Determination of Reference Intervals of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) in Adults

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Objective: *To establish HbA_{1C} reference intervals according to DCCT/NGSP (HbA_{1C} DCCT/NGSP) and IFCC (HbA_{1C} IFCC) in adults.*
Study Design: *The study was a descriptive study.*
Material and Method: *The study was done in 144 subjects, with 99 males and 45 females, aged between 19 to 78 years old. All subjects had normal vital signs, physical examination, chest X-ray. Subjects who had hyperglycemia, renal problem, liver problem, anemia, and/or hemoglobinopathy were excluded from the present study.*
Results: *Reference intervals of HbA_{1C} (DCCT/NGSP) is 5.47% (4.79-6.15) and HbA_{1C} (IFCC) is 3.66% (2.88-4.44). The authors also found very high correlation between HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) of total, male, female, < 35 years old, and ≥ 35 years old, r = 0.9995, 09997, 0.9992, 0.9988, and 0.9999, respectively.*
Conclusion: *The authors found that HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) are not affected by sex but are influenced significantly by age group. Since HbA_{1C} (IFCC) will be widely used in routine diabetes management, the authors recommend all laboratories provide the results of HbA_{1C} in both DCCT/NGSP and IFCC methods during this interim period.*

Keywords: *HbA_{1C} (DCCT/NGSP), HbA_{1C} (IFCC), Reference intervals*

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The measurement of hemoglobin A_{1C} (HbA_{1C}) in blood is meanwhile well established as the most important parameter for monitoring a long-term control of diabetic patients[1,2]. Many different routine methods (>20) claiming to measure HbA_{1C} are currently used by clinical laboratories. Furthermore, there is a very large variability in the performance of those different methods. However, there had not been any internationally reference material, to which the routine assays could be standardized[3] with. Until the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) working group had developed a reference method[4]. To overcome the problem of poor standardization, the working group of IFCC has established a uniform, scientifically well found international standardization. The group decided to develop a reference system in which routine methods can be traced[5,6]. Although the method of determination of HbA_{1C} had not been standardized until the IFCC working group developed a reference method in 2002[4], they had already been studied for several decades by many groups of researchers due to diabetes mellitus (DM) being one of the major health problems that need early management. Thus, the information of studies about DM from the Diabetes Control and Complications Trial Research Group (DCCT)[1], the National Glycohemoglobin Standardization Program (NGSP)[7,8], and UK Prospective Diabetes Study (UKPDS) Group[2] have been provided for worldwide practice for several years before HbA_{1C} according to IFCC (HbA_{1C} IFCC) reference method and reference material had been established. Since the IFCC values can be converted into values of the various established

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national standardization methods, such as DCCT, NGSP, UKPDS, etc, the authors designed to implement the method that standardized against the IFCC reference assays. Furthermore, HbA_{1C} (IFCC) is expected to take place of HbA_{1C} according to DCCT/NGSP (HbA_{1C} DCCT/NGSP) in the near future. In addition, as most biochemical substances in circulation are influenced by many factors, therefore individual laboratories are recommended to establish their own reference intervals of laboratory tests even if the manufacturers have provided them. In order to establish proper reference intervals of laboratory tests for worldwide laboratories, the National Committee for Clinical Laboratory Standards (NCCLS) had provided a standard method to determine reference intervals[9].

In purpose of establishing HbA_{1C} reference intervals according to DCCT/NGSP that have been worldwide used and the method that standardized against the IFCC reference assays for Central Laboratory of King Chulalongkorn Memorial Hospital (KCMH), the authors designed a descriptive study using a recommendation method of NCCLS.

Material and Method

The present study protocol was approved by the Ethical Committee, Faculty of Medicine, Chulalongkorn University. All subjects are checked for vital signs, blood pressure, and physical examined by doctors. Individual with abnormal vital signs or abnormal blood pressure or abnormal doctoral inspection were not included. In addition, chest X-ray (CXR) and laboratory investigation were also added in the exclusion criterion. After informed consent was obtained, blood was collected by venipuncture using EDTA as anticoagulant for HbA_{1C}. Two hundred and seven volunteers were randomly selected, composed of 148 males and 59 females, aged between 19 and 81 years old. Most of them were employees of private and governmental organizations who had enrolled in the annual checkup program, at KCMH, from July to November 2003. All volunteers had normal CXR and were investigated for complete blood count (CBC), fasting plasma glucose (FPG), blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). CBC was analyzed by Advia 120[10], while blood chemistry such as; FPG, BUN, Cr, AST, and ALT were determined by Hitachi 912[11]. In addition, all samples were screened for hemoglobinopathy[12] by automated blood cell analyzer and dichlorophenol-indophenol precipitation test (DCIP) using a reagent kit, KKU-DCIP-Clear[13]. All subjects who had hyperglycemia, renal problem, liver problem, anemia and/or hemoglobinopathy were excluded from the present study. After investigation, only 144 subjects were accepted for the present study, compiled of 99 males and 45 females, aged between 19 and 78 years old. All EDTA samples were kept at -20^0C and analysis of HbA_{1C} was performed in the same batch within 12 weeks. HbA_{1C} was determined by immuno-turbidity using COBAS INTEGRA® 700, standardized against the IFCC reference assays[14]. The results of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) were received for further analysis. Pool of fresh plasma was analysed as intra-assay coefficiency.

To cover for 95% of the population (95% reference intervals or ranges) according to NCCLS recommendation, the reference intervals were calculated using mean $\pm$ 2 standard deviation (mean $\pm$ 2SD) for those laboratory parameters that have Gaussian distribution[5]. Furthermore, study was done by comparison of the results between male and female, as well as age groups (< 35 years old and ≥ 35 years old) using Student's t-test (unpaired samples, two tailed). The p value ≤ 0.05 was considered statistically significant.

Results

The distribution of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) were the same, Gaussian pattern, and shown in Fig. 1 and 2. Characteristics of subjects and results of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) according to sex are shown in Table 1. Characteristics of subjects and results of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) according to age group are shown in Table 2. Reference interval of HbA_{1C} (DCCT/NGSP) was 4.79-6.15% and HbA_{1C} (IFCC) was 2.88-4.44%. The authors found that HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) were not affected by sex, but were influenced by age group. Correlation between HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) of total, male, female, < 35 years old, and ≥ 35 years old are shown in Fig. 3. The intra-assay CV of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) of the present study were 1.3% and 2.4%, respectively. Correlation coefficient (r) between HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) of total, male, female, < 35 years old, and ≥ 35 years old are calculated and shown in Fig. 3 as well.The authors found very high correlation between HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) of total, male, female, < 35 years old, and ≥ 35 years old, r = 0.9995, 09997, 0.9992, 0.9988, and 0.9999, respectively.

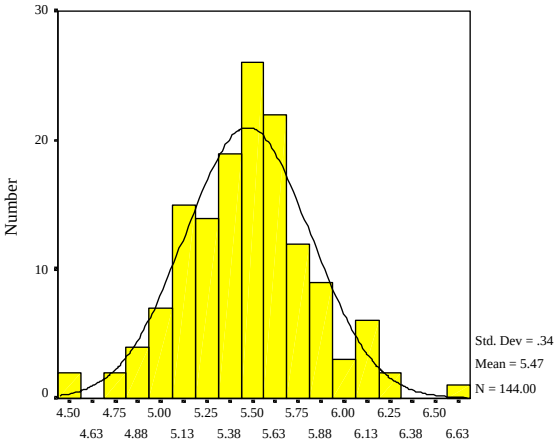

Fig. 1 The distribution pattern of HbA1C (DCCT/NGSP) was Gaussian pattern

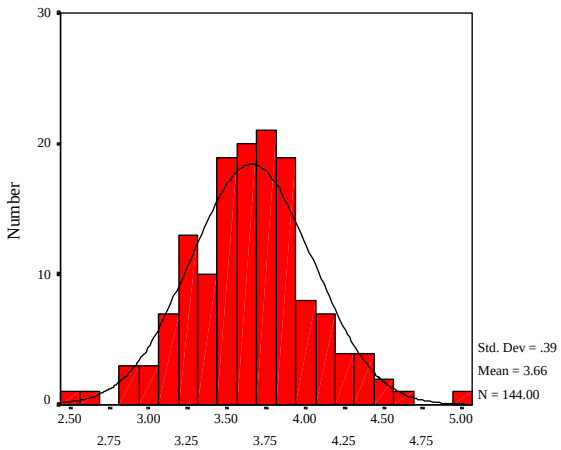

Fig. 2 The distribution pattern of HbA1C (IFCC) was Gaussian pattern

Discussion

The distribution of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) is shown in Fig. 1 and 2. The authors' reference intervals of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) of total, male, female, < 35 years old, and ≥ 35 years old are shown in Table 1 and 2. HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) reference levels of total (n = 144) were 5.47% (4.79-6.15) and 3.66% (2.88-4.44), respectively. The reference values of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) were 5.4% (4.8-5.9) and 3.5% (2.9-4.2)[1,2,11]. From these data, The results are close to the Western data. However, the reference values of HbA_{1C} are slightly higher than reference values in a Western population. These data support

Table 1. Characteristics of subjects and results of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) according to sex

Characteristics	Total	Male	Female	p value
Number	144	99	45	-
Age (years)				
average (±SD)	40 (±13)	40 (±13)	40 (±13)	
range	19-78	19-78	21-66	-
HbA_{1C} (DCCT/NGSP)				
average (± SD)	5.47 (±0.34)	5.44 (±0.33)	5.55 (±0.35)	
interval (average ± 2SD)	4.79-6.15	4.78-6.10	4.85-6.25	0.0795

Table 2. Characteristics of subjects and results of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) according to age

Characteristics	Total	< 35 years	≥ 35 years	p value
Number	144	57	87	-
Age (years)				
average (±SD)	40 (±13)	29 (±4)	48 (±11)	
range	19-78	19-34	35-78	-
HbA_{1C} (DCCT/NGSP)				
average (± SD)	5.47 (±0.34)	5.36 (±0.33)	5.55 (±0.33)	
interval (average ± 2SD)	4.79-6.15	4.70-6.02	4.89-6.21	<0.0007*
HbA_{1C} (IFCC)				
average (± SD)	3.66 (±0.39)	3.52 (±0.37)	3.75 (±0.37)	
interval (average ± 2SD)	2.88-4.44	2.78-4.26	3.01-4.49	<0.0005*

*p value ≤ 0.05 was considered statistically significant

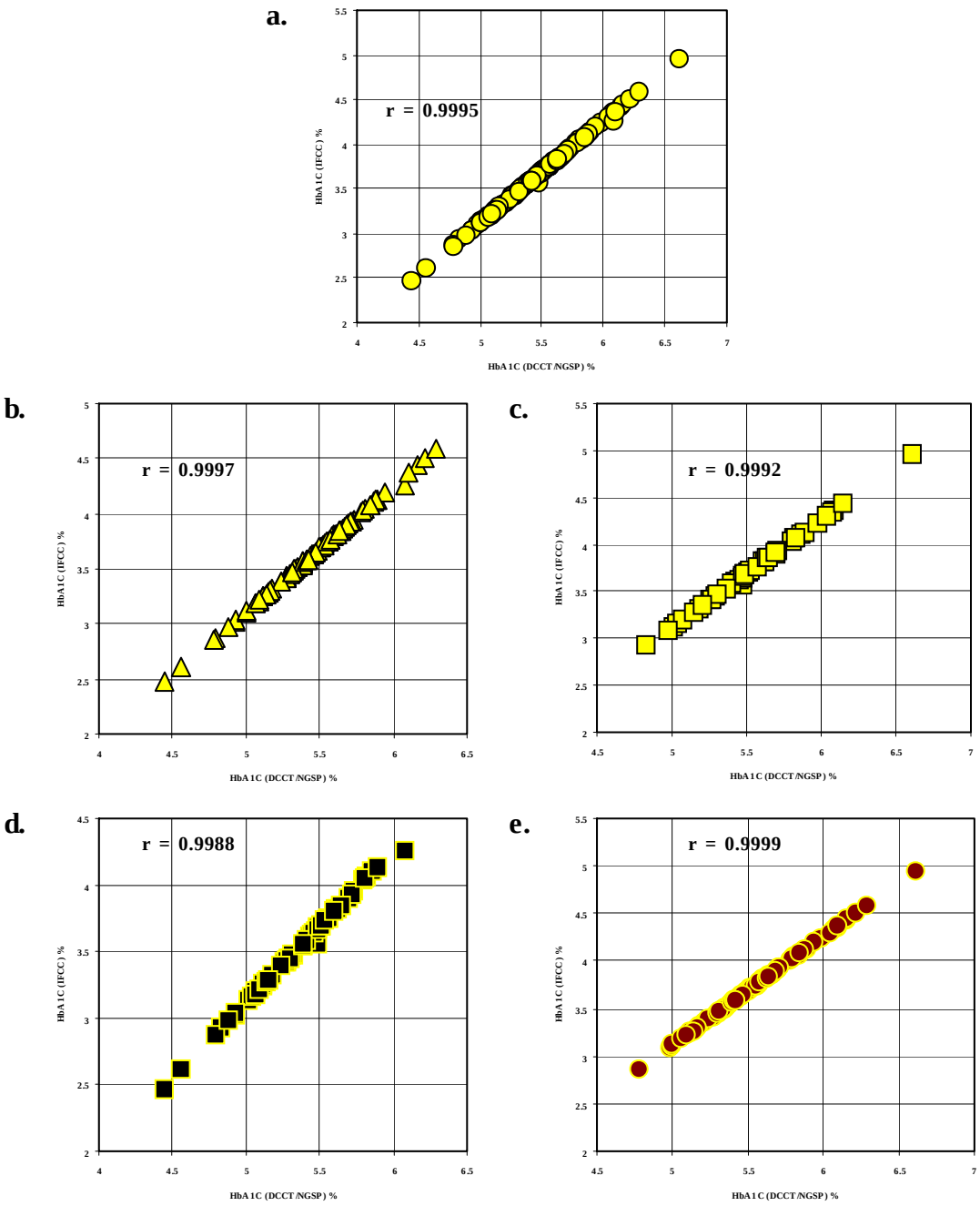

Fig. 3 Correlation between HbA1C (DCCT/NGSP) and HbA1C (IFCC) of a) total (n = 144), b) male (n = 99), c) female (n = 45), d) < 35 years old (n = 57), and e) > 35 years old (n = 87)

the recommendation of NCCLS in encouragement laboratories to establish their own reference intervals of those provided parameters. In addition, the variability of reference intervals of those provided parameters could have occurred from important factors such as; the difference of population, race, and environment[9]. The authors also studied HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) in males, females, < 35 years old, and ≥ 35 years old, the authors found that age group was an important factor effect significantly (p = 0.0007, and 0.0005) to the HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) levels. The authors

found a higher trend level of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) in females compared to males (Table 1). However, the authors could not demonstrate the statistical significance of the difference between reference intervals of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) by sex. The present results are different from a previous study performed by Hitachi 912 and COBAS INTEGRA® 800, which found that age and sex had no statistical significance[15]. The difference may be due to age group classification being different from the present study. That study age group was classified into 5 classes, 18-30 (n = 26), 30-39 (n = 62), 40-49 (n = 24), 50-59 (n = 14), and ≥ 60 (n = 18) years old. However, the results of reference intervals are not notified of difference. They found that HbA_{1C} (DCCT/NGSP) by Hitachi 912 and COBAS INTEGRA® 800 were 4.45% (4.92-6.04), and 5.28% (4.74-5.84), respectively. As well as, HbA_{1C} (IFCC) by Hitachi 912 and COBAS INTEGRA, were 3.63% (3.02-4.30), and 3.43% (2.82-4.01), respectively.

From the present results, all the SD for the reference intervals shown in Table 1 and 2 are less than 0.5% that demonstrated the small deviation of intervals[16]. These suggested that the present result are results are close to the recommendation of NGSP. In order to analyze for precision, the authors found that intra-assay CV of HbA_{1C} (DCCT/NGSP) and HbA_{1C} (IFCC) were 1.3% and 2.4%, respectively. These intra-assay CV were less than 3% according to the recommendation for laboratory analysis in the diagnosis and management of DM[12]. Furthermore, all reference intervals of HbA_{1C} (IFCC) of total, male, female, < 35 years old, and ≥ 35 years old were lower than reference intervals of HbA_{1C} (DCCT/NGSP), these results are not over expectation. The values of all previous routine methods were probably too high since they lacked specificity in various methods[5]. Therefore, the application of the new reference system in laboratory practice will not only effect an analytical problem, but also, a medical problem, because reference ranges for non-diabetics, and recommended values for optimal therapy in diabetic patients have to be revised and adjusted to the new reference values. The various commercial assays have to be adjusted to the new international reference (HbA_{1C} IFCC) system, and new reference ranges and target values have to be introduced into clinical practice. In addition, there is a move towards standardization of analytical systems and harmonization of standards. Nowhere has more evidence than medical laboratory field with recent formation of ISO-TC 212 and international efforts that are underway by IFCC, World Health Organization (WHO), ISO, and NCCLS to further the standardization process. The authors suggested that additional studies in non-diabetics and diabetic patients using HbA_{1C} (IFCC) in Thailand should be further performed to establish our own diabetic management data.

In conclusion, reference intervals of HbA_{1C} are different between age groups, the authors suggested that evaluation of HbA_{1C} level should be considered according to patient age. Because of HbA_{1C} (IFCC) is expected to be used worldwide in the future, the authors recommend all laboratories provide the results of HbA_{1C} in both DCCT/NGSP and IFCC methods to facilitate the clinicians to evaluate the results. In addition, reference intervals should not only be established to standardize the service, but also should support the patients' care. The authors agree with the recommendation of NCCLS[9], ISO[17], etc, that suggested every laboratory should establish its reference intervals. In order to achieve the standard requirement and need of patients' care, laboratory services in developing countries such as Thailand, should consider establishing reference intervals as basic requirement in every laboratory. Supporting Grants to establish reference intervals from the associated or research organizations should be managed and available to the laboratories. In addition, the authors recommend every laboratory should establish reference intervals for all service parameters according to age, and gender. Other sub-populations may be set according to the requirement of each region. The authors also suggest every laboratory update reference intervals of all service parameters at suitable periodic times.

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การหาค่าอ้างอิงของ HbA_{1C} (DCCT/NGSP) และ HbA_{1C} (IFCC) ในผู้ใหญ่

นวพรรณ จารุรักษ์, แอนนา มิลินทากาศ, พรรณดี วัฒนบุญยงเจริญ, โฉมศรี อริยะบุญศิริ

วัตถุประสงค์: *เพื่อหาค่าอ้างอิงของ HbA_{1C} ตามวิธีของ DCCT/NGSP (HbA_{1C} DCCT/NGSP) และวิธีของ IFCC (HbA_{1C} IFCC) ในผู้ใหญ่*

รูปแบบการศึกษา: *การศึกษาเชิงพรรณนา*

วัสดุและวิธีการ: *การศึกษานี้ทำในกลุ่มตัวอย่าง 144 คน เป็นเพศชาย 99 คน เป็นเพศหญิง 45 คน อายุระหว่าง 19 ถึง 78 ปี โดยทั้งหมดเป็นผู้ที่มีผลการตรวจสัญญาณชีพ การตรวจร่างกายโดยแพทย์ การตรวจเอกซเรย์ปอด และผลการตรวจทางห้องปฏิบัติการเป็นปกติ ผู้ที่มีผลการตรวจทางห้องปฏิบัติการผิดปกติ เช่น ภาวะน้ำตาลในเลือดสูง ความผิดปกติทางไต ตับ ภาวะโลหิตจาง และหรือความผิดปกติของฮีโมโกลบิน จะถูกคัดออกจากการศึกษานี้*

ผลการศึกษา: *ค่าอ้างอิงของ HbA_{1C} (DCCT/NGSP) เท่ากับ 5.47 % (4.79-6.15) และHbA_{1C} (IFCC) เท่ากับ 3.66 % (2.88-4.44). คณะผู้วิจัยพบว่าค่าสัมประสิทธิ์สหสัมพันธ์ (r) ระหว่าง HbA_{1C} (DCCT/NGSP) และ HbA_{1C} (IFCC) ของทั้ง 144 คน, เฉพาะเพศชาย, เฉพาะเพศหญิง, ผู้ที่มีอายุ < 35 ปี, และผู้ที่มีอายุ ≥ 35 ปี เท่ากับ 0.9995, 09997, 0.9992, 0.9988 และ 0.9999 ตามลำดับ*

สรุป: *คณะผู้วิจัยพบว่าค่า HbA_{1C} (DCCT/NGSP) และ HbA_{1C} (IFCC) ไม่ถูกกระทบด้วยเพศ แต่ถูกกระทบด้วยกลุ่มอายุ ด้วยแนวโน้มที่ HbA_{1C} (IFCC) น่าจะเป็นวิธีการที่นำมาใช้ต่อไปในอนาคตอันใกล้ คณะผู้วิจัยจึงแนะนำให้ห้องปฏิบัติการทั้งหลายรายงานผลการตรวจ HbA_{1C} ทั้งแบบ DCCT/NGSP และ IFCC ในช่วงระหว่างการเปลี่ยนแปลงนี้*