

## Measurements of HbA1c for Patients with Diabetes Mellitus and Foot Ulceration

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### Abstract

People with diabetes can develop different foot problems. In the blood stream glucose reacts with hemoglobin to make a glycosylated hemoglobin molecule called hemoglobin A1c or HbA1c, the more glucose in the blood the more hemoglobin A1c will be present in the blood. The HbA1c test is currently one of the best ways to check diabetes to be under control. The aim of study is to compare between the blood investigations which includes the fasting blood sugar and HbA1c (glycosylated hemoglobin), and to evaluate the benefit of HbA1c (measurement for diabetic patients with foot ulcer, to be a good indicator for controlling blood glucose). Sixty patients with type2 diabetes mellitus from the outpatient clinic of Baghdad Teaching Hospital, Medical City over the period from Nov. 2006 to Nov. 2008, were included in the study. Follow up was done only to 30 patients with diabetic foot ulcer. Twenty (66.66%) were males and 10(33.33%) were females their age range from (23-75) years (mean age of 52years), and 21 normal subjects as control. A (Glycohemoglobin HbA1-Test/fast Ion-Exchange Resin Separation Method) kit was used. The data finding that there is a greater association between HbA1c level and foot ulceration healing. There is a relationship between the age of the patients and the HbA1c level. The patients who used (Glibenclamide+Metformin) have the lower range of HbA1c, while those who use (Metformin) have the higher level of HbA1c. HbA1c (glycosylated hemoglobin) is most accurate test to determine actual reading over the past 2-3 months, and to evaluating the risk of glycemic damage to the tissues. So, we recommend the HbA1c testing, but it can't be used to monitor day-to-day blood glucose concentration because it's not influenced by fluctuation in blood concentration.

**Key words:** Diabetic foot ulcer, HbA1c

### الخلاصة

المصابين بداء السكري من الممكن ان تتطور لديهم الحالة المرضية الى الاصابة بتقرحات في القدم . في مجرى الدم جزيئات الكلوكرز تلتصق بالهيموغلوبين لتكوين كلايكوسيلانيد هيموغلوبين وتدعى هيموغلوبين A1c او HbA1c وكلما كانت كمية الكلوكرز في الدم اكثر كلما كانت كمية الهيموغلوبين A1c اكثر . لقد وجد في الاونة الاخيرة بان الاستعانة باختبار مستوى HbA1c واحد من افضل الطرق للتحقق من مرض السكري الواقع تحت السيطرة . الغاية من هذه الدراسة للمقارنة بين الطرق المتبعة والمعتمد عليها في السيطرة على نسبة السكر لدى المرضى السكري من النوع الثاني ، مثل اختبار السكري في حالة الصيام ومقارنته باختبار مستوى الكلايكوسيلانيد هيموغلوبين . اجريت الدراسة على 60 شخص مصابين بمرض السكري من النوع الثاني واستمرت الدراسة على 30 شخص منهم مصاب بتقرحات في القدم وبمعدل عمري من 23-75 سنة وكان عدد الذكور 20 (66.66%) والاناث 10 (33.33%) . كما استعين بـ 21 شخص سليم غير مصاب بداء السكري وليس لديه تقرحات (مجموعة سيطرة) وقد وجدنا نتيجة هذه الدراسة بان النتائج المستحصل عليها تفيد بان هناك علاقة كبيرة بين مستوى HbA1c وشفاء تقرحات القدم. كما وجدنا هناك علاقة بين عمر المريض ونسبة الكلايكوسيلانيد هيموغلوبين في الدم ، كذلك وجدنا من خلال هذه الدراسة بان المرضى المعالجين بمجموعة الادوية المخفضة للسكر (ميثفورمين + كليبينكلامايد) قد انخفض مستوى الكلايكوسيلانيد هيموغلوبين اكثر من مجموعة المرضى الذين استعملوا (الميثفورمين) فقط حيث كان مستوى الكلايكوسيلانيد هيموغلوبين اعلى . تستنتج الدراسة بان الكلايكوسيلانيد هيموغلوبين هو اصح اختبار لتعيين القيمة الحقيقية للسكر لمدة 2 - 3 اشهر سابقة ولتقدير الخطورة التي يتعرض لها المصاب . لذلك نوصي باستعمال اختبار الكلايكوسيلانيد هيموغلوبين حتى وان كان لايعطينا قيمة السكر المتغيرة يوميا.

### Introduction

People with diabetes can develop different foot problems<sup>[1,2]</sup>. Foot problems most often occur when there is neuropathy, poor blood flow, or changes in the shape of feet toes<sup>[2-4]</sup>, Ulcers occur most often on the ball of the foot or in the bottom of the big toe. Neglecting ulcers can result in infections, which in turn may lead to loss of a limb<sup>[5]</sup>. Diabetic patients are at higher risk and they

show 2 - 4 times more likely to have heart disease or suffer a stroke than people without diabetes<sup>[5-7]</sup>. In the blood stream glucose reacts to the hemoglobin to make a hemoglobin molecule called hemoglobin A1c (HbA1c), the more glucose in the blood, the more hemoglobin A1c will be formed in the blood<sup>[6,7]</sup>. About 90% of hemoglobin is hemoglobin A<sup>[7]</sup>.

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Although one chemical component accounts for 92% of hemoglobin A, approximately 8% of hemoglobin A is made up of minor components that are chemically slightly different. These minor components include hemoglobin A1c, A1b, A1a1, and A1a2. Hemoglobin A1c is a minor component of hemoglobin to which glucose is bound. Thus it's referred to as glycosylated or glucosylated hemoglobin<sup>[8-10]</sup>. The HbA1c test is currently one of the best ways to check diabetes is under control. The HbA1c level changes slowly, over 10 week, so it can be used as a ((quality control)) test<sup>[11-13]</sup>. Hemoglobin A1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column.<sup>[12]</sup> It was first characterized as a glycoprotein by Bookchin and Gallop in 1968.<sup>[13]</sup> Its increase in diabetes was first described in 1969 by Samuel Rahbar and coworkers the reactions leading to its formation were characterized by Bunn and his co-workers in 1975.<sup>[14, 15]</sup> The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and coworkers<sup>[16]</sup>. In the normal 120-day life span of the red blood cell, glucose molecules react with hemoglobin, forming glycosylated hemoglobin. In individuals with poorly controlled diabetes, the quantities of these glycosylated hemoglobins are much higher than in healthy people<sup>[16, 17]</sup>. Once a hemoglobin molecule is glycosylated, it remains that way. A buildup of glycosylated hemoglobin within the red cell therefore reflects the average level of glucose to which the cell has been exposed during its life cycle<sup>[18, 19]</sup>. Measuring glycosylated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA1c level is proportional to average blood glucose concentration over the previous four weeks to three months<sup>[20]</sup>. Some researchers state that the major proportion of its value is related to a rather shorter period of two to four weeks.<sup>[7]</sup> The 2010 American Diabetes Association Standards of Medical Care in Diabetes added the A1c  $\geq 6.5\%$  as another criterion for the diagnosis of the diabetes<sup>[21]</sup>. There were numbers of laboratories techniques used to measure glycosylated hemoglobin<sup>[22]</sup>:

- high-performance liquid chromatography (HPLC).
- Immunoassay.

## Patients and methods

Patients with type 2 diabetes mellitus with foot ulcers seen in the outpatient clinic of Baghdad Teaching Hospital, Medical City over the period from Nov. 2006 to Nov. 2008. A total number of 30 patients with diabetic foot ulcer and 21 normal subjects.. The age of patients range from 23-75 years (mean age 52), as shown in table (1) and occupation of the patients, in table (2). Diagnosis of the presence of foot ulcers was made by a specialist physician through physical examination and x-ray examination.

For every case, the following had been done,

1. Patient medical history recorded.
2. Full physical examination, a complete blood picture and renal function test.
3. Lab. Investigations:
  - a. Fasting blood sugar: was measured in serum obtained for all subjects blood by a commercial kit obtained from Biomaghreb, using the enzymatic method<sup>(23)</sup>.
  - b. HbA1C (glycosylated hemoglobin): Method of measurement was followed according to the instructions mentioned in glycohemoglobin HbA1-test kit which obtained from Wiesbaden-Germany using blood specimens and EDTA as anticoagulant<sup>(24)</sup>.
4. Request form was given to all patients which include the details of age, sex, occupation, symptoms, site of ulcers, table (3).
5. All 30 patients with diabetic foot ulcer were put on therapy with oral hypoglycemic drugs for 3months (we measured their fasting blood sugar and HbA1C before and after the therapy). These drugs, metformin [500mg, 2/day] Glibenclamide [5mg, 2/day] and combination of Glibenclamide 5mg (2/day)+ Metformin 500mg (2/day)<sup>(25)</sup>, table (5).
6. The relationship of patients age and (HbA1C and FBS) were determined, table (7).
7. A control groups: The control group was represented by 21 apparently healthy persons and their FBS (fasting blood sugar) and HbA1C were measured, table(4).
8. Data were expressed as mean  $\pm$  standard deviation and differences between means were analyzed by paired student's t-test. P value less than 0.05 were considered significantly different.

## Results

The percentages of diabetic foot ulcer were greater over 70 years old and less under 30 years old, table (1). While table (2), Shows the more effected diabetic patients with foot ulcer were laborers and employees and less were students. The biggest sites of foot ulcer were on the bottom of the big toe and less at the ball of the foot, table (3). Table (4), shows significant difference between FBS & HbA1c for diabetic foot ulcer patients and control group. In table (5), Patients who used metformin or Glibenclamide and the combination of them (Glibenclamide + Metformin) the mean±value HbA1C decreased significantly after treatment  $p < 0.05$ . FBS of patients who used Metformin or Glibenclamide and combination of them (Glibenclamide + Metformine) the mean± value of FBS decreased significantly compared with the mean value before treatment, and table (6), shows patients who used combination of (Glibenclamide+Metformin) have higher percentage (53.84%) of healing than patients treated with Glibenclamide 30.0% or Metformin 28.57%. Table (7), Shows the highest level of HbA1C and FBS over 70 years old patients with foot ulcer and the lowest level of HbA1c and FBS under 30 years old. Fig.(1) Show the significant +ve correlation between age and HbA1c%  $r=0.82$ ,  $p < 0.01$ . Fig. (2): Show that age of the patients is proportional to FBS were  $r=0.8$ ,  $p < 0.01$ . For people without diabetes Mellitus, the normal range for the HbA1c  $\leq 6.2\%$ . (70%) of patients whom have HbA1c level less than (9.79%) did not complain from any complications. And (30%) of patients with high HbA1c (9.79-11.07%) shows that they suffered from some of the following complications:

- a. Retinopathy (impairment of vision, exudation and retinal hemorrhage).
- b. Neuropathy (pain, numbness, and loss of sensation).
- c. Nephropathy (pleural effusions, ascites, subcutaneous oedema in legs, high blood urea levels, high serum creatinine levels and albuminuria).
- d. Gastropathy .

**Table 1 : Age distribution**

Age group	Percentage of diabetic foot ulcer
Less than 30	5.5 %
30-39	8.5 %
40-49	7.5 %
50-59	24.5 %
60-69	21.5 %
70-75	32.5%
Total	100 %

N = 30

**Table 2 : The occupation of the patients**

Occupation	Percent
Farmer	15 %
Housewife	24.5 %
Laborers and employees	32.5 %
Student	3.5 %
Retired	24.5 %
Total	100 %

N = 30

**Table 3 : The site of foot ulcers distribution.**

Site	Number of patients	Percent
Ball of the foot	12	40%
Bottom of the big toe	18	60%
Total	30	100%

N = 30

**Table 4: Fasting blood sugar and glycosylated hemoglobin in control subjects and diabetic foot ulcer. Before starting treatment.**

	Control (n=21)	Diabetic foot ulcer (n=30)
<b>FBS (mg/dL)</b>	83 ± 5.04 a	243.83 ± 7.10 b
<b>HbA1c %</b>	3.41 ± 0.17 a	9.25 ± 0.21 b

Data are expressed as mean ± SD.

n=number of patients.

Non-identical superscripts (a,b) represent significant difference,  $P < 0.001$ .

**Table 5 : HbA1c and FBS levels for patients with diabetic foot ulcer after and before treatment**

	HbA1c %		FBS	
	Before	After	Before	After
<b>Metformin (n=7)</b>	9.06 ± 0.2 a	6.64 ± 0.08 a*	238.85 ± 8.08 a	138.98±5.01 a*
<b>Glibenclamide (n=10)</b>	9.64 ± 0.32 a	6.65 ± 0.06 b*	248.08 ± 7.10 a	134.00±5.54 b*
<b>Metformin + Glibenclamide (n=13)</b>	9.05 ± 0.12 a	6.31 ± 0.07 c*	244.56 ± 6.13 a	124.6 ± 3.94 c*

Data are expressed as mean ± SD.

n=number of patients.

\*P<0.05 with respect to pre-treatment value.

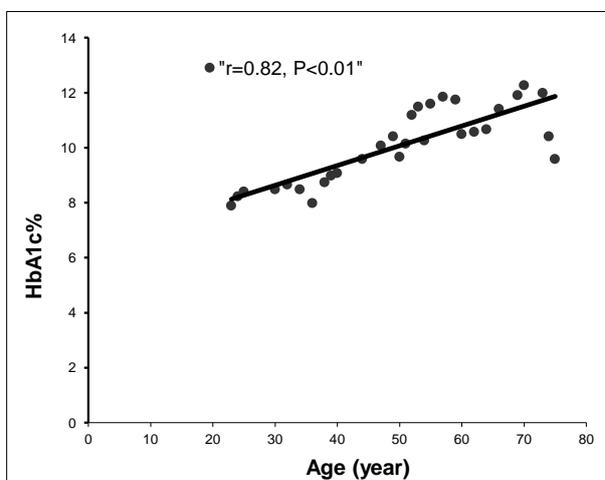
Non-identical superscripts (a,b,c) represent significant difference among groups, P<0.05).

**Table 6 : Percentage of healing after treatment for diabetic foot ulcer patients.**

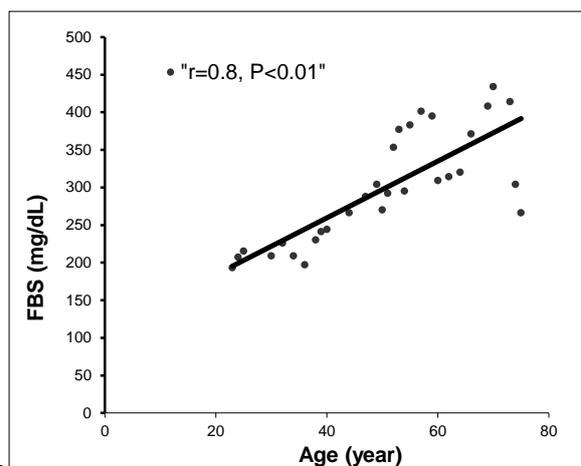
Treatment	Patient No.	Dose	Healing cases	Percentage of healing
<b>Metformin</b>	7	500mg (2/day)	2	28.57%
<b>Glibenclamide</b>	10	5mg (2/day)	3	30.0%
<b>Glibenclamide + Metformin</b>	13	5mg (2/day) + 500mg (2/day)	7	53.84%

**Table 7 : Distribution of mean HbA1c and FBS among the patients with foot ulcer.**

Age group	HbA1c%	FBS (mg/dl)
<30	8.18 ± 0.12	205 ± 5.25
30-39	8.56 ± 0.13	218.66 ± 6.67
40-49	9.79 ± 0.29	275.5 ± 13.07
50-59	10.99 ± 0.29	345.75 ± 18.46
60-69	11.01 ± 0.27	344.4 ± 19.39
>70	11.07 ± 0.64	354.5 ± 41.07



**Figure 1 : The relationship between HbA1c and Age.**



**Figure 2: The relationship between FBS and Age.**

**Discussion and conclusion**

The present study reflecting the recommended tests and examinations, to assess the diabetic care<sup>(26,27)</sup>. Diabetes is most commonly associated with many micro and macrovascular abnormalities. One of these serious complications is the foot ulcer development in patients with poor glucose level control. HbA1c is the a useful indicator of how well the blood glucose level has been controlled in the recent past and may be used to monitor the effects of diet, exercise and drug therapy on blood glucose in diabetic patients ..Most patients in this study (60%) have ulcer in bottom of the big toe, while 40% have ulcer

in ball of foot. This may be due to the low rate of blood circulation in these two sites, table (3)<sup>(16)</sup>. The groups of the patients were treated with either Metformin, Glibenclamide or a combination of (Glibenclamide + Metformin) showed no significant difference among them in respect to HbA1c and FBS before treatment. The mean value of the HbA1c and FBS after treatment in all groups decreased significantly  $P < 0.05$ , table (5). However, combination therapy (Glibenclamide+Metformin) showed high percentage of healing than other patients who were on the Metformin or Glibenclamide alone. So this combination was the best treatment to control the HbA1c thus controlling the glucose level table(6), hence faster healing of foot ulcers. In this study we found that the older patients have higher HbA1c and FBS levels. That means older patients were with less compliance than younger patients, table (7). fig. (1), fig. (2). The higher HbA1c level was detected in ages higher than 70 years old, table (1)<sup>(28)</sup>, indicating bad glycemic control. More percent of the patients were those with occupation as laborers and employees. This may be due to their hard work with defective circulation due to their diabetes, table (2). In July,2009, an international expert committee published a report that made the case for using the hemoglobin A1c assay to diagnose type2 diabetics<sup>(29)</sup>. Moreover as of January 2010, the American Diabetes Association included A1c as an appropriate diagnostic test<sup>(21)</sup>. It has been reported that high HbA1c levels increase the development and progression of eye, kidney and nerve complications in diabetes mellitus poor glucose control also increases the risk of short-term complications of surgery such as poor wound healing<sup>(30-32)</sup>. In our study patients with high HbA1c levels (9.79-11.07%) suffered from eye, nerve or kidney complications, while patients with HbA1c level less than (9.79%) did not suffer from complications. Since HbA1c is not influenced by daily fluctuations in blood glucose concentration. We recommended that people with diabetes should keep their HbA1c level less than (6.63%) by following diet and drug instructions, diabetes out of control could result in complications .Patients with diabetes mellitus should make HbA1c test every three months to determine whether their blood glucose have reached the target level of the control. Patients who have glucose level under good control may be able to wait longer between the blood tests, but it's recommended to be checked at least 2 times a year<sup>(33)</sup>.

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