Clinical Hemorheology and Microcirculation 64 (2016) 1–5
DOI 10.3233/CH-152033
IOS Press

Hemorheological parameters better classify metabolic syndrome than novel cardiovascular risk factors and peripheral vascular disease marker

Prajwal Gyawali[a,*], Ross S. Richards[a], Paul Tinley[b], Ezekiel Uba Nwose[c] and Phillip T. Bwititi[d]

[a]*Biomedical Sciences, School of Community Health, Charles Sturt University, NSW, Australia*
[b]*Podiatry, Charles Sturt University, NSW, Australia*
[c]*School of Community Health, Charles Sturt University, NSW, Australia*
[d]*School of Biomedical Sciences, Charles Sturt University, NSW, Australia*

Abstract. The present study compares the association of Metabolic Syndrome (MetS) with hemorheological parameters, oxidative stress, inflammation and peripheral arterial disease markers. 100 participants were recruited and participants were divided into three groups on the basis of absence or presence of MetS and its components. Odds ratio for correctly predicting MetS was highest for erythrocyte aggregation followed by erythrocyte deformability. ROC curve analysis demonstrated that all the hemorheological components significantly classified MetS participants. Area Under Curve was higher for the hemorheological parameters (erythrocyte aggregation and erythrocyte deformability) than for the oxidative stress, inflammation and peripheral arterial disease markers. The possibilities of the hemorheological components to be identified as better cardiovascular risk markers due to their strong association with MetS cannot be precluded from the present findings.

Keywords: Hemorheology, oxidative stress, inflammation, ROC curve

1. Background

Hemorheological parameters are altered in metabolic syndrome (MetS) and its components [8–13]. Oxidative stress and chronic inflammation present in MetS are shown to be responsible for hemorheological changes to certain extent [7, 8]. In this brief report, we have presented the data that compare the association of MetS with hemorheological parameters (erythrocyte aggregation, erythrocyte deformability and whole blood viscosity (WBV)), oxidative stress (urinary isoprostanes), inflammation (high sensitivity C-reactive protein (hsCRP)), coagulopathy (D-dimer) and peripheral arterial disease (toe brachial pressure index (TBPI)).

2. Materials and methods

Erythrocyte deformability and erythrocyte aggregation was measured by RheoScan-AnD 300 system (RheoMeditech Inc., Korea). WBV measurement was carried out using a Brookfield DV-II+

*Corresponding author: Dr. Prajwal Gyawali (PhD), Biomedical Sciences, School of Community Health, Charles Sturt University, NSW 2640, Australia. Tel.: +61414932057; E-mail: clbioprajwal@gmail.com.

1386-0291/16/$35.00 © 2016 – IOS Press and the authors. All rights reserved

programmable viscometer (MA, USA), using a CP40 spindle at 37°C at a shear rate of 150 s^{-1}. Erythrocyte morphology was studied by scanning electron microscopy (JCM 5000, Benchtop SEM, Neoscope). All the rheological measurements were performed within two hours of blood collection after adjusting EDTA anticoagulated whole blood to the hematocrit of 40%. TBPI was measured by using SysToe (ATYS Medical). MetS was defined by National Cholesterol Education Program, Adult Treatment Panel III definition [6]. Inflammatory markers high sensitivity C-reactive protein (hsCRP) and thrombotic marker D-dimer were measured in the day of collection in a commercial clinical pathology laboratory. 15-isoprostanes F2t was measured in urine sample (NWLSS™) and was expressed as ng of isoprostanes per mmol of urinary creatinine (Cayman chemical). The details of instrumentation and demographic characteristics of the participants have been published elsewhere [7–9, 13]. Briefly, 100 participants were recruited from a rural town of Australia from June–Dec 2013. Pregnant women, non-ambulatory patients, and children under 18 years of age were excluded from the study. Recruited participants were divided into three groups on the basis of absence or presence of MetS and its components. Group I consists of the participants without any positive components of MetS (healthy controls); group II consists of the participants with one or two positive components; and group III consists of participants with three or more positive components. Participants in groups I and II are non- MetS whereas participants of group III are with MetS.

3. Results

Of the 100 participants, 36 participants had MetS, 33 had one or two positive components and 33 were healthy controls.

3.1. Binomial logistic regression analysis

Binomial logistic regression analysis (adjusted for age and sex) was performed to predict the chances of having MetS by altered hemorheological parameters; urinary isoprostanes, hsCRP, D-Dimer and TBPI. All of the markers were divided into quartiles and the odds of having MetS after increase or decrease (EI_{max}, TBPI) in one quartile of the markers was estimated. The results show that all of the markers significantly predicted MetS and the Odds ratio was highest for erythrocyte aggregation followed by erythrocyte deformability.

3.2. ROC Curve analysis

The values of odds ratio obtained in the regression analysis depend on the range of data and the scaling. The regression coefficient represents the expected change in y (Mets/non-MetS) for a one unit change in × (the predictor: markers), hence, the magnitude of that coefficient is partly determined by the magnitude of the units used. Therefore, to confirm the outputs of logistic regression analysis, ROC curve was used to compare the association of different markers with MetS. The ROC curve shows the diagnostic performance of a test, or the accuracy of a test to discriminate two groups (MetS and non-MetS [14] and the area under the ROC curve (AUC) is a measure of how well a parameter can distinguish between two groups [14]. ROC curve analysis demonstrated that all the hemorheological components significantly classified MetS participants (P-values for all curves were < 0.0005). AUC was higher for the hemorheological parameters (erythrocyte aggregation and erythrocyte deformability) than for the TBPI or other oxidative stress and inflammatory markers (Table 2 and Fig. 1).

Table 1
Age and sex adjusted odds ratio for predicting MetS by hemorheological parameters, oxidative stress and inflammatory markers and TBPI

Parameters	Odds Ratio	95% CI	*P*-Value
Critical stress (quartile)	3.896	2.174 to 6.985	<0.0005
EI_{max} (quartile)	2.840	1.666 to 4.830	<0.0005
WBV (quartile)	1.823	1.030 to 1.114	0.009
TBPI (quartile)	1.828	1.059 to 3.154	0.030
Urinary isoprostanes (quartile)	1.715	1.096 to 2.683	0.018
hsCRP (quartile)	2.090	1.297 to 3.370	0.002
D-dimer (quartile)	1.639	1.035 to 2.595	0.035

Table 2
AUC and 95% CI obtained from ROC curve analysis for differentiating MetS from non-MetS

Parameters	AUC	95% CI	*P*-value
Critical stress	0.818	0.715 to 0.922	<0.0005
EI_{max}	0.782	0.688 to o.876	<0.0005
TBPI	0.774	0.679 to 0.869	<0.0005
WBV	0.719	0.616 to 0.821	<0.0005
Urinary isoprostanes	0.706	0.603 to 0.809	0.001
D-dimer	0.695	0.583 to 0.807	0.001
hsCRP	0.661	0.549 to 0.774	0.008

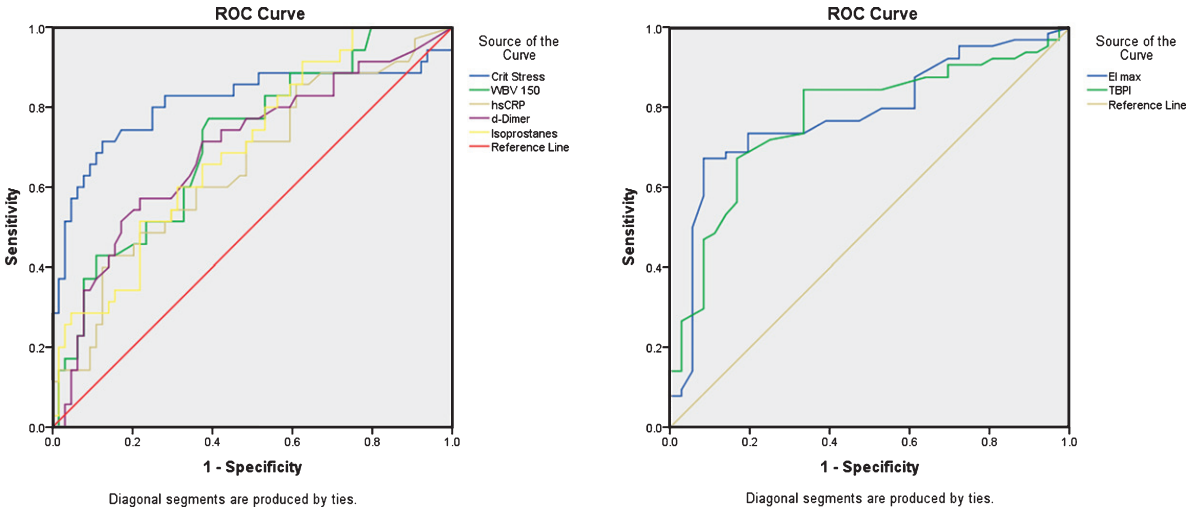

Fig. 1. ROC curve for haemorheological parameters, novel cardiovascular risk factors and peripheral vascular diseases marker for correctly classifying MetS.

4. Conclusions

Age and sex adjusted odds ratio for predicting MetS was higher for hemorheological parameters when compared to TBPI. The ROC curve analysis also showed that two of the three haemorheological

parameters (critical stress and EI_{max}) better classified MetS than TBPI. The finding suggests that hemorheology better identifies with MetS than macrovascular circulation abnormalities. Microvascular dysfunction (lower functional capillary density) has been shown in MetS participants [5]. Superiority of the hemorheological parameters in predicting MetS than that of peripheral arterial disease marker further emphasises the importance that should be given to rheological changes occurring in the MetS along with macrovascular assessment. The present findings also suggests that rheological changes may occur earlier or more frequently than the peripheral vasculopathy in MetS and its early identification may provide clinical benefits to the MetS patients.

Insulin resistance is generally considered as a major factor for the pathogenesis of MetS [15]. Insulin resistance is associated with increased erythrocyte aggregation [4]. Brun JF et al. suggested that increased erythrocyte aggregation is an early phenomenon that characterises insulin resistance at an initial stage where it is compensated by an increase in insulin secretion [4] and the increased erythrocyte aggregation could be considered as a major hemorheological alteration of insulin resistance [3]. Moreover, increased erythrocyte aggregation has been reported among the obese subjects who are not under the state of MetS [2] signifying that role of adipocytokines and adiposity in hemorheological alterations. Similarly, in the present study, the AUC for erythrocyte aggregation (critical stress) was found to be higher than that of hsCRP and urinary isoprostanes. Also, since erythrocyte aggregation is significantly associated with oxidative stress and chronic inflammation generated in MetS, it could be included as a component of MetS. No studies have reported the ROC curve analysis of hemorheological parameters for the correct prediction of MetS making it difficult to make comparisons. However, it has been shown that increased erythrocyte aggregation correctly classified patients with vascular disease [1]. Furthermore, from the ROC curve analysis, AUC of erythrocyte aggregation for the correct classification of vascular disease was shown to be higher than that of ESR, fibrinogen and hsCRP [1]. Similarly, it has been shown that although conventional cardiovascular risk parameters such as triglyceride, HDL-C, LDL-C, total cholesterol, BMI and fibrinogen did not significantly predicted cardiac death, haematocrit/WBV significantly predicted the same ($AUC = 0.716$; $P = 0.028$) [16]. The possibilities of the hemorheological components to be identified as better cardiovascular risk markers due to their strong association with MetS cannot be precluded from present findings.

References

[1] S. Berliner, O. Rogowski, S. Aharonov, T. Mardi, T. Tolshinsky, M. Rozenblat, D. Justo, V. Deutsch, J. Serov, I. Shapira and D. Zeltzer, Erythrocyte adhesiveness/aggregation: A novel biomarker for the detection of low-grade internal inflammation in individuals with atherothrombotic risk factors and proven vascular disease, *Am Heart J* **149** (2005), 260–267.

[2] J.-F. Brun, E. Varlet-Marie, C. Fedou and d. M.E. Raynaud, Are metabolically healthy obese patients also hemorheologically healthy? *Clin Hemorheol Microcirc* **61** (2015), 39–46.

[3] J.F. Brun, E. Varlet-Marie and E. Raynaud de Mauverger, Relationships between insulin sensitivity measured with the oral minimal model and blood rheology, *Clin Hemorheol Microcirc* **51** (2012), 29–34.

[4] J.F. Brun, E. Varlet-Marie, E. Raynaud de Mauverger and J. Mercier, Minimal model-derived insulin sensitivity, insulin secretion and glucose tolerance: Relationships with blood rheology, *Clin Hemorheol Microcirc* **51** (2012), 21–27.

[5] S. Czernichow, J.R. Greenfield, P. Galan, F. Jellouli, M.E. Safar, J. Blacher, S. Hercberg and B.I. Levy, Macrovascular and microvascular dysfunction in the metabolic syndrome, *Hypertens Res* **33** (2010), 293–297.

[6] 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, treatment of high blood cholesterol in adults (adult treatment panel III), *JAMA* **285** (2001), 2486–2497.,

[7] P. Gyawali and R.S. Richards, Association of altered hemorheology with oxidative stress and inflammation in metabolic syndrome, *Redox Report* (2014).

[8] P. Gyawali, R.S. Richards, P.T. Bwititi and E.U. Nwose, Association of abnormal erythrocyte morphology with oxidative stress and inflammation in metabolic syndrome, *Blood Cells Mol Dis* **54** (2015), 360–363.

[9] P. Gyawali, R.S. Richards, P.T. Bwititi and E.U. Nwose, The association of dyslipidemia with erythrocyte aggregation, *Clin Lipidol* **10** (2015), 129–135.
[10] P. Gyawali, R.S. Richards, D.L. Hughes and P. Tinley, Erythrocyte aggregation and metabolic syndrome, *Clin Hemorheol Microcirc* **57** (2014), 73–83.
[11] P. Gyawali, R.S. Richards and E.U. Nwose, Erythrocyte morphology in metabolic syndrome, *Expert Rev Hematol* **5** (2012), 523–531.
[12] P. Gyawali, R.S. Richards, E.U. Nwose and P.T. Bwititi, Whole-blood viscosity and metabolic syndrome, *Clin Lipidol* **7** (2012), 709–719.
[13] P. Gyawali, R.S. Richards, P. Tinley and E.U. Nwose, Hemorheology, Ankle brachial pressure index (ABPI) and toe brachial pressure index (TBPI) in metabolic syndrome, *Microvasc Res* **95** (2014), 31–36.
[14] J.A. Hanley and B.J. McNeil, The meaning and use of the area under a receiver operating characteristic (ROC) curve, *Radiology* **143** (1982), 29–36.
[15] S.R. Kashyap and R.A. Defronzo, The insulin resistance syndrome: Physiological considerations, *Diab Vasc Dis Res* **4** (2007), 13–19.
[16] P. Kenyeres, I. Juricskay, P. Tarsoly, G. Kesmarky, D. Mühl, K. Toth and L. Bogar, Low hematocrit per blood viscosity ratio as a mortality risk factor in coronary heart disease, *Clin Hemorheol Microcirc* **38** (2008), 51–56.

Copyright of Clinical Hemorheology & Microcirculation is the property of IOS Press and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.