Stability and reference interval of serum phosphoglucomutase (PGM)

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Summary We examined the stability and reference interval of serum phosphoglucomutase (PGM) in healthy subjects. Serum obtained from venous blood was stored at -80°C and 4°C, and the stability of PGM activity was evaluated by measuring the passage time for both samples. PGM activity was stable for 3 days after collection with storage at 4°C. It was stable for a long periods of time with storage at -80°C.

PGM activity was measured using serum from 298 healthy adults (110 men and 187 women). There was a significant difference between men and women (p <0.001), and the distribution was log-normal. The reference interval was 5.4 – 26.6 U/L in men and 4.8 – 20.3 U/L in women.

Key words: phosphoglucomutase(PGM), Acute myocardial infarction (AMI), Reference interval

1. Introduction

PGM is a glucose metabolism-related enzyme present in the cell supernatant fraction, which catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate. However, the clinical significance of PGM activity has not been established, routine measurement has not been achieved. Akabane et al reported that the PGM activity is increased in acute myocardial infarction (AMI)4. In addition, Nishinari et al. carried out a detailed study and reported that PGM activity was elevated at an earlier period and had high clinical utility in patients admitted with suspected myocardial infarction5-10.

Mortality from heart disease in 2010 was 15.8%, second only to malignant neoplasms. Acute myocardial infarction accounts for 22.5% of heart disease mortality, or one of 4-5 peoples who die from heart

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disease. Currently, myocardial infarctions diagnosed with comprehensive test results including measurement of ECG and biochemical cardiac markers, and clinical findings. Widely used markers of myocardial damage markers, include troponin T\(^6\), creatine kinase MB isoenzyme (CK-MB)\(^3\), myoglobin\(^6\), heart-type fatty acid-binding protein (H-FABP)\(^6,11\), and pentraxin-3\(^2\). These markers appear in the blood at different times after myocardial infarction, 2-4 hours after myocardial injury\(^3\). Although myoglobin and H-FABP have been measured as markers that rise early, myoglobin exists in skeletal muscle and there is doubt as to its specificity, and H-FABP has the drawback of being elevated in renal failure\(^4\). The significance of early detection of myocardial infarction, is that an increase in patients with chronic heart failure due to left ventricular remodeling can be prevented by prophylaxis, early diagnosis, and reperfusion therapy. We believe that markers which can be detected early are essential, and focused on PGM as a marker that may be able to fulfill that purpose.

To promote the clinical use of PGM, we conducted an investigation with the idea that it is necessary to establish a reference interval. In addition, the preservation state of specimens is important for consideration of the reference interval, and the stability of PGM activity was also examined.

2. Material and methods

1. Sample

Informed consent was obtained from each patient. In addition, the procedure was approved by the Kitasato University ethics committee, and the study was performed according committee guidelines [B Ethics 09-19].

2. PGM activity measurement methods and principles

PGM activity with a modified method\(^1\) of King et al\(^9\). Reagent was adjusted to 6 mmol/L EDTA-Mg, 0.24 mmol/L NAC, 0.48 mmol/L NADP, 15 mmol/L G1P, 96 \(\mu\) mol/L G1, 6P, 2.4 U/mL G6PD, 100 mmol/L Tris-HCl buffer pH 8.0. Enzyme activity was measured using an AU640 (Beckman Coulter).

The rate of increase of NADPH was measured at 340 nm.

3. Stability of PGM activity

Serum obtained by normal blood sampling was used as a sample. The samples were stored at -80°C and 4°C, and the day samples were obtained was taken as day 0. Measurements were made with samples stored 5 days from the date of collection.

4. Adsorption to hydrophobic substances in PGM activity

Platelet sediment was added in solution having PGM activity, and the PGM activity was measured after incubation for ten minutes. The added platelets were then collected again, the surfactant Tween 20 was added, and PGM activity was measured after incubation for ten minutes.

5. Reference interval for PGM activity

Among people who underwent specific physical examination the Health Science Center Foundation from 30 May 2011 to 07 September 2011 and consented to the study, those who satisfied all of the following items were selected as healthy patients: waist circumference less than 85 cm in men and less than 90 cm in women, HDLC over 40 mg/dL, TG less than 150 mg/dL, FBS less than 100 mg/dL, systolic blood pressure below 130 mmHg, diastolic blood pressure less than 85 mmHg, and BMI less than 25. Smokers and people who were taking any medication were excluded. Samples were stored at -80°C within three days after blood collection. The subjects for measurement were 110 men (average age 49.3 years old), and 187 women (average age 51.5 years old). They included 53 subjects in their 30s (17 men, 36 women), 94 subjects in their 40s (33 men, 61 women), 82 subjects in their 50s (31 men, 51 women), 60 subjects in their 60s (24 men, 36 women), and 8 subjects 70 years old or more (5 men, 3 women).

3. Results

1. Stability of PGM activity

PGM activity did not decrease during 3 days with
the storage condition of 4°C. With the storage condition of -80°C, no decrease the activity value was observed and the activity was the same even after 27 days (Fig. 1). Activity tended to rise in some samples during the 3 days after collection.

2. Reference interval of PGM activity

The mean for each item is shown for men and women in Table 1. Subjects more than 70 years old were grouped together because of the small number of subjects. There were 4 persons in their 70s and 1 person in his 80s among men, and 3 persons in their 70s among women. The aggregate PGM activity for each group is shown in Figure 2. A histogram of PGM activity is shown in Fig. 3. Outliers, kurtosis and skewness were 0.0045, 0.78, -0.11 in men and 0.0293, 1.18, 3.44 in women.

4. Discussion

In clinical examinations, the storage situation of specimens is an important factor. Test values are affected by storage condition, such as frozen storage, refrigerated storage, and room temperature storage depending on the test item.

For the clinical application of PGM activity, evaluation of the stability of the value according to the

![Fig. 1 Stability of PGM activity. (A: 4°C, B: -80°C)](image)

![Fig. 2 PGM activity of each ages.](image)

<table>
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<th>Age</th>
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<th>TG</th>
<th>FBS</th>
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M: Male; F: Female
BMI: body mass index, HDL-C: high density lipoprotein cholesterol, TG: triglyceride,
FBS: fast blood sugar, SAP: systolic arterial pressure, DBP: diastolic blood pressure
state of preservation is very important. Activity gradually decreased after 5 days in the specimens stored at 4°C. On the other hand, the measured values did not change in the specimens stored at -80°C even after 27 days. For these reasons, our assessment was that serum stored at 4°C is stable within 3 days and that serum stored at -80°C is stable over a long period.

PGM is known to be present in platelets and white blood cells. Therefore, once solidified in order to fractionate serum, there is a possibility that PGM activity is increased. Therefore, the same samples were measured by CTAD tubes with added platelet protecting reagent commercially available from B & D, and EDTA tubes. Although the activity of the samples collected in the CTAD tubes was low, there was no significant difference.

In setting the number of subjects in the reference interval, there are a number of considerations when calculating the NCCLS standards that embody the concept of the reference interval recommended by the IFCC (International Federation of Clinical Chemistry). One of them, is that the number of subjects should not be less than 120. From among the approximately 7,000 people who underwent a medical examination, 297 people (110 men and 187 women) were selected excluding those who were smokers, drinkers, exceeded metabolic syndrome criteria, taking medication, or suffering from any chronic disease, and PGM activity was measured. The target number of people to make an assessment by gender was not met, but a number close to that could be collected.

NCCLS stipulates that measurements should exclude factors that could cause measured values to become abnormal. The risk of ischemic heart disease is elevated with increases in the number of people having risk factors for metabolic syndrome, and it is reported that groups with 3-4 factors have a 31-fold increasedon risk of developing ischemic heart disease. Nakamura et al. reported that the risk of myocardial infarction is 8 times higher risk in those with 3 to 4 accumulated risk factors, such as obesity, hyperglycemia, hypercholesterolemia, and hypertension, than in those without. In any event, accumulation of risk factors for coronary atherosclerosis has been shown to promote onset of ischemic heart disease. Risk factors for metabolic syndrome are smoking, hypertension, obesity, hyperglycemia, and dyslipidemia. According to a JACC Study, among people who smoke the risk of death from ischemic heart disease increases 2.51-fold in men and 3.35-fold in women. Among smokers the risk of death from cardiovascular disease, including heart disease, subarachnoid hemorrhage, cerebral infarction, and ischemic stroke, was reported to be 1.6-fold higher in men and 2.06-fold higher in women. In addition, smoking more than two packs every day increases risk for myocardial infarction more than 4-fold. In addition, from the young to the elderly, lower blood pressure has been reported to be associated with lower subsequent cardiovascular mortality. Therefore, we added the diagnostic standard values of the Metabolic Syndrome Committee to the exclusion criteria. A diagnosis of metabolic syndrome, requires a waist circumference of more than 85 cm in men and more than 90 cm in women. However, the accumulation of risks has been reported to be dangerous even in lean people, and persons who fit all diagnostic criteria for metabolic syndrome are treated as subjects.

Serum samples were stored in ultra-low temperature freezer at -80°C until being assayed within 3
days. Because the samples were stored in a refrigerator at 4°C before being put in the ultra-low temperature freezer, the stability of the samples is considered to be sufficient. Distribution type was evaluated by histogram, and skewness and kurtosis were determined in comparison with the normal distribution. When kurtosis is "3" spikes will be equal to the normal distribution, with steep cusps when kurtosis is greater and loose cusps when kurtosis is smaller. The spread of the tail was gradual compared to normal distribution. Skewness is an indicator of symmetry, with "0" indicating perfect symmetry. The value is greater with distortion to the right side and smaller with distortion to the left side. The distribution of PGM was thought to be distorted to the right since it was greater than "0."

In observations of men and women by age, men tended to be higher. In addition, a decreasing tendency was seen with age. Considering a log-normal distribution, and gender difference, the reference interval was 5.4 - 26.6 U/L, in men and 4.8 - 20.3 U/L in women. PGM has been reported to be bound to the Z band of striated muscle[27], and there is a possibly that it is dependent on muscle mass. This may be a cause of the existence of a gender difference and the tendency for lower values with age.

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References

1) Toshiaki Akahane: Usefulness of phosphoglucomutase assay on acute myocardial infarction. report of graduate school of Kitasato university, 2006.


14) Hafidh A Al-Hadi, Brent William et al.: Serum Level of Heart-Type Fatty Acid-Binding Protein in Patients with Chronic Renal Failure. SQU MED J, DECEMBER 2009, VOL.9, ISS. 3, PP.311-314, EPUB. 19 DEC 2009.


