# Glycosylated hemoglobin and its correlation with both vitamin D and cortisol

*Hemoglobina glicosilada y su correlación con la vitamina D y el cortisol*

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## Abstract

This study was conducted to evaluate the relationship and correlation between the levels of glycosylated hemoglobin, vitamin D, and cortisol. The sample was composed of 40 patients (20 male and 20 female), and the control group was composed of 40 healthy persons (20 male and 20 female). The markers measured in this study were fasting blood sugar (FBS), glycosylated hemoglobin (HbA1C), vitamin D (Vit D), and cortisol (Cort).

Our results show a significant increase ($P<0.05$) in the levels of FBS and Cort, and HbA1C, and a significant decrease ($P<0.05$) in the levels of serum Vit D in patients as compared with healthy persons. Also, this study shows a positive correlation of HbA1C with both FBS and Cort and a negative correlation with Vit D.

**Keywords**: Blood sugar (glucose), Glycosylated hemoglobin, Cortisol, Vitamin D.



## Resumen

Este estudio evaluó la relación y correlación entre la hemoglobina glicosilada, la vitamina D y el cortisol. La muestra estuvo conformada por 40 pacientes (20 hombres y 20 mujeres), y grupo control compuesto por 40 personas sanas (20 hombres y 20 mujeres). Los marcadores medidos en este estudio fueron azúcar en sangre en ayunas (FBS), hemoglobina glicosilada (HbA1C), vitamina D (Vit D) y cortisol (Cort). Se observó un aumento significativo ($P<0,05$) de los niveles de FBS y Cort, y HbA1C, y una disminución significativa ($P<0,05$) de los niveles de Vit D sérica en pacientes en comparación con personas sanas. Además, este estudio muestra una correlación positiva de HbA1C con FBS y Cort y una correlación negativa con Vit D.

**Palabras clave**: Azúcar en sangre (glucosa), Hemoglobina glicosilada, Cortisol, Vitamina D.

## Introduction

The pancreas is a vital endocrine-exocrine organ that produces several hormones and enzymes[1]. Once insulin is released into the blood, it stimulates the entrance of glucose into skeletal muscles and, to a lesser extent, liver, and adipose tissue via special transporters, thus controlling glucose homeostasis[2]. Today, Diabetes mellitus (DM) is a major worldwide health problem leading to markedly increased mortality and serious morbidity[3]. It is a metabolic disorder characterized by elevated levels of glucose in the blood[4] that is associated with long term damage dysfunction and eventually the failure of organs especially the eyes, kidneys, nerves, heart, and blood vessels[5]

Diabetes mellitus (DM) is divided into two types, type 1 and type 2 diabetes. Type 2 diabetes is characterized by insulin resistance and disturbance of pancreatic ß- cells[6].

The need for accurate and precise measurement of blood glucose has made Glycosylated hemoglobin (HbA1c) become the gold standard for monitoring glycemic control in patients with DM from primarily fasting plasma. HbA1c is an index of estimated average blood glucose over the preceding three months, giving an estimate of long-term glycemic status. Its value is used both for the diagnosis and monitoring of the blood glucose control of patients with diabetes mellitus. Glycated hemoglobin levels >7.4% or <5.6% are associated with elevated all-cause mortality in patients with diabetes. In persons without diabetes, a level between 5.0% and 6.5% is optimal[7,8].

Vitamin D is a steroid hormone and the active form of it is 1,25-dihydroxy vitamin $D_3$ (calcitriol). It is synthesized endogenously from the precursor molecule cholesterol when the skin is exposed to ultraviolet rays from sunlight[9]. In addition to the main function of vitamin D in the control of calcium metabolism, it controls gene expression after interaction with its intracellular receptor and also regulates immunity, cell division, and apoptosis[11,12]. In addition, an association between vitamin D levels and glycemic control has been reported in several studies, but the association is not consistent. There is evidence suggesting that vitamin D has a direct effect on improved insulin secretion and insulin action, and subsequently, improved glucose homeostasis through activation of the vitamin D receptor on the β-cell of the pancreas islet of Langerhans[13].

Cortisol is a glucocorticoid (corticosteroid) hormone that is produced by the suprarenal gland cortex from the precursor molecule cholesterol. Cortisol is one of many hormones responsible for changes in body physiology, due to its aid in the control of stress response, blood glucose levels, inflammatory responses, and blood pressure[14-16].

**The aim of the study**

This study was conducted to detect the correlation between glycosylated hemoglobin with vitamin D and also with cortisol hormone.

**Subjects and Methods**

This study included 80 persons [40 patients with diabetes (20 male and 20 female), and 40 healthy people (20 male and 20 female)]. This study was conducted from April 2019 to March 2020 in Tikrit city (general hospital of Tikrit and central laboratory of Tikrit university).

The instruments used in this study were a spectrophotometer for the determination of fasting blood glucose and AFIAS6 (AFIAS-automated fluorescent immunoassay system) for the determination of glycosylated hemoglobin, vitamin D, and cortisol.

**Biochemical Analysis**

**1- Estimation of Fasting Blood Glucose**

Evaluation of serum glucose levels was conducted by enzymatic colorimetric method using a Biolabo kit (Maizy, France). The determination of glucose is based on the enzymatic oxidation by glucose oxidase. The reaction of hydrogen peroxide with phenol and 4-aminophenazone which is catalyzed by peroxidase yields a quinone imine dye (red-violet) as the indicator.

**Determination of glycosylated hemoglobin**

A1c-glycosylated in the human blood sample was determined using sandwich immunoassays, used to detect antibodies in the buffer which bind to the antigen in the sample to form antigen-antibody complexes, which are then moved through nitrocellulose matrix to captured by other immobilized-antibody on the test strip. The antigen-antibody complex is formed according to the quantity of antigens present in the sample, which is proportional to the intensity of fluorescence signal to detection antibody, which reflects the content of glycated hemoglobin in the blood sample.

**Determination of vitamin D**

The competitive immunodetection method was used to estimate vitamin D. The target material in this sample binds to the fluorescence (FL)-labeled antibody in the detection buffer, and this leads to the formation of a complex mixture. This complex moves through the nitrocellulose matrix where the covalent linkage of 25(OH)D3 and bovine serum albumin (BSA) is immobilized on a test strip, which interferes with the linking of target material and FL-labeled antibody. The target material that exists in blood inversely correlates with antibody accumulation, resulting in less fluorescence signal.

**Determination of cortisol**

Cortisol was determined by the use of the competitive immunodetection method. The complex mixture is formed by the binding of target material in the sample with fluorescence (FL)-labeled antibody in detection buffer. This complex migrates through the nitrocellulose matrix where the covalent linkage of cortisol and bovine serum albumin (BSA) is immobilized on a test strip and interferes with the linking of target material and FL-labeled antibody. Target material and detection antibodies were inversely correlated which resulted in a less fluorescence signal.

**Biostatistical analysis**

Results were expressed as mean ± SD. The student's t-test and bivariate correlation [Pearson correlation coefficient (r)] were used for the analysis of the results for both patients with control groups. A significant variation was established when the P-value was less than 0.05.

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## Results

**1-** The relation of FBS, HbA1C, Vitamin D, and Cortisol between diabetic patients and healthy subjects.

There was a significant ($P<0.05$) increase in serum levels of FBS, cortisol, and blood HbA1C, while serum vitamin D was significantly ($P<0.05$) decreased in patients with diabetes mellitus when compared with healthy persons. There were no significant changes in age between these two groups.

**2-** The relation of FBS, HbA1C, Vitamin D, and Cortisol between male and female diabetic patients.

Serum FBS, cortisol, and blood Hba1C levels showed a tendency to increase however it was not statistically significant, while serum vitamin D was significantly ($P<0.05$) higher in male patients as compared to females.

**3-** Correlation of vitamin D and cortisol with both FBS and HbA1C.

There was a significantly negative correlation between vitamin D with both FBS and HbA1C, and there was a non-significant positive correlation between cortisol with both FBS and HbA1C.

**Table 1. Age, FBS, HbA1C, Vit D, and Cort in diabetic patients and healthy persons.**

| | Age (years) ±S.D | FBS (mg/dL) ±S.D | HbA1C (%) ±S.D | Vit.D (ng/mL) ±S.D | Cort (mmo/L) ±S.D |
|---|---|---|---|---|---|
| Patients with diabetes mellitus | 54.70 ±5.32 | 234.15 ±38.40 | 9.32 ±0.98 | 13.45 ±4.09 | 206.90 ±73.47 |
| Normal subjects | 54.83 ±6.10 | 98.43 ±8.52 | 5.29 ±0.42 | 37.76 ±15.39 | 146.15 ±43.78 |
| $P_{value}$ | 0.867 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

**Table 2. Age, FBS, HbA1C, Vit D, and Cort in male and female diabetic patients.**

| | Age (years) ±S.D | FBS (mg/dl) ±S.D | HbA1C (%) ±S.D | Vit.D (ng/ml) ±S.D | Cort (mmol/L) ±S.D |
|---|---|---|---|---|---|
| Male Patients | 54.23 ±5.04 | 235.73 ±40.04 | 9.32 ±1.05 | 15.4 ±3.83 | 215.64 ±76.98 |
| Female Patients | 55.28 ±5.74 | 232.22 ±37.35 | 9.32 ±0.91 | 11.06 ±3.03 | 196.22 ±69.58 |
| $P_{value}$ | 0.855 | 0.704 | 0.970 | 0.003 | 0.109 |

**Table 3. Correlation of FBS and HbA1C with vitamin D and cortisol.**

| Patient type | Markers | Vitamin D | | Cortisol | |
|---|---|---|---|---|---|
| | | r | $P_{value}$ | r | $P_{value}$ |
| Diabetic Patients | FBS | -0.539 | 0.0001 | 0.267 | 0.096 |
| | HbA1C | -0.626 | 0.0001 | 0.269 | 0.093 |



**Figure 1. The association between blood HbA1C and serum vitamin D in patients with diabetes mellitus.**

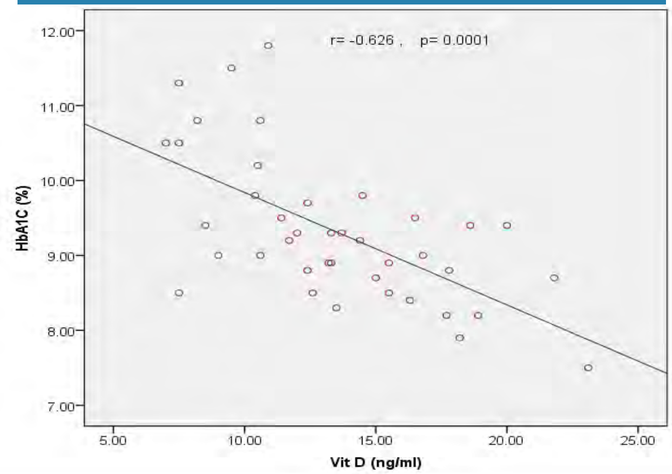
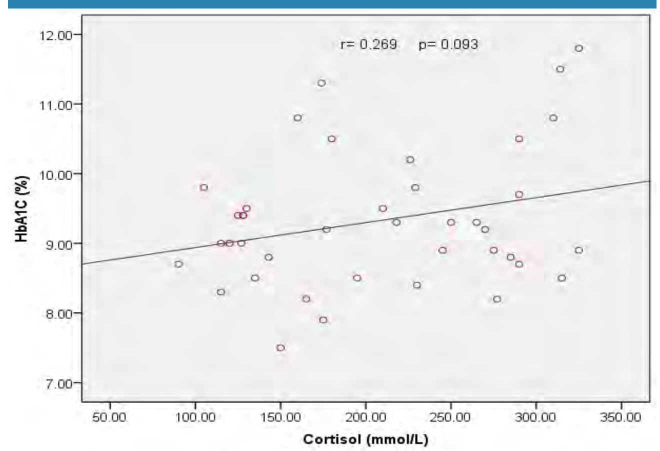



**Figure 2. The association between blood HbA1C and serum cortisol in patients with diabetes mellitus.**




## Discussion

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[17,18]. Glycosylated hemoglobin is increased over a long period in diabetes[19-20].

The results demonstrate a significant increase in levels of serum FBS, cortisol, and blood HbA1C and a significant decrease in vitamin D in diabetic patients as compared with healthy persons. Kamba[6] mentioned that the increase of serum cortisol is significantly associated with decreased insulin secretion. Also, Garcia-Bailo[21] and Chiodini[22] noted that increased levels of cortisol secretion are associated with diabetes complications, and this may give the cortisol the focal point for researching diabetes complications.

Angellotti[10] and Mohanad[23] showed that the decreased levels of vitamin D correlated to diabetes mellitus and increased levels of HbA1C, therefore; supplementation with vitamin D is necessary for increasing immunity and increasing insulin secretion and sensitivity. Serum FBS, cortisol, vitamin D, and blood HbA1C levels were increased in diabetic males in relation to diabetic females, however; Manal et al.[24], show an increase in FBS and HbA1c and a decrease in vitamin D in male diabetic patients as compared with female diabetics. Aya et al.[25] also mentioned that cortisol was higher in a significant value in diabetic males as compared with females.

**Recommendations**

1- Perform a periodic measurement of HbA1c for control of diabetes.

2- In the case of diabetes, especially if the presence of decreased immunity, is recommended to improve diet or vitamin D supplementation with added exposure to sunlight at the optimal sun exposure time.

3- Cortisol should be measured at some times, and if there is a problem give the suitable treatment.

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