it has been demonstrated that aggregation of erythrocytes reduces the surface-area-to-volume ratio of the cells for oxygen release [29]. The circulatory impairment due to increased erythrocyte aggregation could be one of the pathophysiological bases of cardiovascular risk due to dyslipidemia. By contrast, an alternative view has been proposed that suggests some beneficial effect of erythrocyte aggregation on microvascular perfusion [31]. Erythrocyte aggregation has been considered to enhance axial accumulation of erythrocyte [32] by increasing plasma skimming [33] and Fahraeus effect [34]. This lowers the microvascular hematocrit increasing tissue blood f ow [31]. The net effect of erythrocyte aggregation on microcirculation

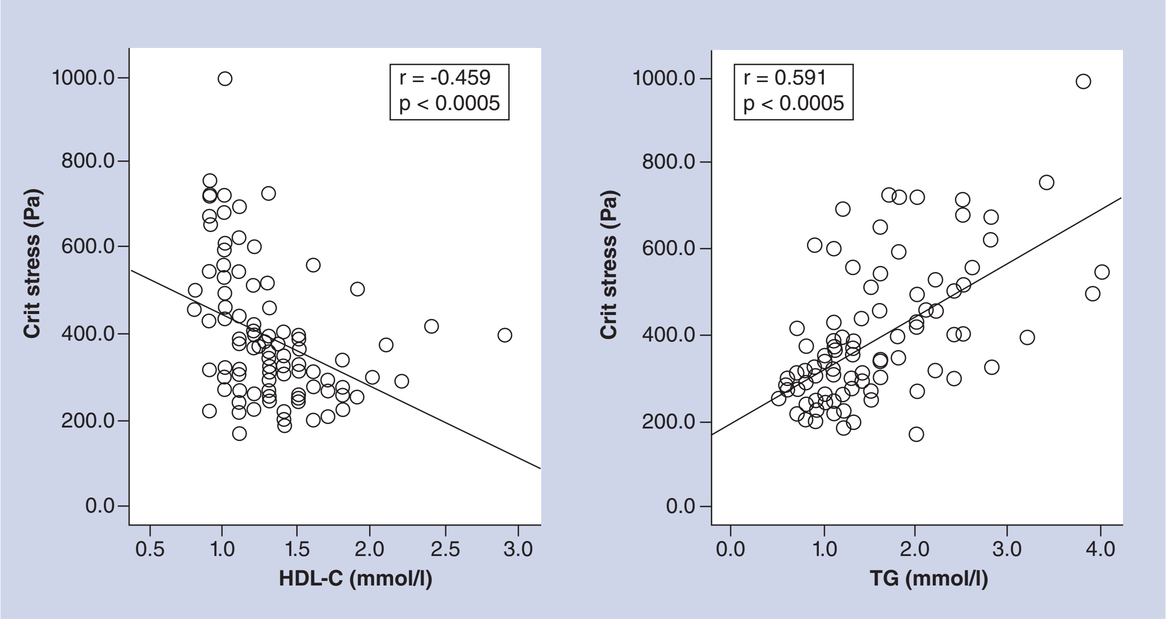

Figure 1. Correlation of critical stress with triglyceride and HDL-cholesterol level.
Crit: Critical; HDL-C: HDL-cholesterol; TG: Triglyceride.

Table 4. Area under the curve and 95% CI obtained from receiver operating characteristics curve analysis for classifying hyperaggregation by lipid parameters.

Parameters	AUC	95% CI	p-value
TG	0.831	0.740–0.922	<0.0005
HDL-C	0.800	0.694–0.906	<0.0005
LDL-C	0.259	0.137–0.380	<0.0005
TC	0.324	0.196–0.452	0.010

AUC: Area under the curve; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TC: Total cholesterol; TG: Triglyceride.

could possibly depend on the interplay of the various complexities underlying the particular diseases in dyslipidemic participants.

Conclusion

This study has shown the association of dyslipidemia with erythrocyte aggregation and has also demonstrated that the association of TG (positive) and HDL-C (negative) to be stronger than other lipid parameters. The strong association of plasma TG and HDL-C with erythrocyte aggregation in the present study possibly emphasizes on the role of altered hemorheology in the cardiovascular system. Dyslipidemia is present in most of the metabolic diseases and the abnormal microcirculation present in metabolic diseases could possibly be due to the effect of dyslipidemia on erythrocyte aggregation.

Future perspective

Larger studies should be undertaken to establish the causal relationship between hemorheological parameters, such as erythrocyte aggregation, and dyslipidemia. Some of the effect of dyslipidemia on the cardiovascular system could be due to the abnormalities in erythrocyte rheological properties. It is high time studies be focused toward the clinical importance of hemorheological parameters. The significance of erythrocyte aggregation as a marker of CVD certainly deserves more attention.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

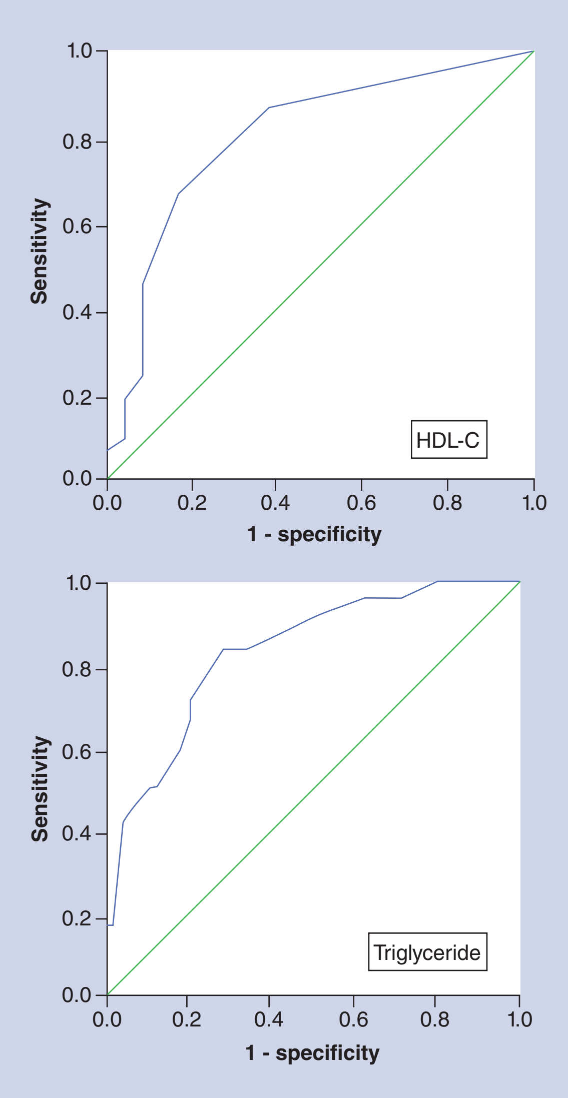

Figure 2. Receiver operating characteristics curve showing the area under the curve for correctly predicting hyperaggregation (critical stress >476.9 Pa). HDL-C: HDL-cholesterol.

Executive summary

- The nature of the dietary lipids and the lipid composition of the erythrocytes influence the aggregation properties of erythrocytes.
- Increased erythrocyte aggregation is associated with the adverse effect in the cardiovascular system.
- Erythrocyte aggregation was associated with the abnormalities in lipid parameters with the association of HDL-cholesterol and triglyceride (TG) being stronger than for LDL-cholesterol and total cholesterol in the present study.
- There are some underlying processes related to dyslipidemia that are relevant to erythrocyte aggregation.
- The stronger association of TG among lipid parameters with erythrocyte aggregation in the present study suggests that the possible effect of TG in the cardiovascular system should not be ignored.
- Some of the effect of dyslipidemia on the cardiovascular system could be due to the increased erythrocyte aggregation.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Expert Panel on Detection Evaluation Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* 285(19), 2486–2497 (2001).

2 Dougherty RM, Galli C, Ferro-Luzzi A, Iacono J. Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. *Am. J. Clin. Nutr.* 45(2), 443–455 (1987).

3 Kanakaraj P, Singh M. Influence of cholesterol-enrichment under *in vivo* and *in vitro* conditions on the erythrocyte membrane lipids and its deformability. *Indian J. Biochem. Biophys.* 26(6), 381–385 (1989).

4 Gyawali P, Richards RS, Hughes DL, Tinley P. Erythrocyte aggregation and metabolic syndrome. *Clin. Hemorheol. Microcirc.* 57(1), 73–83 (2014).

• **This review article provides all the important and latest evidence to emphasize the importance of erythrocyte aggregation in metabolic syndrome.**

5 Stoltz J, Donner M. Hemorheology-importance of erythrocyte aggregation. *Clin. Hemorheol.* 7(1), 15–23 (1987).

6 Neumann FJ, Katus HA, Hoberg E *et al.* Increased plasma viscosity and erythrocyte aggregation: indicators of an unfavourable clinical outcome in patients with unstable angina pectoris. *Br. Heart J.* 66(6), 425–430 (1991).

7 Gyawali P, Richards RS, Tinley P, Nwose EU. Hemorheology, ankle brachial pressure index (ABPI) and toe brachial pressure index (TBPI) in metabolic syndrome. *Microvasc. Res.* 95, 31–36 (2014).

8 Zhao H, Wang X, Stoltz J. Comparison of three optical methods to study erythrocyte aggregation. *Clin. Hemorheol. Microcirc.* 21(3), 297–302 (1999).

9 Hou JX, Shin S. Transient microfluidic approach to the investigation of erythrocyte aggregation: comparison and validation of the method. *Korea–Australia Rheology J.* 20(4), 253–260 (2008).

10 Gyawali P, Richards RS. Association of altered hemorheology with oxidative stress and inflammation in metabolic syndrome. *Redox Rep.* (2014) http://dx.doi.org/10.1179/1351000214Y.0000000120. (Epub ahead of print).

11 Simon E, Razavian SM, Lebeyec J *et al.* Influence of lipoprotein subfractions on dextran- and fibrinogen-induced erythrocyte aggregation. *Clin. Hemorheol.* 15(4), 667–676(1995).

12 Crowley JP, Metzger J, Assaf A, Carleton RC, Merrill E, Valeri CR. Low density lipoprotein cholesterol and whole blood viscosity. *Ann. Clin. Lab. Sci.* 24(6), 533–541 (1994).

13 Sloop GD, Garber DW. The effects of low-density lipoprotein and high-density lipoprotein on blood viscosity correlate with their association with risk of atherosclerosis in humans. *Clin. Sci.* 92(5), 473–479 (1997).

14 Maeda N, Cicha I, Tateishi N, Suzuki Y. Triglyceride in plasma: prospective effects on microcirculatory functions. *Clin. Hemorheol. Microcirc.* 34(1–2), 341–346 (2006).

15 Cicha I, Suzuki Y, Tateishi N, Maeda N. Enhancement of red blood cell aggregation by plasma triglycerides. *Clin. Hemorheol. Microcirc.* 24(4), 247–255 (2001).

• **Demonstrates the effect of triglyceride on erythrocyte aggregation.**

16 Abrashkina ED, Shaalali N, Pakhrova OA, Shutemova EA, Nazarova OA. Effect of atorvastatin on hemorheological parameters in patients with arterial hypertension with dyslipidemia. *Kardiologiia* 50(5), 25–28 (2010).

17 Babu N. Alterations in aggregation parameters of erythrocytes due to hyper cholesterol in Type-2 diabetes mellitus. *Open Circ. Vasc. J.* 2, 10–14 (2009).

18 Zhao T, Guo J, Li H *et al.* Hemorheological abnormalities in lipoprotein lipase deficient mice with severe hypertriglyceridemia. *Biochem. Biophys. Res. Commun.* 341(4), 1066–1071 (2006).

19 Vayá A, Falcó C, Réganon E *et al.* Influence of plasma and erythrocyte factors on red blood cell aggregation in survivors of acute myocardial infarction. *Thromb. Haemost.* 91(2), 354–359 (2004).

• **Deals with the plasmatic factors affecting erythrocyte aggregation.**

20 Hulley SB, Rosenman RH, Bawol RD, Brand RJ. Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary heart disease. *N. Engl. J. Med.* 302(25), 1383–1389 (1980).

21 Miller M, Stone NJ, Ballantyne C *et al.* Triglycerides and cardiovascular disease: a scientific statement From the American Heart Association. *Circulation* 123(20), 2292–2333 (2011).

•• **Highlights the effect of triglyceride on cardiovascular system.**

22 Boden WE. High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Am. J. Cardiol.* 86(12), 19–22 (2000).

•• **Focuses on the independent effect of low HDL-cholesterol in cardiovascular system.**

23 Miller M, Cannon CP, Murphy SA, Qin J, Ray KK, Braunwald E. Impact of triglyceride levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the PROVE IT-TIMI 22 trial. *J. Am. Coll. Cardiol.* 51(7), 724–730 (2008).

24 Barter P, Gotto AM, Larosa JC *et al.* HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N. Engl. J. Med.* 357(13), 1301–1310 (2007).

25 Chapman MJ, Ginsberg HN, Amarenco P *et al.* Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur. Heart J.* 32(11), 1345–1361 (2011).

26 Sigdel M, Yadav BK, Gyawali P *et al.* Non-high density lipoprotein cholesterol versus low density lipoprotein cholesterol as a discriminating factor for myocardial infarction. *BMC Res. Notes* 5(1), 640 (2012).

27 Ergun-Cagli K, Ileri-Gurel E, Ozeke O *et al.* Blood viscosity changes in slow coronary f ow patients. *Clin. Hemorheol. Microcirc.* 47(1), 27–35 (2011).

28 Yedgar S, Koshkaryev A, Barshtein G. The red blood cell in vascular occlusion. *Pathophysiol. Haemost. Thromb.* 32(5–6), 263–268 (2002).

29 Tateishi N, Suzuki Y, Cicha I, Maeda N. O2 release from erythrocytes f owing in a narrow O2-permeable tube: effects of erythrocyte aggregation. *Am. J. Physiol. Heart Circ. Physiol.* 281(1), H448–H456 (2001).

30 Boland EJ, Nair PK, Lemon DD, Olson JS, Hellums JD. An *in vitro* capillary system for studies on microcirculatory O2 transport. *J. Appl. Physiol.* 62(2), 791–797 (1987).

31 Baskurt OK, Meiselman HJ. Erythrocyte aggregation: basic aspects and clinical importance. *Clin. Hemorheol. Microcirc.* 53(1), 23–37 (2013).

32 Bishop JJ, Popel AS, Intaglietta M, Johnson PC. Effects of erythrocyte aggregation and venous network geometry on red blood cell axial migration. *Am. J. Physiol. Heart Circ. Physiol.* 281(2), H939–H950 (2001).

33 Pries A, Secomb T, Gaehtgens P. Biophysical aspects of blood f ow in the microvasculature. *Cardiovasc. Res.* 32(4), 654–667 (1996).

34 Fahraeus R. The suspension stability of blood. *Physiol. Rev.* 9, 241–274 (1929).