Influence of different methods for measuring HbA1c on health checkups in a rural town in Hokkaido, Japan

Junko Oikawa¹ · Koshi Nakamura¹ · Shigekazu Ukawa¹ · Tomoko Kishi¹ · Akinobu Nakamura² · Akiko Tamakoshi¹

Received: 18 August 2014 / Accepted: 20 February 2016 / Published online: 9 March 2016
© The Japan Diabetes Society 2016

Abstract Using data on health checkups performed in one Japanese town, we investigated the effect on health checkups of the methods used to measure hemoglobin A1c (HbA1c). The study included 337 participants undergoing health checkups at two facilities. At facility 1, HbA1c was measured by high-performance liquid chromatography (HPLC) in 2012 and by immunoassay (IA) in 2013, while at facility 2, HbA1c was measured by HPLC in both years. At facility 1, the mean HbA1c was significantly decreased from 2012 to 2013 (5.83 vs 5.50 %, respectively; $P < 0.001$), although the mean fasting plasma glucose (FPG) was significantly increased from 2012 to 2013 (91.7 vs 95.2 mg/dL, respectively; $P = 0.02$). Of the 202 participants at facility 1, 97 who had an HbA1c of ≥ 5.6 % in 2012 had an HbA1c of < 5.6 % in 2013. At facility 2, the mean HbA1c marginally increased, while there were similar FPG levels in both years. An additional study of single blood samples from 27 healthy participants who were tested at the same facility using both HPLC and IA found that the mean HbA1c was significantly lower for IA than for HPLC (5.19 vs 5.50 %, respectively; $P < 0.001$). In summary, we found a substantial decrease in the mean HbA1c and the prevalence of impaired glucose tolerance and diabetes mellitus in study participants who underwent

health checkups for two consecutive years when different methods were used to measure HbA1c. The lack of standardization of HbA1c measurement methods may have a large effect on health checkups.

Keywords Health checkup · Hemoglobin A1c · High-performance liquid chromatography · Immunoassay · Measurement

Introduction

Glycated hemoglobin [i.e., hemoglobin A1c (HbA1c)] is widely used to monitor glycemic control in patients with diabetes mellitus (DM), mainly to prevent diabetic complications [1]. HbA1c levels provide treating physicians with the mean glycemic control of DM patients over the 2–3 months previous to measurement [2, 3]. In Japan, HbA1c is also used as a screening tool for identifying patients with DM and impaired glucose tolerance (i.e., borderline DM) at health checkups [4]. Health checkups are mandatory for all Japanese residents aged 40–74 years, and focus on abdominal obesity and associated metabolic disorders, including DM, because of a new healthcare access law for the elderly [5, 6].

High-performance liquid chromatography (HPLC) is a standard commercially available method for measuring HbA1c in Japan. However, an immunoassay (IA) and other methods that can measure HbA1c more rapidly and less expensively than HPLC [7] have been developed [7]. IA is now being used more frequently by clinical laboratories throughout Japan to meet the dramatically increased demand for HbA1c tests to screen for DM at the health checkups that a large number of people undergo on any given day [8].

✉ Akiko Tamakoshi
tamaa@med.hokudai.ac.jp

¹ Department of Public Health, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo, Hokkaido 060-0812, Japan

² Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

HPLC and IA methods are quite different analytical techniques, and some but not all reports have indicated that IA provides slightly lower HbA1c values than HPLC [7, 9–12]. This possible discrepancy may be somewhat acceptable at a clinical practice where physicians treat previously diagnosed DM patients. However, it is unclear whether the possibly discrepant results between IA and HPLC are acceptable for health checkups that are conducted to assess the health of apparently healthy people and for epidemiologic studies. The aim of our study was to investigate this issue at health checkpoint facilities using different methods to measure HbA1c.

Methods

The study consisted of longitudinal observations for two consecutive years, 2012 and 2013. It included participants aged 40–74 years who were residents of a single town with a population of approximately 7000 people of all ages in Hokkaido, Japan. The participants were enrolled in the National Health Insurance scheme. In general, all Japanese residents are required to enroll in one of three insurance groups, with the eligibility for each group depending on the individual's age and occupation (i.e., “health insurance for all”) [13, 14]. All residents 75 years of age or older are enrolled in the Advanced Elderly Medical Service (coverage rate 12 %), while those 74 years of age or younger are enrolled in either the Employee's Health Insurance scheme (58 %) or the National Health Insurance scheme (30 %), based on their occupation [13]. The Employee's Health Insurance scheme covers employees and their dependents, while the National Health Insurance scheme covers the remaining population, including self-employed individuals (e.g., farmers and fishermen) and retirees and their dependents. The Employee's Health Insurance and National Health Insurance schemes provide annual health checkups for beneficiaries aged 40–74 years, with the objective of screening for metabolic syndrome [5, 6]. In the target town investigated in our study, National Health Insurance beneficiaries were encouraged to undergo an annual health checkpoint at one of three examination facilities. Health checkpoint data were obtained from the two examination facilities (facilities 1 and 2) that accounted for 95 % or more of the beneficiaries undergoing annual health checkups. The data were anonymized to remove all matching links to personal data.

A total of 337 beneficiaries who underwent health checkups at the same facility for the two consecutive years (202 beneficiaries at facility 1 and 135 beneficiaries at facility 2) were included in the primary analysis, with four beneficiaries lacking data on fasting plasma glucose (FPG).

The body weight and height of each participant were measured, and the body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Fasting blood samples were obtained after an overnight fast to measure HbA1c and FPG levels. At facility 1, HbA1c was measured by HPLC in 2012 and by IA in 2013, while at facility 2, HPLC was used to measure HbA1c in both years. Both facilities had their own HPLC analyzer. Quality control of the HbA1c measurements was performed using the standard certified by the Japan Diabetes Society (JDS). The HbA1c values were converted to the values of the National Glycohemoglobin Standardization Program (NGSP) using the formula provided by the JDS: $\text{HbA1c (NGSP) (\%)} = 1.02 \times \text{HbA1c (JDS) (\%)} + 0.25$ [15]. FPG was measured enzymatically at each facility.

Because the FPG data showed a skewed distribution, the FPG data were normalized by natural logarithmic transformation for analysis. The unpaired *t* test or chi-square test was used to compare the study participants' demographics and measured parameters from facilities 1 and 2 within each year. The paired *t* test or McNemar test was used to compare the participants' characteristics between the years of 2012 and 2013 within each facility. The repeated-measures analysis of variance was used to test whether the one-year change in log-transformed FPG and HbA1c differed between the two facilities. Furthermore, the Bowker symmetry test was used to compare the distributions of participants classified into subgroups of FPG and HbA1c between 2012 and 2013 within each facility. HbA1c was categorized into the following four subgroups: <5.6, 5.6–5.9, 6.0–6.4, and ≥ 6.5 % [4]. FPG was categorized into the following four subgroups: <100, 100–109, 110–125, and ≥ 126 mg/dL [4]. Normal glucose tolerance was defined as $\text{FPG} < 100$ mg/dL and $\text{HbA1c} < 5.6$ %, while DM was defined as $\text{FPG} \geq 126$ mg/dL and/or $\text{HbA1c} \geq 6.5$ %. A sensitivity analysis was conducted after excluding participants who were taking medication for DM in 2012 and/or 2013.

All statistical analyses were performed using JMP Pro 11.1.1 software for the Mac (SAS Institute Inc., Cary, NC, USA). The significance level was set at $P < 0.05$.

Additional study

Although facility 1 determined the HbA1c values by HPLC and IA for each study participant, each value was measured in separate specimens for each year. Therefore, we performed an additional investigation of the differences in HbA1c values measured by the two methods at facility 1 on the same blood specimen. In April 2015, the participants of this additional study were recruited from employees who worked at our university departments in Sapporo,

Hokkaido, Japan. The study included 27 healthy participants (7 men and 20 women) aged between 20 and 59 years without a history of DM. Informed consent was obtained from all the participants. In order to protect their privacy, their names were deleted from the data.

Fasting blood samples were obtained by cubital venipuncture after an overnight fast of at least 10 h. The samples were transported to the laboratory of facility 1. HbA1c was measured by HPLC using an automated analyzer (HA-8160; ARKRAY, Inc., Kyoto, Japan) and by IA using another automated analyzer (JCA-BM6010; JEOL Ltd., Tokyo, Japan) that measured the FPG enzymatically.

We compared the distributions of data on HbA1c measured by HPLC and IA in the 27 participants. The Pearson correlation coefficient was calculated for the HbA1c values measured by each method. The paired *t* test was used to compare the HbA1c values determined by the two methods.

Results

The demographics and parameters of the 337 study participants grouped according to facility and year are shown in Table 1. There were almost no differences within each year between the characteristics of the participants at each facility for age, proportion of males, BMI, and the proportion of participants using medication for DM. At both facilities, there were significant increases in the mean BMI and the proportion of participants on medication for DM from 2012 to 2013.

Table 2 shows the mean ln(FPG) and HbA1c values in the participants grouped according to facility and year. Although the mean ln(FPG) level was significantly increased from 4.51 in 2012 to 4.54 in 2013 among the

participants at facility 1, the mean HbA1c was significantly decreased from 5.83 % in 2012 to 5.50 % in 2013. Among the participants at facility 2, the mean HbA1c marginally but significantly increased from 5.77 % in 2012 to 5.89 % in 2013, while there were similar mean ln(FPG) levels for 2012 and 2013 (4.56 and 4.56). The 1-year change in ln(FPG) and HbA1c significantly differed between facilities 1 and 2 [$P = 0.01$ for ln(FPG), and $P = 0.005$ for HbA1c]. Notably, the two facilities showed opposite directions of one-year change in HbA1c.

The FPG category distribution (Table 3) was in accordance with symmetry (no significant alteration in tendency) at the two facilities. However, the HbA1c category distribution (Table 4) deviated significantly from symmetry at the two facilities ($P < 0.001$ at facility 1, and $P < 0.001$ at facility 2). Notably, the direction for the asymmetry was diverse between facilities 1 and 2. Although 40 (30 %) of the 135 participants at facility 2 were in the higher category in the follow-up year, there was only 1 (0.5 %) of the 202 participants at facility 1 in the higher category in the follow-up year. Conversely, the number of participants who were in the lower category in the following year were 117 (58 %) at facility 1 and just 9 (7 %) at facility 2.

When diabetic status was assessed according to FPG and HbA1c, the proportion of the 202 participants at facility 1 who were found to have normal glucose tolerance increased substantially from 19.8 % ($n = 40$) in 2012 to 61.4 % ($n = 124$) in 2013 (data not shown). On the other hand, the proportion of the 135 participants at facility 2 who were found to have normal glucose tolerance decreased slightly from 31.1 % ($n = 42$) in 2012 to 19.3 % ($n = 26$) in 2013 (data not shown).

Similar patterns were observed after excluding participants who were taking medication for DM in 2012 and/or 2013 (data not shown).

Table 1 Characteristics of the study participants who underwent health checkups in a rural town in Hokkaido, Japan, by facility and year

	Facility 1			Facility 2			<i>P</i> value ^a
	2012	2013	<i>P</i> value ^b	2012	2013	<i>P</i> value ^b	
Number of participants	202			135			
Age (years)	62.1 ± 9.0			61.6 ± 8.8			0.27
Males	84 (41.6 %)			66 (48.9 %)			0.18
Body mass index (kg/m ²)	23.0 ± 3.5	23.2 ± 3.5	<0.001	23.4 ± 3.1	23.6 ± 3.2	<0.001	0.26
Medication for DM	9 (4.5 %)	17 (8.4 %)	0.005	13 (9.6 %)	11 (11.1 %)	<0.001	0.06
							0.54

Variables are presented as the mean ± SD or as the number (percentage)

DM diabetes mellitus

^a Unpaired *t* test or chi-square test was used to compare each variable between the two facilities within each year

^b Paired *t* test or McNemar test was used to compare each variable between the two years within each facility

Table 2 Levels of fasting plasma glucose and hemoglobin A1c in the study participants who underwent health checkups in a rural town of Hokkaido, Japan, by facility and year

	Facility 1			Facility 2			<i>P</i> value ^a	<i>P</i> value ^c
	2012	2013	<i>P</i> value ^b	2012	2013	<i>P</i> value ^b		
Number of participants	202			135				
FPG (mg/dL)	89 (84–95)	91 (86–98)		94 (88–101)	94 (87–102)			
ln(FPG)	4.51 ± 0.14		0.002	4.56 ± 0.12		0.41	<0.001	0.01
		4.54 ± 0.17			4.56 ± 0.13		0.24	
HbA1c (HPLC) (%) ^d	5.83 ± 0.60		<0.001	5.77 ± 0.44		<0.001	0.33	0.005
					5.89 ± 0.50		<0.001	
HbA1c (IA) (%) ^d		5.50 ± 0.66						

Variables are presented as the mean ± SD or as the median (interquartile range)

FPG fasting plasma glucose, HbA1c hemoglobin A1c, HPLC high-performance liquid chromatography, IA immunoassay

^a Unpaired *t* test was used to compare each variable between the two facilities within each year

^b Paired *t* test was used to compare each variable between the two years within each facility

^c Repeated-measures analysis of variance was used to test whether the one-year change in each variable differed between the two facilities

^d At facility 1, HbA1c was measured by HPLC in 2012 and by IA in 2013; at facility 2, HbA1c was measured by HPLC in both years

Table 3 Distributions of study participants in the fasting plasma glucose subgroups who underwent health checkups in a rural town in Hokkaido, Japan, by facility and year

	FPG (mg/dL) in 2013				Total
	<100	100–109	110–125	≥126	
Facility 1					
FPG (mg/dL) in 2012					
<100	152 (90.5 %)	14 (8.3 %)	1 (0.6 %)	1 (0.6 %)	168 (100 %)
100–109	8 (42.1 %)	10 (52.6 %)	1 (5.3 %)	0 (0 %)	19 (100 %)
110–125	0 (0 %)	0 (0 %)	5 (55.6 %)	4 (44.4 %)	9 (100 %)
≥126	1 (33.3 %)	0 (0 %)	1 (33.3 %)	1 (33.3 %)	3 (100 %)
Total	161	24	8	6	199
Facility 2					
FPG (mg/dL) in 2012					
<100	83 (88.3 %)	10 (10.6 %)	0 (0 %)	1 (1.1 %)	94 (100 %)
100–109	9 (40.9 %)	11 (50.0 %)	1 (4.5 %)	1 (4.5 %)	22 (100 %)
110–125	0 (0 %)	6 (54.5 %)	4 (36.4 %)	1 (9.1 %)	11 (100 %)
≥126	0 (0 %)	1 (14.3 %)	3 (42.9 %)	3 (42.9 %)	7 (100 %)
Total	92	28	8	6	134

Variables are presented as the number of subjects (percentage)

FPG fasting plasma glucose

The Bowker test was used to compare the distributions of participants in FPG subgroups in 2012 and 2013: *P* = 0.49 for facility 1, and *P* = 0.47 for facility 2

Additional study

Among 27 healthy participants with a mean age of 36.1 years (Table 5), none had DM with an HbA1c value of 6.0 % or greater according to both HPLC and IA. Twenty-three of the participants (85.2 %) had an HbA1c value of <5.6 % by IA, and of these, 15 (55.6 %) had an HbA1c value of <5.6 % by HPLC. Twelve participants (44.4 %)

had an HbA1c value between 5.6 and 5.9 % by HPLC; one of those participants had a normal-high FPG value (108 mg/dL) while four of those participants (14.8 %) had an HbA1c value between 5.6 and 5.9 % by IA. The Pearson correlation coefficient was 0.947 (*P* < 0.001) for the HbA1c values determined by HPLC and IA. The mean HbA1c level was significantly lower for IA than for HPLC (5.19 vs 5.50 %, respectively; *P* < 0.001) (Table 5).

Table 4 Distributions of study participants in the hemoglobin A1c subgroups who underwent health checkups in a rural town in Hokkaido, Japan, by facility and year

	HbA1c (IA) (%) ^a in 2013				Total
	<5.6	5.6–5.9	6.0–6.4	≥6.5	
Facility 1					
HbA1c (HPLC) (%) ^a in 2012					
<5.6	43 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	43 (100 %)
5.6–5.9	90 (76.3 %)	27 (22.9 %)	1 (0.8 %)	0 (0 %)	118 (100 %)
6.0–6.4	4 (13.8 %)	16 (55.2 %)	9 (31.0 %)	0 (0 %)	29 (100 %)
≥6.5	3 (25 %)	2 (16.7 %)	2 (16.7 %)	5 (41.7 %)	12 (100 %)
Total	140	45	12	5	202
Facility 2					
HbA1c (HPLC) (%) ^a in 2012					
<5.6	26 (53.1 %)	21 (42.9 %)	2 (4.1 %)	0 (0 %)	49 (100 %)
5.6–5.9	4 (8.9 %)	32 (71.1 %)	9 (20.0 %)	0 (0 %)	45 (100 %)
6.0–6.4	0 (0 %)	4 (11.8 %)	22 (64.7 %)	8 (23.5 %)	34 (100 %)
≥6.5	0 (0 %)	0 (0 %)	1 (14.3 %)	6 (85.7 %)	7 (100 %)
Total	30	57	34	14	135

Variables are presented as the number of subjects (percentage)

HbA1c hemoglobin A1c, *HPLC* high-performance liquid chromatography, *IA* immunoassay

The Bowker test was used to compare the distributions of participants in HbA1c subgroups in 2012 and 2013: $P < 0.001$ for facility 1, and $P < 0.001$ for facility 2

^a At facility 1, HbA1c was measured by HPLC in 2012 and by IA in 2013; at facility 2, HbA1c was measured by HPLC both years

Table 5 Characteristics of the 27 healthy nondiabetic participants

Number of participants	27	
Age (years)	36.1 ± 10.5	
Males	7 (35.0 %)	
FPG (mg/dL)	87 (79–92)	
HbA1c (HPLC) (%)	5.50 ± 0.21	$P < 0.001$
HbA1c (HPLC) <5.6 %	15 (55.6 %)	
HbA1c (IA) (%)	5.19 ± 0.25	
HbA1c (IA) <5.6 %	23 (85.2 %)	

Variables are presented as the mean ± SD, the median (interquartile range), or as the number (percentage)

Paired *t* test was used to compare HbA1c between HPLC and IA

FPG fasting plasma glucose, *HbA1c* hemoglobin A1c, *HPLC* high-performance liquid chromatography, *IA* immunoassay

Discussion

We evaluated data on Japanese participants who underwent health checkups at two facilities in a rural town in Hokkaido, Japan, for two consecutive years. At the first facility, where the method used to measure HbA1c was changed from HPLC in the first year to IA in the second, we observed substantial decreases in the mean value of HbA1c and the prevalence of impaired glucose tolerance and DM despite a significant increase in BMI during the second year as compared to the first year. The other characteristics of the study participants at this facility that we

extracted from the data were unlikely to be the cause of the improved diabetic status of the participants assessed. By contrast with the first facility, at the second facility, where HbA1c was measured by HPLC in both years, the mean HbA1c was marginally increased in the second year, although the increase in HbA1c was partly attributable to the increase in BMI. An additional study of healthy volunteers without DM demonstrated that the values of HbA1c measured by IA were significantly lower (by approximately 0.3 %) than those measured by HPLC, despite the high correlation between the values measured by these two methods. Taken together, the discrepant HbA1c values obtained by these two methods may have resulted in the misclassification of a considerable number of people who underwent health checkups for diabetic status. To the best of our knowledge, this is the first report to describe the influence of different methods for measuring HbA1c on the results of health checkups that a large number of Japanese people underwent.

Some investigations have shown that HPLC and IA provide similar HbA1c values [7, 9]. Other investigations have found that these two methods provide discrepant HbA1c values. They reported that IA provides slightly lower (by approximately 0.2–0.3 %) HbA1c values than HPLC, even though the maintenance and calibration of the devices used for HbA1c measurement were performed appropriately [10–12]. The results of our study are in accordance with the results of those reports. In clinical

practice, testing for HbA1c is mostly performed to assess the glycemic control of patients already diagnosed with DM, and physicians focus on each individual DM patient. Therefore, it may be true that the discrepancy between the HbA1c values provided by the two methods is negligibly small for clinical practice. However, our results suggest that this discrepancy is not negligible from the perspective of health checkups that are used to screen a population in order to identify patients who have DM or are at risk of it.

Currently, the Japanese national government requires that all Japanese residents aged 40–74 years undergo an annual health assessment and attend a lifestyle modification educational program that focuses on metabolic syndrome [5, 6]. In this program, an individual is initially assessed according to the findings of a physical checkup and a questionnaire, and an FPG level ≥ 100 mg/dL and/or an HbA1c value ≥ 5.6 % (without specifying the method of measurement) is regarded as indicating an increased risk of lifestyle-related disease [6]. Depending on the individual's health risk, information on health, motivational advice for lifestyle modification, or an intensive lifestyle modification program is provided [6]. As our data show, most people who undergo health checkups have a normal or normal-high HbA1c value (i.e., 5.0–5.9 %). Small variations in HbA1c level (e.g., 0.2–0.3 %) caused by the use of different methods of HbA1c measurement may result in a large number of people being categorized as having low or high levels of HbA1c. Some of those people might then be misclassified as having normal or impaired glucose tolerance, and the misclassification may result in a substantial increase or decrease in the number of people who require advice for lifestyle modification. Individuals might also be misclassified as having impaired glucose tolerance or DM, which might result in a substantial increase or decrease in the numbers of people who require frequent examinations and medical treatment for DM. An overestimation of impaired glucose tolerance and DM might increase the financial burden on insurance organizations as well as insurance beneficiaries. An underestimation might result in inadequate management of patients with impaired glucose tolerance and DM. Accordingly, our results provide important implications for the current public health strategy used in Japan. Furthermore, our data suggest that the lack of standardization of HbA1c measurement methods results in difficulties in evaluating individuals annually for changes in diabetic status and in comparing diabetic status among populations living in different regions.

This study has several limitations. First, the study participants were limited to National Health Insurance beneficiaries belonging to self-employed groups in a single rural area. The socioeconomic status and lifestyle of these beneficiaries may have had an effect on their health status, including diabetic status. Therefore, caution should be

exercised when generalizing the results of our study. Second, there are other possible reasons for the interfacility difference in consecutive HbA1c levels than the adoption of IA at facility 1, although the data from the additional study that measured HbA1c in the same blood sample using the two different methods support our interpretation of the results from facility 1. The study participants included only those who underwent health checkups at the same facility for the two consecutive years; those who underwent health checkups at different facilities in the two consecutive years and those who underwent health checkups only in the first year were excluded. Those excluded people may have received different interventional lifestyle modification advice from the different facilities, which could also have affected the HbA1c in the second year among the study participants. In addition, any differences in sample conditions between the two facilities until the assay could affect the HbA1c measurement results.

In conclusion, the lack of standardization of the methods used to measure HbA1c may have a large effect on health checkups, resulting in the misclassification of a large number of people as having normal glucose tolerance, impaired glucose tolerance, or DM. If the data from health checkups show that the proportion of people who have normal glucose tolerance, impaired glucose tolerance, or DM has changed substantially, physicians and public health nurses should investigate whether the method used to measure HbA1c was changed from what was used previously.

Acknowledgments The authors express their sincere appreciation to the staff of the health centers of the target town for their generous cooperation.

Compliance with ethical standards

Conflicts of interest J. Oikawa, K. Nakamura, S. Ukawa, T. Kishi, A. Nakamura, and A. Tamakoshi declare that they have no conflicts of interest.

Human rights statement and informed consent The study was approved by the Ethics Committee of the Hokkaido University Graduate School of Medicine. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revisions. Informed consent was obtained from all study participants who were included in the additional study.

References

1. Nathan DM, Buse JB, Davidson MB, et al. Management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. *Diabetologia*. 2009;52:17–30.
2. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. *Diabetologia*. 2007;50:2239–44.

3. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, A1c-Derived Average Glucose (ADAG) Study Group. Translating the A1C assay into estimated average glucose values. *Diabetes Care*. 2008;31:1473–8.
4. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *J Diabetes Investig*. 2010;1:212–28.
5. Mizushima S, Tsushita K. New strategy of prevention and control of noncommunicable lifestyle-related diseases focusing on metabolic syndrome in Japan. In: Muto T, Nakahara T, Eun WN, editors. *Asian perspectives and evidence on health promotion and education*. Tokyo: Springer; 2011. p. 31–9.
6. Ministry of Health, Labour and Welfare of Japan. The basic guideline for health checkups and healthcare advice with a particular focus on the metabolic syndrome (final edition). Tokyo: Ministry of Health, Labour and Welfare of Japan; 2007 (**in Japanese**).
7. Nakanishi K, Ishida A, Mori N, et al. Evaluation of HbA1c by HPLC and immunoturbidimetry latex agglutination on calibrator JCCLS CRM-004a (JDS Lot3). *Jpn J Clin Lab Autom JJCLA*. 2009;34:233–6.
8. National Federation of Industrial Health Organization. An annual report of accuracy control surveillance for clinical laboratory test. http://www.zeneiren.or.jp/research_list/index.html Accessed 28 Sep 2015 (**in Japanese**).
9. Vogeser M, Parhofer KG. Head-to-head comparison of an automated immunometric and an automated HPLC method for the quantification of HbA1c. *Clin Biochem*. 2008;41:1410–2.
10. Imoto S, Kido T, Nakanishi S, Yamano C. HbA1 assay by auto clinical chemistry analyzer. *Annu Rep Kyoto City Inst Health Environ Sci*. 2005;71:118–9.
11. Matteucci E, Cinapri V, Rossi L, Lucchetti A, Giampietro O. Glycated hemoglobin measurement: intermethod comparison. *Diabetes Nutr Metab*. 2001;14:217–9.
12. Ishiguro A, Yamauchi T, Imada R, et al. Differences in hemoglobin A1c (HbA1c) levels due to measurement methods. *Igakukensa*. 2014;63:767–72 (**in Japanese**).
13. Health and Welfare Statistics Association. 2014/2015 Kokumin Eisei no Doko (Trend for national health and hygiene, Japan). Tokyo: Health and Welfare Statistics Association; 2014 (**in Japanese**).
14. Nakamura K, Miura K, Nakagawa H, et al. Treated and untreated hypertension, hospitalization, and medical expenditure: an epidemiological study in 314 622 beneficiaries of the medical insurance system in Japan. *J Hypertens*. 2013;31:1032–42.
15. Kashiwagi A, Kasuga M, Araki E, Committee on the Standardization of Diabetes Mellitus-Related Laboratory Testing of Japan Diabetes Society, et al. International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. *Diabetol Int*. 2012;3:8–10.