Original Article

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# DETERMINATION OF GLYCOSYLATED HEMOGLOBIN REFERENCE RANGE IN ADULT POPULATION ATTENDING A MILITARY CARE SET UP IN RAWALPINDI

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## ABSTRACT

***Objective:*** To determine the glycosylated hemoglobin reference range in healthy adult population attending a military care setup in Rawalpindi in accordance with National Glycohemoglobin Standardization Program (NGSP) and International Federation of Clinical Chemistry (IFCC)

***Study Design:*** Descriptive cross-sectional study

***Place and Duration of Study:*** Armed Forces Institute of Pathology Rawalpindi from May - Oct 2010

***Subjects and Methods:*** A total of 254 healthy adults (18-80 years) comprising 169 males and 85 females, were included by non probability consecutive sampling from Rawalpindi. History and clinical examination were carried out. Blood HbA1c was analyzed high performance liquid chromatography on biorad D-10. Data were analyzed by SPSS-17.

***Results:*** Total 254 subjects consisting of 169 males and 85 females were recruited from Rawalpindi Pakistan. The reference range of total population was found 4.6–6.56% and 2.69–4.81 mmol/mol in accordance with NGSP and IFCC, respectively. When compared in gender, HbA1c levels were not significantly changed. However, the healthy elderly population had higher HbA1c levels. Reference range for HbA1c based on NGSP in individuals <40 years was found 4.52-6.4% while for individuals aged > 40 years, it was 4.8-6.68%..

***Conclusion:*** The reference ranges for healthy individuals and the recommended values for optimal therapy in diabetic patients have to be revised and adjusted. Population based reference ranges should be established to provide better patient care services.

**Keywords:** HbA1C, Healthy Population.

## INTRODUCTION

The glycosylated hemoglobin (HbA1c) assay has become the gold-standard for monitoring of glycemic control in diabetes mellitus over two decades[1]. Plasma glucose level is directly associated with HbA1c[2]. It is easy to interpret, cost effective and routinely used to monitor long-term glycemic control[3] as it is directly related to the risks for diabetic complications[4]. By performing HbA1c test, patient's average glycemic control over the preceding 2–3 months can be measured[3]. The relationship between HbA1c and estimated average glucose has been established recently[5]. HbA1c has less intra-individual variation and more reproducibility than fasting plasma glucose and oral glucose tolerance test (OGTT)[6]. Studies showed the value of 2 standard deviation (SD) above the normal mean is significant in identifying high proportion of undiagnosed diabetic individuals[6]. This advocates use of HbA1c as an important screening and diagnostic test for diabetes mellitus[7]. National Glycohemoglobin Standardization Program (NGSP) and International Federation of Clinical Chemistry (IFCC) have standardized HbA1c assays[8]. It is suggested that HbA1c should be reported in both NGSP (%) and IFCC (mmol/mol) units[9].

HbA1 c levels variation based on gender, age and ethnic groups in healthy population have been reported [10-12]. In Thai healthy individuals, reference range of HbA1c were found 4.79% -6.15%[11]. In a study conducted in USA , mean HbA1c levels were varied among different ethnic populations; 4.93% (+ 0.04) in non-Hispanic whites and 5.05% (+ 0.02) in Mexican-Americans to 5.17% ( +0.02) in non-Hispanic blacks 12. In another study conducted in Kuwaiti population, the mean HbA1c level was 4.8% with a standard deviation of 0.5%[13]. Thus to use HbA1c as diagnostic tool in a local population, it is also recommended that each

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laboratory should establish its own reference range of HbA1c for non diabetic individuals[8].

We are using the reference range which is based on foreign data and may not represent our non diabetic range. This is the first study to determine HbA1c reference range in Pakistani population. There exists a need of establishing a reference range for HbA1c in our medical set up so that it can be used for diagnosis, screening and monitoring of our diabetic patients.

## SUBJECTS AND METHOD

The present study protocol was approved by the Ethics Committee, Armed Forces Institute of Pathology Rawalpindi. It was a descriptive cross-sectional study conducted on a total of 254 healthy subjects at Armed Forces Institute of Pathology, Rawalpindi from May 2010 - Oct 2010. The healthy adults aged between 18 and 80 years comprising of males (n=169) and females (n=85), inhabitants of Rawalpindi , coming for routine annual medical checkup in military health care setup , were selected by non probability consecutive sampling and included in the study. Medical check up of all the subjects was done. Individuals having history of any acute or chronic illness, fasting plasma glucose > 5.6 mmol/L, anemia, hemoglobinopathy, pregnancy or individuals taking any medication were excluded from the study. After the informed consent was obtained, whole blood samples were obtained for analysis of HbA1c. HbA1c was determined by high performance liquid chromatography (HPLC) using Biorad D-10. The results were obtained according to NGSP and afterward NGSP results were converted to IFCC using the master equation [14].

NGSP = (0.915 X IFCC) +2.15. Mathematically, it can also be written as

IFCC = (NGSP - 2.15) / 0.915.

The reference range was evaluated using mean ± 2 SD in accordance with National Committee on Clinical Laboratory Standards (NCCLS) recommendation15

### Statistical Analysis

The data were analyzed using SPSS version 17. Descriptive study was used to describe the data. Independent sample t-test was used to compare HbA1c between males and females and between different age groups . The $p$ value < 0.05 was considered statistically significant. Three levels of controls were analyzed for intra assay coefficient of variation (CV).

## RESULTS

Total 254 subjects consisting of 169 (66.5%) males and 85(33.5%) females were recruited . Baseline characteristics of the participants are shown in Table-1. The distribution pattern of HbA1c based on NGSP was Gaussian (Figure). The healthy volunteers had mean + SD of HbA1c 5.58±0.49% based on NGSP while according to IFCC, it was 3.75±0.53. The reference range of total population was found 4.6–6.56% and 2.69–4.81 mmol/mol in accordance with NGSP and IFCC, respectively.

HbA1c levels were not significantly changed in males as compared with females (Table-2). However, the healthy elderly population had higher HbA1c levels (Table-2). Reference range for HbA1c based on NGSP in individuals <40 years was found 4.52 - 6.4 % while for individuals aged > 40 years, it was 4.8 - 6.68%. According to IFCC, it was 2.6 – 4.64 mmol/mol and 2.88 – 4.92 mmol/mol for age groups <40 years and >40 years old, respectively. The intra-assay total CVs of HbA1c were 2.6%, 1.9% and 1.6% for low, normal and high levels of controls, respectively.

## DISCUSSION

This is the first study in Pakistan for the evaluation of reference range for HbA1c and the reference range of HbA1C for total population was found to be 4.6 – 6.56% and of 2.69–4.81 mmol/mol, in accordance with NGSP and IFCC, respectively. The reference range of HbA1c is slightly higher than that of Western[12] and Kuwaiti[13] population. However, our data was more in coherence with data from Thai[11] population. This data support the recommendation of NCCLS to encourage

**Table-1: Baseline characteristics of the participants**

| | **Total** (Mean±SD) | **Male** (Mean±SD) | **Female** (Mean±SD) |
|---|---|---|---|
| **Age** (Years) | 41.07±15.69 | 40.82±16.93 | 42.74±15.51 |
| **BMI** (kg/m$^2$ ) | 24.67±0.52 | 24.6 ±0.56 | 24.8±0.43 |
| **Blood Pressure** (mm Hg) | | | |
| Systolic | 120.40±0.8 | 120.3 ± 0.81 | 120.5±0.75 |
| Diastolic | 80.1±0.85 | 80.1±0.81 | 79.9±0.91 |
| **Fasting plasma glucose** (mmol/l) | 4.0 ± 0.49 | 4.0 ± 0.52 | 3.9 ± 0.44 |

**Table-2: Comparison of HbA1c levels in relation with age and gender**

| **Independent variable** | **HbA1c (NGSP%) Mean ±SD** | **HbA1c (IFCC mmol/mol) Mean ±SD** |
|---|---|---|
| **Age ( years)** | | |
| < 40 ( n = 143) | 5.46 ± 0.47 | 3.62 ± 0.51 |
| > 40 ( n = 111) | 5.74 ± 0.47 | 3.9 ± 0.51 |
| p – value* | 0.001 | 0.001 |
| **Gender** | | |
| Males ( n = 169) | 5.61 ± 0.51 | 3.78 ± 0.56 |
| Females (n =85) | 5.54 ± 0.43 | 3.7 ± 0.47 |
| p – value* | 0.298 | 0.298 |

*p value < 0.05 was considered statistically significant

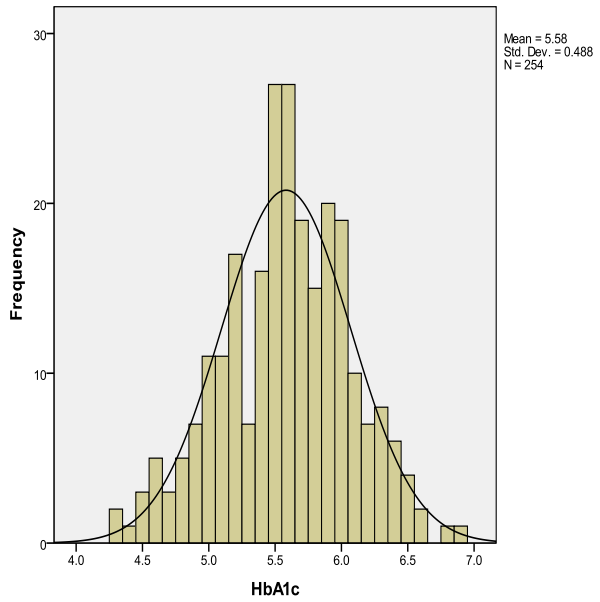


**Figure: The distribution pattern for HbA1c.**

laboratories in establishing their own reference ranges of provided parameters[15]. In addition, the variability of reference ranges of provided parameters could have occurred from factors such as; the difference of population, race, and environment[15]. The authors also studied HbA1c levels in males, females and age groups < 40 years old and > 40 years old, and found that HbA1c levels are independent of gender ($p$=0.298). However, it varied among age groups. In our data HbA1c levels are higher among elderly age group ($p$=0.001).

The present results are different from a previous study performed in Thai population which found that gender had statistical significance[11]. In our study, the intra-assay CV of HbA1c were 2.6%, 1.9% and 1.6% for low, normal and high levels of controls , respectively. These intra-assay CVs were less than 3% according to the recommendation for laboratory analysis in the diagnosis and management of diabetes mellitus[16].

The reference ranges for healthy individuals and the recommended values for optimal therapy in diabetic patients have to be revised and adjusted in accordance to the new reference range. "The various commercial assays need to adjust to the new international reference system for reference ranges and target values and to pace with the move towards standardization of analytical systems and harmonization of standards". It is recommended that all the results of HbA1c should be reported in both NGSP and IFCC methods not only to facilitate the physicians but also to avoid confusions in reported results. In addition, population based reference ranges should be established to provide better patient care services and to achieve the standardization requirements according to NCCLS recommendations[14].

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