GLYCATED HEMOGLOBIN REFERENCE LIMITS OBTAINED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN ADULTS AND PREGNANT WOMEN

ERİSKİNLERDEKİ VE GEBE KADINLARDAKİ YÜKSEK PERFORMANSLI SIVI KROMATOĞRAFİSİ İLE ÖLÇÜLMÜŞ GLİKOLİZE HEMOGLOBIN REFERANS ARALIKLARI

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ABSTRACT

Purpose: The measurement of serum glycated hemoglobin levels (HbA1C) is a useful index in the management of diabetes mellitus. Many authors have shown a variety of reference values obtained using different forms of high performance liquid chromatography (HPLC); this reinforces the importance of determining reference values for each clinical laboratory. The aim of this study was to determine the reference values of HbA1C in adults and to assess the influence of sex and pregnancy on them. Methods: We investigated 60 (30 females and 30 males) healthy adults and 68 pregnant women admitted to Gazi University Medical School. HbA1C levels were measured by HPLC. Results: Reference limits for HbA1C were determined as 3.31-5.39% for the whole adult population, 3.23-5.63% for men, 3.42-5.14% for women and 1.96-4.06% for pregnant women (2.5 and 97.5 percentiles). The results showed that there was no difference between females and males (p>0.05). HbA1C levels were found to be significantly different between pregnant and nonpregnant women (p<0.01). Conclusion: Ethnic and socioeconomic differences between populations influence HbA1C reference values. In addition, HbA1C concentrations are lower in pregnancy.

Key Words: Glycated Hemoglobin, Reference Limits, High Performance Liquid Chromatography.

INTRODUCTION

The measurement of glycated hemoglobin (HbA1c) is one of the cornerstones of diabetes mellitus management. Most physicians use HbA1c values in the assessment of patient blood glucose values. Good glycemic control is associated with lower rates of microvascular complications in both type 1 and type 2 diabetic patients (1).

ÖZET


Anaf tar Kelimeler: Glikolize Hemoglobin, Referans Aralığı, Yüksek Performanslı Svi Kromatografi.
Gestational diabetes mellitus (GDM) includes any degree of glucose intolerance occurring with the onset or first recognition of pregnancy (2). Causes of GDM frequently return to normal postpartum. However, this disease is associated with increased perinatal complications and an increased risk for the development of diabetes mellitus in later years (3). Untreated GDM can cause macrosomia of the fetus, increased risk of birth trauma, hyperbilirubinemia, hypercalciemia, hypoglycemia or respiratory distress syndrome, gestational hypertension and preeclampsia. It is important to check glycemic levels in order to avoid maternal-fetal complications (4).

Various authors have investigated the relationship between HbA1c levels and gestational complications (5). Although the results are controversial, some studies have shown a relationship between high levels of HbA1c and macrosomia, fetal malformation, newborn respiratory problems and vascular complications (6).

Many authors have shown a variety of reference limits obtained by using different high performance liquid chromatography (HPLC) equipment, and this reinforces the importance of determining reference values for each clinical laboratory (7).

The aim of this study was to establish the reference intervals for HbA1c in two groups of healthy populations and pregnant women and to verify if these intervals were different. This was accomplished by using HPLC methodology.

PATIENTS AND METHODS

In this study, we investigated 60 (30 females and 30 males) healthy Turkish adults and 68 pregnant women admitted to Gazi University Medical School.

The pregnant women were between 20 and 35 years and healthy adults were between 25 and 45 years. They had no history of disease. The pregnant women who volunteered to participate in this study were selected from a group of healthy women under prenatal medical care in the hospital. They were all in their third trimester (28th week). Fasting blood samples were collected from all participants in tubes containing EDTA. The samples were hemolyzed using a hemolyzing buffer solution. They were incubated at 37°C for 20 min and they were then centrifuged at 3000 rpm for 5 min. They were analyzed by HPLC in the Shimadzu automated system. This system has an exchange cation column kept at 25°C. Hemoglobin A1a, A1b, F and A1c were eluted sequentially from the column (8). The HbA1c ranges were calculated by the parametric method. The differences between men and women were compared using the Mann-Whitney U test. Adult means were compared with pregnant group means by using an unpaired t test; p<0.05 was considered significant.

RESULTS

The means for male and female groups were compared using the Mann-Whitney U test and no significant statistical difference was determined (p>0.05). The lower and upper reference limits were then recalculated for the whole adult population and reference limits for HbA1c were determined to be 3.31-5.39% (2.5 and 97.5 percentiles) (Table 1).

The HbA1c reference limits were 1.96-4.40% for the pregnant women group (2.5 and 97.5 percentiles). Turkish adult means were compared with the mean of the pregnant group by using an unpaired t test. Extremely significant statistical differences were observed in all comparisons (p<0.0001). HbA1c was also found to be significantly different between pregnant and nonpregnant women (p<0.001). However, there was no difference between the HbA1c levels of males and females, as shown in Fig. 1 (p>0.05).

DISCUSSION

Diabetes mellitus is prevalent in the population and it brings about a high rate of premature death. The determination of reference values for glycated hemoglobin is very important in monitoring diabetic patients to analyze the glycemic status of individuals. Using this critical analysis can prevent premature death.

The reference limits for HbA1c obtained in
Table 1: Mean, standard deviation, and lower and upper limits of HbA1c levels (%) in adults and pregnant women.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Lower limit**</th>
<th>Upper limit**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=30)</td>
<td>4.43</td>
<td>0.60</td>
<td>3.23</td>
<td>5.63</td>
</tr>
<tr>
<td>Women (n=30)</td>
<td>4.28</td>
<td>0.43</td>
<td>3.42</td>
<td>5.14</td>
</tr>
<tr>
<td>Men + Women (n=60)</td>
<td>4.35</td>
<td>0.52</td>
<td>3.31</td>
<td>5.39</td>
</tr>
<tr>
<td>Pregnant Women (n=68)</td>
<td>3.18*</td>
<td>0.61</td>
<td>1.96</td>
<td>4.40</td>
</tr>
</tbody>
</table>

* p<0.05 compared to men and women.
** The lower and upper reference values correspond respectively to the 2.5 and 97.5 percentiles.

4.39% and pregnant: 3.2-4.3% (2).

These findings show that HbA1c concentrations are lower in pregnancy despite the fact that plasma glucose concentration levels are not significantly different. The lower values can be explained by a hemodilution tendency during pregnancy.

In this study, a Shimadzu analyzer was also employed. It utilizes HPLC technology, which is a highly sensitive, precise and accurate analytical method (6, 7), but it is not easy to operate correctly and requires specialized personnel.

The reference limits obtained in our study of the pregnant group (1.96-4.90) were lower and statistically different from those obtained for the adult group. Similar results were shown in Brazil by Parentoni et al. (adult: 3.49-

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REFERENCES


