

The Role of a Novel

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The Role of a Novel Digital Microcapillary Instrument in Detecting Blood and Plasma Hyperviscosity

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ABSTRAK

Tujuan: menguji presisi dan akurasi mikrokapiler digital dalam mengukur viskositas darah dan plasma. **Metode:** sebanyak 40 sampel darah untuk uji presisi diambil dari pasien Medical Check-Up di RS Cipto Mangunkusumo (RSCM) pada bulan Desember 2011. Uji akurasi menggunakan desain potong lintang, melibatkan 135 subjek stroke iskemik akut yang diambil baik dari pasien rawat inap maupun pasien poliklinik di Departemen Neurologi RSCM, RS Fatmawati Jakarta, RS Prikasih Jakarta, dan RS Bhakti Yuda Depok. Baku emas yang digunakan adalah viskometer Brookfield LV-DV III. Uji presisi dilakukan dengan menghitung coefficient of variation (CV), interrater variability Crohnbach Alpha, dan coefficient reliability Bland Altman. Uji akurasi dilakukan dengan uji diagnostik. Baku emas yang digunakan untuk uji tersebut adalah viskometer Brookfield LV-DV III. **Hasil:** hasil uji presisi adalah: CV = 0,04; interrater variability dari viskositas darah dan plasma = 0,94 dan 0,82; mean difference Bland Altman = -0,19. Hasil uji akurasi adalah: sensitivitas untuk pemeriksaan viskositas darah dan plasma = 88,9% dan 100%; spesifisitas untuk pemeriksaan viskositas darah dan plasma = 88,9% dan 84%. **Kesimpulan:** mikrokapiler digital memiliki presisi dan akurasi yang tinggi, karena itu, alat ini dapat dipertimbangkan untuk digunakan sebagai pemeriksaan skrining dalam mengukur viskositas darah dan plasma.

Kata kunci: viskositas darah, viskositas plasma, stroke.

ABSTRACT

Aim: to test the precision and accuracy of a Digital Microcapillary instrument in measuring blood and plasma viscosity. **Methods:** about 40 blood samples were drawn for precision test. The samples were obtained from patients admitted for Medical Check-Up at CiptoMangunkusumo Hospital (RSCM) in December 2011. Accuracy test was evaluated using cross-sectional design and involving 135 patients with acute ischemic stroke. The patients underwent either inpatients or outpatients care at Department of Clinical Pathology, Department of Neurology, and Emergency Unit of RSCM, Fatmawati Hospital Jakarta, Prikasih Hospital Jakarta, and Bhakti Yuda Hospital Depok. The precision test was evaluated by calculating the coefficient of variation (CV),

1 interrater variability of Cronbach Alpha, and reliability coefficient of Bland Altman. The accuracy of the test was evaluated with a diagnostic test. The gold standard used for these tests was Brookfield LV-DV III viscometer. **Results:** the results of precision test were: $CV = 0.04$; interrater variability of blood and plasma viscosity = 0.94 and 0.87, **10** respectively; the Bland Altman mean difference = -0.19 . The results of accuracy test **10** re: sensitivity of blood and plasma viscosity measurement were 88.9% and 100%, respectively; specificity of blood and plasma viscosity measurement were 88.9% and 84%, respectively. **Conclusion:** the digital microcapillary has high sensitivity and specificity; therefore the instrument can be considered to be used as screening test tool to measure blood and plasma viscosity.

Key words: digital microcapillary, blood viscosity, plasma viscosity, acute ischemic stroke.

6 INTRODUCTION

Blood viscosity is the resistance of blood to flow caused by friction due to blood movement through the lamina along the axis of blood vessels due to the differences in blood flow speed.¹ Blood viscosity is a laboratory variable in hemorheology, a field that studies blood flow and its properties, including the nature and cellular components of plasma and their relationship with the circulation.

This hemorrheological approach can be used by clinicians (including neurologist, internist, and cardiologist) to understand clinical manifestations associated with vascular condition and blood circulation.² Many studies shows the correlation between blood flow—with blood viscosity as its component—and hemostasis and thrombosis. An increase in blood viscosity is associated with prothrombotic condition.^{3,4}

Blood viscosity value varies between individuals, and tends to be higher in populations with risk factors, such as old age, smoking, hypertension, diabetes melitus, ischemic heart disease, and dyslipidemia.^{3,6} Therefore, blood viscosity value can describe other systemic conditions, and is associated with risk factors of many circulatory system diseases as well as metabolic syndromes. Blood viscosity value may also play an important role in transient ischemic attack (TIA) and ischemic stroke since it is increased in some of these patients (34% and 48%, respectively).⁷ The pathophysiology of cerebral ischemia is highly associated with blood flow, including the collaterals, the blood vessels, and the blood viscosity. Blood hyperviscosity is found in most patients with acute ischemic stroke and it contributes to the severity of outcomes.⁸

Several studies in many countries, including Indonesia, have been conducted to assess the

role of blood viscosity in acute ischemic stroke patients. Meliala⁹ conducted a case control study of 75 subjects aged 35 years or older, diagnosed with ischemic stroke, and had their first attack. In this study, they found that blood hyperviscosity contributed as a risk factor of stroke. Using the adjusted odds ratio (OR) and multivariate analysis, the relationship between hyperviscosity and acute stroke was found to be statistically significant ($p < 0.02$, $OR = 2.32$, 95% Confidence Interval = 1.07–5.11; $p < 0.001$). Rasyid¹⁰ in his study of 160 acute ischemic stroke subjects with up to 3 days onset reported that 65 subjects (40.6%) were found to have blood hyperviscosity. Szaparyl¹¹ studied 297 ischemic stroke subjects, and found that there were increase in hematocrit, fibrinogen, erythrocyte aggregation level, and blood viscosity. Ott¹² in **23** study stated that blood viscosity was increased in more than 40% subjects with acute ischemic stroke in 24 hours.

Blood viscosity represents vascular and circulatory conditions, and also plays a major role in ischemic stroke. Therefore, assessment of this value is very important especially in patients with risk factors, and measurement in acute settings is crucial for stroke patients as it determines the treatment. To date, the assessment can only be done in large laboratory setting and the results cannot be obtained instantly. We have designed a simple and portable instrument to measure blood and plasma viscosity that produces immediate results. The instrument, digital microcapillary (DM), was analyzed to determine its precision and accuracy in its application for healthy patients, patients with risk factors, as well as acute ischemic stroke patients by comparing the values measured with DM to those of conventional instrument.

METHODS

The Principle of Viscosity Measurements

The principles of viscosity measurements in DM was adapted from microcapillary principles. When the blood or plasma is touched by the tip of microcapillary, it is forced by capillary suction force and flows through the tube. The blood or plasma along the tube will pass through two sensors. The first sensor will turn on the counter; while the second provides signal to the counter to stop counting. The blood or plasma passing time will be recorded and converted to blood or plasma viscosity (**Figure 1**).

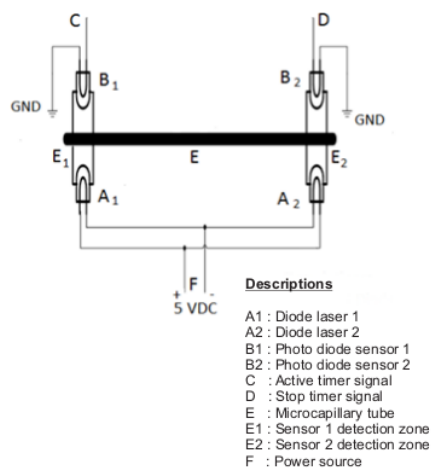


Figure 1. Digital microcapillary.
(Source: Rasyid A. Modifikasi Mikrokapiler Digital untuk Mengukur Nilai Viskositas Darah dan Viskositas Plasma. Kementerian Hukum dan Hak Asasi Manusia R.I. Direktorat Jenderal Hak Kekayaan Intelektual Direktorat Paten P00201300222, Mar. 2013)

Procedure

The instruments used in this study included 3 mL K3EDTA vacuum tubes, 2 mL tubes filled with anticoagulant, alcohol pad, plaster, blood tube shelves, and a digital microcapillary instrument. The venous blood sample was collected and put into a tube containing anticoagulant ethylene potassium ethylene diamina tetra acetic acid (K3EDTA) with the total volume of 5 mL (3 mL sample and 2 mL K3EDTA). The sample was then used for blood and plasma viscosity measurements (2 mL) and hemoglobin, hematocrit, erythrocytes,

leukocytes, and platelets laboratory tests (3 mL). To obtain plasma, the blood sample was centrifuged at 300 rpm speed for 20 minutes. The plasma was subsequently mixed with eosin (0.1 mL eosin for 0.5 mL plasma).

Subjects

This study was based on laboratory examination to test the precision and accuracy of DM. The precision value was obtained by calculating the coefficient of variation (CV), interrater variability of Cronbach Alpha, reliability coefficient of Bland Altman. The number of samples was calculated using Wang's equation.¹³ The precision test used 40 blood samples withdrawn from patients aged 17 – 60 years old who were admitted in Department of Clinical Pathology, Cipto Mangunkusumo Hospital (RSCM) for medical check-up in December 2011.

For the precision test, we excluded patients with fever and abnormal laboratory tests (hemoglobin, leukocytes, platelets, erythrocytes, and total cholesterol).²

An additional test was performed to assess the effects of temperature on blood and plasma viscosity. The temperature in this study represented the tropical climate, i.e. 26 °C, 32 °C, and 37 °C. For this study, 20 samples were used.

The accuracy was assessed using repeated cross-sectional study design. Blood and plasma viscosity values measured by DM was compared with the values measured by the gold standard. We used Brookfield LV-DVIII as the gold standard for this test. With such design, the sensitivity, specificity, likelihood ratio, and predictive value of DM could be obtained. As many as 135 accuracy test samples were obtained from patients who underwent either inpatients or outpatients care, aged 35 – 74 years old, were diagnosed with acute ischemic stroke with up to 3 days onset, and were admitted in Department of Neurology in RSCM, Fatmawati Hospital Jakarta, Prikasih Hospital Jakarta, and Bhakti Yuda Hospital Depok since March 2013 until the number of samples was considered adequate. The samples excluded in accuracy test were patients with transient ischemic attack history and patients with disorders affecting blood and plasma viscosity (anemia, polycythemia, dengue

hemorrhagic fever with diagnostic criteria of WHO 1997, trauma with massive bleeding/ first class bleeding classification, and diarrhea with diagnostic criteria of diarrhea without dehydration).

At the beginning of the study written informed consents were obtained. Our study has been approved by Ethics Committee of Faculty of Medicine, Universitas Indonesia, Jakarta. The data were analyzed using Statistical Product and Service Solution (SPSS) version 17 software.

RESULTS

Precision Test

CV was calculated by measuring the microcapillary passage time of two different samples: red-stained samples and blood samples. (Table 1)

Table 1. Microcapillary passage time

	Red-stained samples	Blood samples
N	10	10
x (seconds)	2.88	5.82
SD	0.12	0.23
CV	0.04	0.04

Each of the samples was tested ten times. The measured microcapillary passage time values were subsequently converted into viscosity values. Table 1 shows that the test has a high precision, in accordance to the narrow CV (0.04).

To convert the blood and plasma viscosity values measured by DM to the gold standard unit (poise), we used correlation test between DM and the gold standard. Linear regression was used to work out the prediction (y) formula as listed in Table 2.

According to Table 2, we concluded the formula as follows: For blood viscosity, $y = 0.846x + 0.614$; for plasma viscosity, $y = 1.072x + (-0.160)$.

These formulas were used to calculate the interrater variability of Cronbach Alpha, with the results of 0.94 and 0.82, respectively. The calculation of reliability coefficient of Bland Altman was used not only to measure the precision, but also to assess the stability of

Table 2. Prediction formula of blood and plasma viscosity

Model	Standardized Coefficient		Unstandardized Coefficient		P
	B	Std. Error	Beta	T	
Plasma					
Constant	-0.160	0.063		-2.542	0.020
Test	1.072	0.031	0.993	34.629	0.000
Blood					
Constant	0.614	0.429		1.431	0.170
Test	0.846	0.099	0.896	8.554	0.000

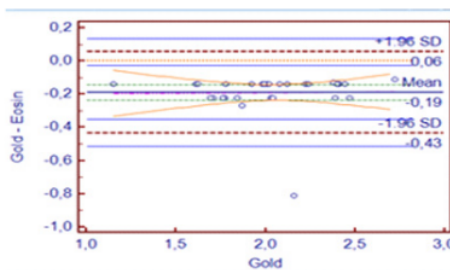


Figure 2. Reliability coefficient of Bland Altman test result. The mean difference of Bland Altman was -0.19.

plasma viscosity value as the samples were red-stained by eosin. (Figure 2)

Modification of Viscosity Measured by Digital Microcapillary in Accordance to Temperature Changes

The effect of temperature on blood and plasma viscosity was assessed by modifying the temperature of an incubator that corresponded to the incubator where DM was used. The temperature tested represented the temperature in tropical climate, which were 26 °C, 32 °C, and 37 °C. The measurement categorized by gender was also done to evaluate the effect of sex differences on blood and plasma viscosity. The results are listed in Table 3.

From the analysis using general linear model (GLM), [24] found that temperature had effects on blood viscosity ($p=0.002$) and plasma viscosity ($p=0.014$) and that sex differences had effect on blood viscosity ($p=0.035$) [19] which were statistically significant. However, there was no significant effect of sex differences on plasma viscosity ($p=0.517$). The interaction between

Table 3. Blood and plasma viscosity measured by DM in various temperature

Viscosity	Male			Female			p value
	26°C	32°C	37°C	26°C	32°C	37°C	
Blood, Mean±SD (poise)	5.09±0.49	5.01±0.16	4.81±0.12	5.09±0.47	4.67±0.23	4.58±0.36	0.002*
Plasma, Mean±SD (poise)	1.84±0.32	1.74±0.28	1.99±0.16	1.80±0.36	1.71±0.15	1.94±0.12	0.014**

*p value of the effect of temperature on blood viscosity. **p value of the effect of temperature on plasma viscosity

temperature and sex differences did not have significant effect on blood and plasma viscosity, with χ^2 value 0.297 and 0.982, respectively.

There was no significant difference of blood viscosity between 26 °C and 32 °C ($p=0.061$), and between 32 °C and 37 °C ($p=0.539$), but there was a significant difference between 26 °C and 37 °C ($p=0.01$). As for plasma viscosity, there was no significant difference between 26 °C and 32 °C ($p=0.770$), and between 26 °C and 37 °C ($p=0.201$), but there was a significant difference between 32 °C and 37 °C ($p=0.012$).

Accuracy Test

The optimal cut-off value, sensitivity, and specificity were determined by analyzing receiver operator characteristic (ROC) curve.

Figure 3 shows the optimal cut-off value, which is described as follows. The optimal cut-off value of blood viscosity in female was 4.70 (sensitivity=97.3%, specificity=86.7%). The optimal cut-off value of blood viscosity in male

was 5.09 (sensitivity=97.1, specificity=89.6%). The optimal cut-off value of plasma viscosity in female was 2.10 (sensitivity=91.4%, specificity=70.6%). The optimal cut-off value of plasma viscosity in male was 2.11 (sensitivity=92%, specificity=90%). The viscosity exceeded these cut-off value is categorized as hyperviscosity.

The diagnostic test results for blood viscosity are described as follows: sensitivity was 88.9%, specificity was 88.9%, positive likelihood ratio was 8.01, negative likelihood ratio was 0.12, positive predictive value was 90.1%, negative predictive value was 87.5%, prevalence was 53% and accuracy was 91%. The diagnostic test results for plasma viscosity are: sensitivity was 100%, specificity was 84%, positive likelihood ratio was 6.25, negative likelihood ratio was 0.00, positive predictive value was 91.3%, negative predictive value was 100%, prevalence was 62%, and accuracy was 90%.

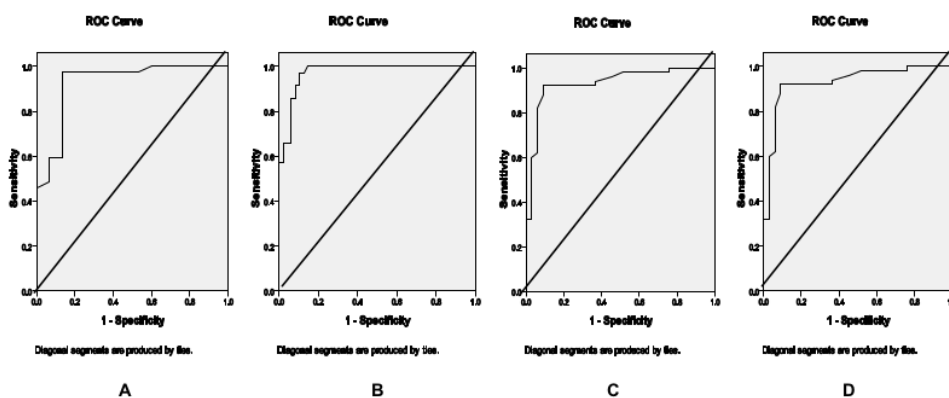


Figure 3. A: ROC curve of blood viscosity in female; B: ROC curve of blood viscosity in male; C: ROC curve of plasma viscosity in female; D: ROC curve of plasma viscosity in male

DISCUSSION

The precision¹ of DM was assessed by calculating the CV, interrater variability of Cronbach Alpha, and reliability coefficient of Bland Altman.

The obtained CV, which was 0.04 (less than 5%), indicated that the precision is good, as a good test is indicated by a narrow range of CV, while the less one is indicated by a wider range of CV.¹⁴ The correlation coefficients of blood and plasma viscosity were 0.81 and 0.99, respectively. It demonstrated that the blood and plasma passing time values in DM were almost the same with those in gold standard.¹⁵

DM also has high internal validity. Gliem¹⁶ stated that high Cronbach Alpha result shows that the test has a good internal consistency/precision. The Bland Altman mean difference, which was -0.19 (close to 0), also indicates that the precision is good and eosin can be used to stain the samples for plasma viscosity measurement. According to the CV, interrater variability of Cronbach Alpha, and mean difference of Bland Altman, we conclude that the DM has a high precision in measuring the blood and plasma viscosity.¹⁷

Additional test was done to show the effects of temperature and sex differences on blood and plasma viscosity, so that in the application of DM, both of these factors could be taken into account. This test shows that the effects of temperature on blood and plasma viscosity were significant, with p value 0.002 and 0.014, respectively. There was an increase of blood viscosity either in male or female subjects from 37 °C to 26 °C. The result of our study is somewhat similar to the study conducted by Cinar.¹⁸ He observed the effect of temperature on blood viscosity value measured by Capillary Tube Viscometer in 37 healthy subjects. He also found that the increase in viscosity occurred when the temperature was decreased from 37 °C to 25 °C; while reduced viscosity occurred when the temperature was elevated from 37 °C to 39 °C. The effect of sex differences on blood viscosity was statistically significant, which was showed by p value=0.035; however, the effect of sex differences on plasma viscosity was insignificant, with p value=0.517. The result had some impacts in determining

the cut-off value of viscosity, which should be different between male and female. The study conducted by Li¹⁹ in 31 subjects showed that there was a correlation between blood viscosity and sex differences. Nemeth²⁰ studied the correlation between erythrocytes aggregation and sex differences in rats, and found that it was statistically significant. In contrast, Kesmarky²¹ in his study stated that plasma viscosity was not affected by sex differences. A study conducted by Haliman²² also showed that sex differences had no effect on plasma viscosity.

As the temperature significantly affected viscosity, setting one temperature as a standard temperature for DM would be necessary, which in this case, was 37 °C. The temperature was selected as it is the normal temperature of the human body; therefore, we expect that the viscosity measured by DM could represent the real viscosity in vivo. Also, in the gold standard we found a temperature controller, which was set to 37 °C.²³ Subsequently, we put a temperature controller in DM to maintain the standard temperature of 37 °C during the viscosity measurement.

The diagnostic test shows that DM had high sensitivity and specificity values for both blood and plasma viscosity measurements. It also indicates that DM has high precision in addition to its high accuracy.

CONCLUSION

Diabetes mellitus is a simple and a novel diagnostic instrument to measure blood and plasma viscosity which has high precision and accuracy. The test also indicates high sensitivity and specificity; therefore the instrument can be considered to be used as screening test tool to measure blood and plasma viscosity.

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