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Original article

Glycated hemoglobin assay in a Tlemcen population: Retrospective study

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ABSTRACT

The understanding of the relationship between the standard values of glycated hemoglobin (HbA1c) and related parameters of the subject (age, sex, BMI, and complications etc . . .) could be a good track for following and the screening of diabetes. In this side, we recruited ten non diabetic subjects as witnesses and ninety diabetic type 2. Diabetic subjects were assayed for their blood glucose and glycated hemoglobin levels and a calculation of their body mass index. Our results showed that the diabete is more common in women than men. The most affected age group by diabetes is between 45 and 55 years for women (24.44%), while among men it over 65 years (20%). Obesity affects 31.11% of women and 889% of men in the studied population. We also found an increase in HbA1c values with age in both sexes. The correlation study between the values of HbA1c and blood glucose in diabetic patients shows the existence of a moderate positive correlation (r = 0.68). Finally we found that diabetes related complications are more common in females than males. Knowledge of the range of reference corresponding patients to better interpret diabetes is important for clinicians. The correlation HbA1c / blood glucose level allow better control of glycemic control.

1. Introduction

Clinical entity and multiple etiologies aspects, diabetes is defined as a state of persistent hyperglycemia in normal supply conditions and in the absence of intercurrent disease or drug intake may induce a transient hyperglycemia. The frequency of the disease and the severity of its complications are in it a major public health problem, especially as he knows, in all parts of the globe, an alarming increase in its prevalence [1]. It is widespread throughout the world where there are 5–7% of the world population [2–5].

According to WHO, the incidence of diabetes is about 12% among the population of the Maghreb. In Algeria, the prevalence of diabetes is approximate of 4 million people suffering from this disease. Experts differ on the quantification of diabetes, the fourth cause of death for us. The national study conducted multiple indications by the Ministry of Health, Population and Hospital Reform, in collaboration with the National Statistics Office and UN representations in Algiers pathology class of diabetes in second, after arterial hypertension. According to these data, the number of people with diabetes is increasing. It jump to 0.3% in those aged under 35 years to 4.1% among 35–59 years and 12% for over 60 years [6]. In Tlemcen, diabetic prevalence at the University Hospital of Tlemcen is around 570 during two years 2012 and 2013 [Epidemiology and Preventive Medicine register, University Hospital of Tlemcen].

All recommendations are reporting the interest of HbA1c for monitoring of glucose control of diabetics [7,8]. This setting is indeed very convenient since roughly reflects the average blood glucose levels of the last three months. Its standardization by validated techniques allows, at least in the hexagon, to have a reliable indicator that imposes few constraints for patients since it is not necessary to fast to get to laboratory.

No one therefore challenges the best interests of HbA1c in monitoring glycemic control many of these patients, but everyone recognizes her limitations. Overall, various official recommendations put HbA1c targets of 6.5–7%. However, a lesser requirement is required in case of old and already complicated diabetes, particularly in the elderly [8].

The aim of our work is to see the distribution of diabetes according to gender, age, weight and complications, then search for a possible correlation between the values of HbA1c and those rates of glucose fasting in diabetic patients.

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2. Materials and methods

2.1. Sampling and data collection

The study included 150 subjects of the Tlemcen region, 10 non-diabetic subjects (controls) and 90 diabetics. The investigations were made during the period of February-March 2016. For each subject were noted his identification, age, weight, height, type of diabetes, hypertension, and possible complications. The subjects were the subject of their glucose level and HbA1c assay.

It was excluded from our sample persons:

- Not having the HbA1c assay results.
- Lacking the results of blood sugar.
- Incomplete files.
- The results of which were not complying with quality control.

Control subjects are identified as such if their blood glucose level (normal) is less than 1.26 g/ l and HbA1c less than 6%, have reference values in non-diabetics compared with diabetics.

2.2. Study protocols

The venous blood sample was collected into tubes with EDTA and fluoride-heparin as anticoagulants. EDTA has been used for HbA1c and heparin was used to fasting blood sugar.

2.3. Blood sugar assay

The blood glucose assay was performed with a semi automatic spectrophotometer apparatus biosystem BTS-310″using the Bio-maghreb reagents. The procedure was as fellow:

- Centrifuge whole blood at 2000 rpm / min for 10 min.
- Pipette 10 μl of plasma in a test tube and add 1000 μl of the working solution.
- Pipette 10 μl of standard (standard), and add 1000 μl working solution.
- The preparation of white was conducted with 1000 μl of the working solution. This tube is used to adjust the spectrophotometer.
- Adjust the spectrophotometer against the reagent blank.
- Read the absorbance.

2.4. HbA1c

The assay was performed using chromatography cation exchange resin. The formation of HbA1c in erythrocytes is irreversible and progressive manner throughout their normal service life (120 days). Being stable throughout the life of the erythrocyte, the concentration of HbA1c reflects the average glucose levels in the blood to 4–6 weeks prior to dosing. The procedure was as fellow:

The blood was mixed with a lysing agent containing a detergent and a high concentration of borate ions. The elimination of the labile Schiff base has been completed and during hemolysis. The hemolyzed blood was mixed for 5 min, with low cation exchange resin. During this time, the HbA0 is connected to the resin. After mixing all, a special separator was used to remove the resin from the supernatant which contains HbA1c. The proportion of HbA1c is given in percentage of total Hb in the sample and this by assaying the HbA1c fraction and total Hb at 415 nm in comparison with the standard of the HbA1c assay obtained during the reaction.

Step 1: Preparation of the lysis

Pipette 100 μl in CUP (or clotted) labeled for each sample (whole blood), standard (STD), normal Hb control (GCN) or control pathogenic Hb (GCA) and add 0.5 ml lysis (mix well prior to use) in each tube following mix and incubate at 15–25 °C for 5 min.

Step 2: Determination of HbA1c:

Pipette 100 μl of the hemolysate from Step 1 in labeled GTA (micro-column contains 2.5 ml of resin).

Insert SEP (empty micro-column) so that the rubber is about 1 cm above the resin level of suspension, shake for 5 min. push SEP to down until the resin is firmly packed. Pour the supernatant into a tank.

Read the absorbance at 415 nm of HbA1c STD / sample / control.

Step 3: Determination of total Hb:

Pipette 20 μl of the hemolysate from Step 1 in labeled tubes.

Add 5 ml of distilled water and mix thoroughly.

Read absorbance A total Hb STD time / sample / control.

- Determination of F factor :

$$F = \frac{A_{\text{Hb total STD}} \times \%\,\text{HbA1}_{\text{STD}}}{A_{\text{HbA1 STD}}}$$

- Determination of HbA1 in samples:

$$\%\,\text{HbA1}_{\text{sam}} = F \times \frac{A_{\text{HbA1 sam.}}}{A_{\text{Hb total sam}}}$$

2.5. Statistical analysis

Statistical analysis of the data (calculation of averages, standard deviation and correlation) was performed with the computer software Microsoft Excel 2007 version.

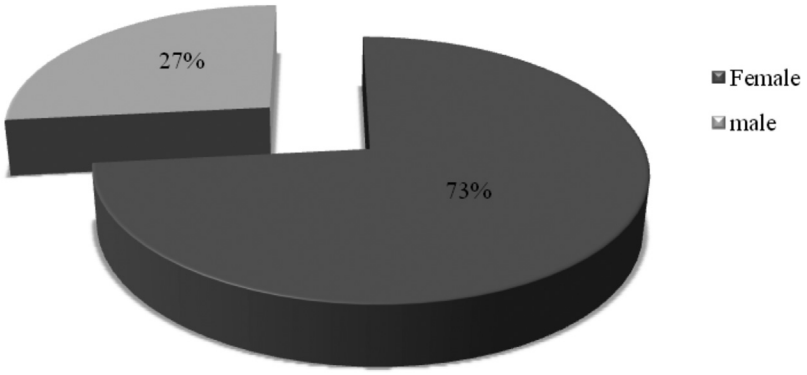

Fig. 1. Distribution of diabetic subjects by gender.

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