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Evaluating Homocysteine, By HPLC, HBA1C And Fasting Blood Glucose Levels In Patients With Type 2 Diabetes Mellitus

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ABSTRACT

Background: The objective of the study was to evaluate the levels of Homocysteine, HBA1C and fasting plasma glucose in serum of type 2 diabetic patients as a parameter and correlation coefficient between Homocysteine and each of HBA1Cand fasting blood glucose (FPG).
Materials and Methods: The present study was applied on 30of type 2 diabetic patients and 30 healthy controls of same age and gender. Fasting blood samples were collected from each patient and control and assessed by measurement of serum Homocysteine (HPLC assay), FPG (Glucose Oxidation Peroxidase method) and HbA1c (Immunoturbidimetric method) levels.
Result and discussion: : In this project, the mean value of serum Homocysteine in diabetic patients was 26.5 ± 1.73 µmol/L. It was increased significantly compared to controls (0.98 ± 0.11 µmol/L, p=0.000). The mean value of FBG in diabetic patients was 230.3 ± 6.73 µmol/L. Also, it was increased significantly compared to controls (89.5 ± 4.7 mg/dL, p=0.000). Similarly, the mean value of HbA1c in diabetic patients was 11.5 ± 0.99 µmol/L. It was increased significantly compared to controls (5.1 ± 0.13 mg/dL, p=0.000). Our findings demonstrated significant positive correlation between serum Homocysteine and fasting blood glucose levels (r=0.811,p=0.001), and also between serum Homocysteine and HbA1c levels (r=0.324, p=0.02).

Keywords: Homocysteine, HbA1C, FPG, Diabetes Mellitus, HPLC

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is turning in to most prevalent worldwide defince to the health of people. Globally, the incidence of T2DM elevated from 0.7 % to 9.7 % between 1980–2010 [1]. The main reasons of rapid elevated statistics of T2DM patients are dramatic urbanization, aging, chaning of life style, and over weighting. In rural world, shortage in awareness, treatment and control has accelerated the incidence of T2DM [2].

Diabetes mellitus (DM) is a common endocrine disorder manifiested by hyperglycemia. It is a serious worldwide health care problem. The main metabolic abnormalities of DM are diabetic ketoacidosis and non-ketotic hyperosmolar coma which lead to several pathological impacts on the vision, renal system, nervous system, circulation and gastrointestinal tract [3].

Increased level of serum Homocysteine is involved in generating Homocysteine disulfides and Homocysteine thiolactone, which in turn lead to endothelial cell injury (sulfation of collagen) and aggravating the development of thrombosis and arteriosclerosis . Plasma homocysteine levels are increased either in type 2 diabetic patients and pre-diabetic subjects with insulin resistance. In this condition, plasma Homocysteine levels is influenced by the insulin concentrations and anti-diabetic therapies including; metformin, glitazones or insulin . these therapies can increase or lower the plasma Homocysteine levels . Insulin resistant, hyperinsulinemic, and T2DM patients with healthy pancreatic β-cell function are tested with Hyperhomocysteinemia [4].

Patients with damaged pancreatic β-cells might have a reduction in plasma Homocysteine concentrations. Non-diabetic persons who have insulin-resistance syndrome showed higher plasma Homocysteine levels which confirms the link between elevated plasma Homocysteine and insulin levels [5].

Evaluating the level of glycated hemoglobin (HbA1c) is a widely approperiated method for evaluating glycemic control [1]. Poor glycemic control is associated with greater possibility of chronic outcomes including micro-vascular pathologies (retinopathy, nephropathy, and neuropathy) and cardiovascular diseases (coronary artery disease, peripheral arterial disease, and stroke) . HbA1c levels can be considered by physicians to evaluate glycemic control of diabetic patients and making changes in therapy. Precision in evaluating and interpreting results of HbA1c reflect the importance of this marker [6].

Several risk factors for DR have been identified, including diabetic nephropathy[7], arterial hypertension[8], and dyslipidemia The most frequent consequences of DR that have been reported due to long-term hyperglycemic exposure were diabetic neuropathy (7), hypertension (8) and dyslipidemia . Optimizing glycemic control according to the American Diabetes Association at normal ranges is necessary to reduce the harmful effects and development of DR. On other hand, the velocity of decrease and magnitude of HbA1c in accordance with uncontrolled T2DM are crucial for diabetic patients. In addition, registeration of HbA1c values for at least 3 previuos years should be documented in order to assess the statement patients. If the reduction rate between two consecutive HbA1c values is more than -3%, the patients will classified as rapid declinors [10].

Hemoglobin A1c (HbA1c) is a marker reflecting the average of blood glucose levels over a interval of 2 to 3 months [11]. HbA1c was discovered by late 1960s, it was

employed to clinical practice in the 1970s-1980s and globally approved in the 2000s .. At present, we have a new challenge,overuse of diagnostic possibilities such as analytical methods, number of tests and processing time spending for blood speciements collection to a final laboratory report . However, there has been a shortage of evidence to judge directly referring to laboratory overuse. On other hand, up to 70% of high-throughput laboratory tests are possibly useless with ambiguous importance [12].

The hyperglycemia could impair neurological function . It is also closely correlated with cerebrovascular diseases . However, there are few studies on hyperglycemia and CVT, Zuurbier et al., showed that increased random blood glucose (RBS) levels which were significantly associated with poor outcome and mortality of CVT . Previously, a lot of studies demonstrated that FBG could be a more accurate marker of blood glucose status than RBS [13]. Early identifying of CVT status could be improved the prognosis of CVT patients and life-saving for them [14].

Atrial fibrillation (AF) is a main risk factor of cardiovascular disease, ischemic stroke, and mortality . Impaired fasting glucose (IFG) and T2DM provoked AF development . Duration of T2DM-induced microvascular consequences correlated with the incidence and risk of AF. Therefore, the time table of AF development should be monitored in T2DM patients [15].

MATERIAL AND METHODS

Sample collection & preparation:

Blood specimens were collected from patients hospitalized in Marjan hospital, Babylon province, Iraq. The sera were separated by adding 10% trichloroacetic acid (TCA) in order to precipitate plasma proteins. Centrifugation at 10000 rpm/5 min. was applied. The supernatant was removed.By using syringe filter with a 0.45 µm pore size, filtration was done.The reagents used obtained at high purity grade. Mixtures were prepared by adding 250 µl of sample and 500 µl of methanol). After mixing, incubation was performed at laboratory temperature for 5 minutes. Then, centrifugation at 5000 rpm/5 min. was done, 250 µl of the supernatant was collected and mixed well with 100 µl of borate buffer. 50 µl of OPA solution was added to the final solution and eventually, incubation was carried out for 2 min. at room temperature [16].

HPLC assay:

The HPLC tests were done in the Ministry of Science and Technology, Department of Environment and Water Laboratories, Baghdad province, Iraq. Using The High-Performance Liquid Chromatography Technique (HPLC), model SYKAMN German, the method provided by (....) Mobile phase Consists of acetonitrile: buffer: DW (60:10:30), at a Flow rate (1 ml/min) and Column separation was C18-NH2 (25 cm* 4.6 mm) The Detector= Florescence, Ex=365 nm, Em= 445 nm [16].

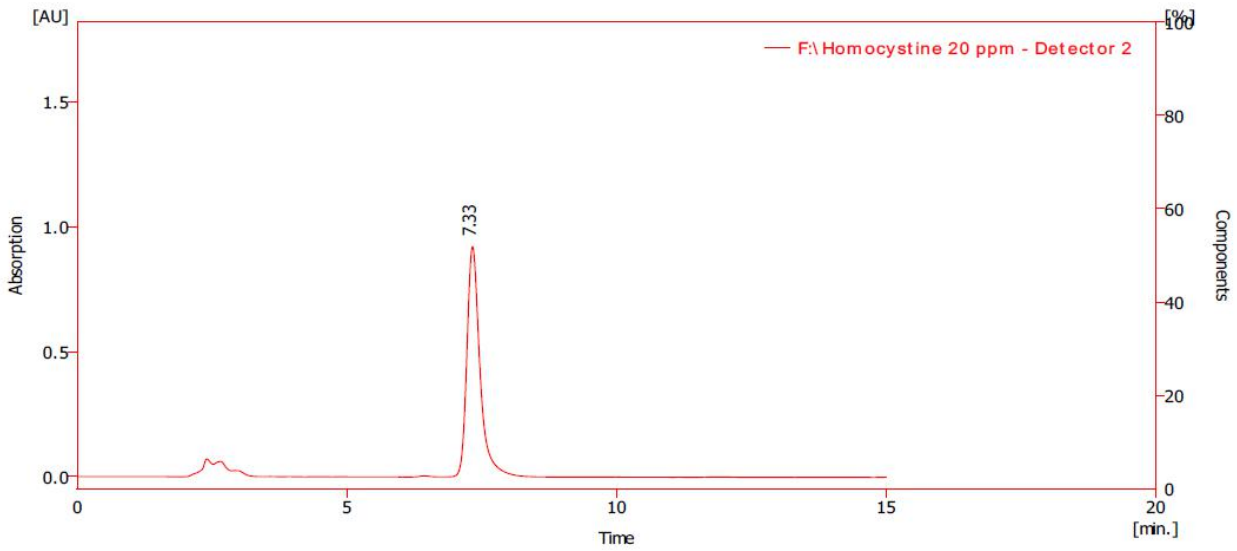

Figure 1: standard curve of Homocysteine in HPL

THE RESULTS

Table 1: Results of all parameters and comparison between groups.

Parameters	Diabetic Mean ± SD	Non-Diabetic Mean ± SD	p - value
Homocysteine (µmol/L)	26.5 ±1.73	0.98±0.11	0.000
HbA1c (%)	11.5 ±0.99	5.1±0.13	0.000
FPG (mg/dl)	230.3 ±6.73	89.5±4.7	0.000

Table 2: Correlation of Homocysteine with FBG and HbA1c

Correlation	r-value	p-value
Homocysteine and FPG	0.811	0.001
Homocysteine and HbA1c	0.324	0.02

Homocysteine levels:
The mean value of serum Homocysteine in diabetic patients was 26.5±1.73 µmol/L It was significantly higher compared to the controls (0.98±0.11 µmol/L,p=0.000), all of Homocysteine results got by HPLC and it were shown in below chart.

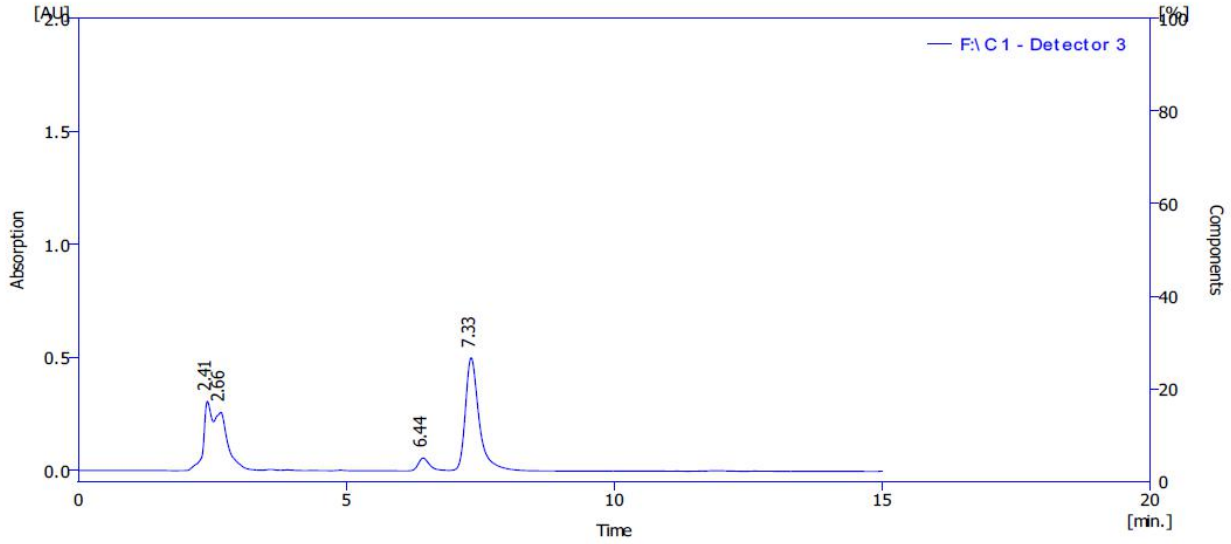

Figure 2: the concentration of Homocysteine for control by HPLC

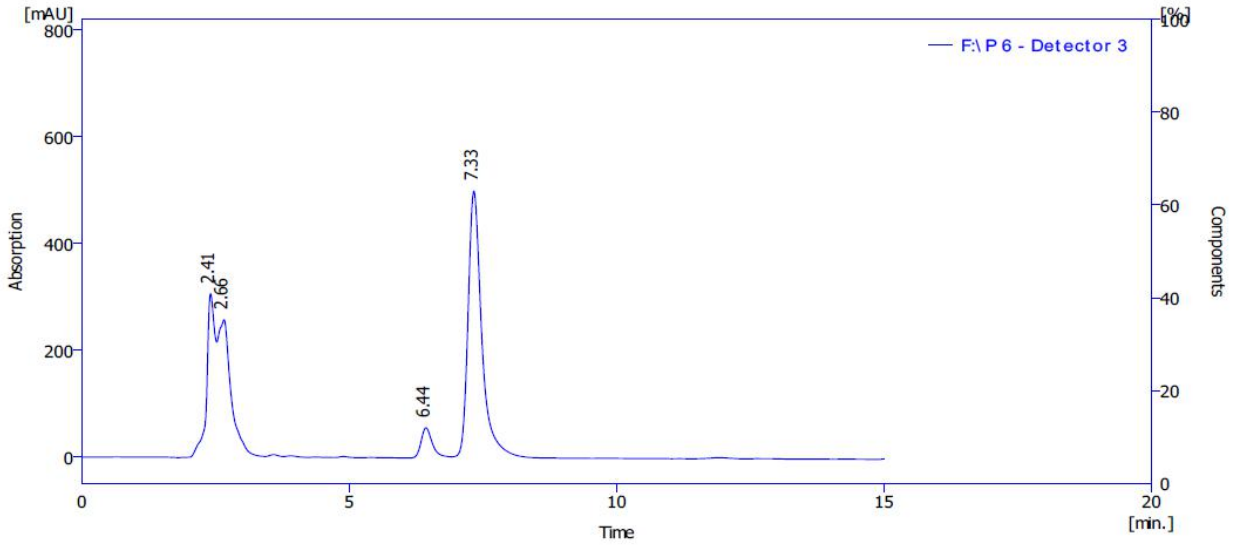

Figure 3: the concentration of Homocysteine for control by HPLC

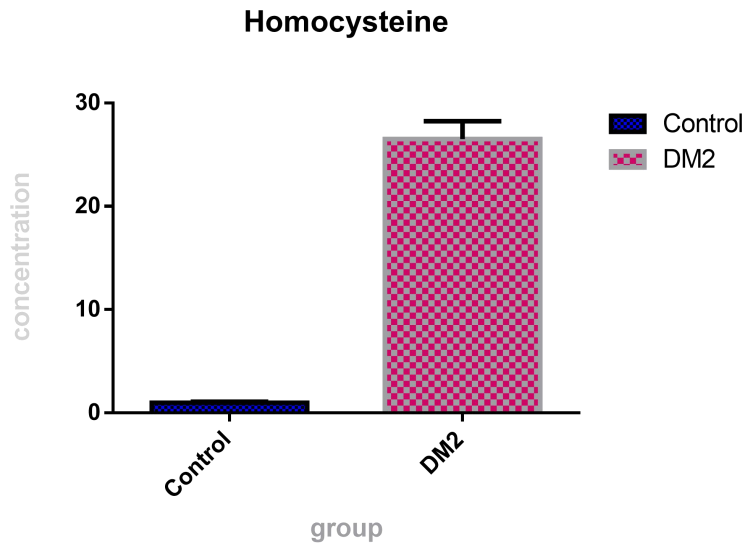

Figure 4: Comparison of Homocysteine level between study groups

HbA1c levels:
The mean value of HbA1C diabetic patients was 11.5 ±0.99 µmol/L. It was significantly higher compared to controls (5.1±0.13 mg/dL, p=0.000). Also, significant positive correlation was seen between serum Homocysteine levels and FBG (r=0.811, =0.001).

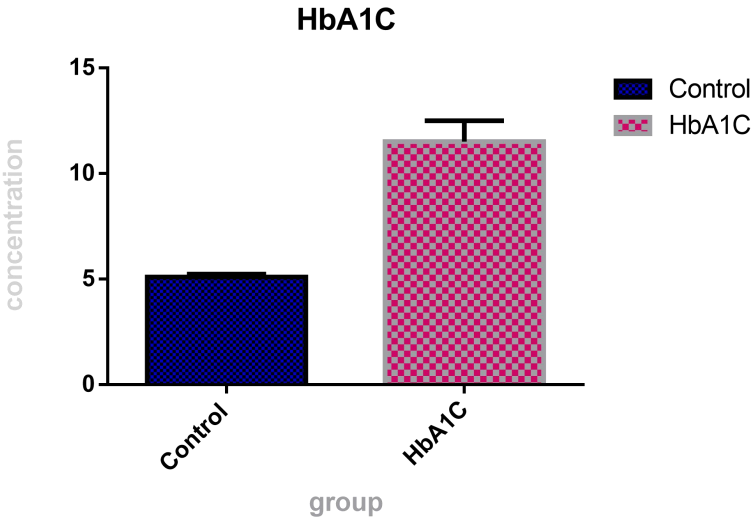

Figure 5: Comparison of HbA1C level between study groups

FPG levels:
The mean value of serum FPG in diabetic patients was 230.3±6.73 µmol/L. It was significantly higher comared to controls (89.5±4.7 mg/dL, p=0.000). Also, significant positive correlation was seen between serum Homocysteine levels and FBG (r=0.324, p=0.02).

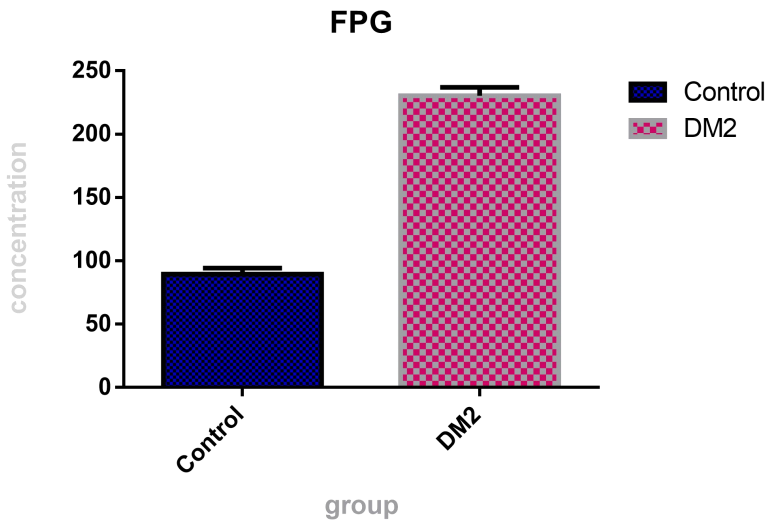

Figure 6: Comparison of FPG level between study groups

DISCUSSION

Diabetes Mellitus is provoked as a result of series of metabolic disorders that follow a similar hyperglycemic effect. There are many distinct forms of diabetes mellitus with distinct etiological variables, such as variations in genetic , environmental and life styles. Different factors can affect hyperglycemia, depending on the etiology, through different pathways featured with low insulin secretion, impaired use of glucose and increased levels of blood glucose . Ssecondary pathophysiological consequences could affect multi-organ systems and lead to a heavy burden on diabetic patients and the health care organizations.

Atherosclerosis is a chronic inflammatory condition induced by Diabetes mellitus disease. Accordingly, serum Homocysteine can be used as a predictor for homocysteine CAD-leading atherogenesis . Our findings showed that increased serum Homocysteine levels (26.5 ±1.73) in T2DM patients. Otherwise, its' levels were 0.98 ± 0.11 µmol/L in non-diabetic individuals.

Pervious studies have shown altered serum concentrations of Homocysteine in patients with T2DM . Insulin resistance and hyperinsulinemia can induce hyperhomocysteinemia in T2DM patients with healthy pancreatic β-cell function . But in situation of dysfunctioned pancreatic β-cells, plasma Homocysteine levels could decrease . In non-diabetic(people with insulin resistance syndrome), Higher plasma Homocysteine levels also were seen. This indicates the relation between high plasma Homocysteine concentrations and increased plasma insulin concentrations .

When comparing serum Homocysteine levels with HbA1c and FPG (diabetes metabolic control), our results were substantial (P=0.05) suggesting that serum Homocysteine levels significantly increase and highly significant associated (p=0.001) with FPG and HbA1c among diabetic patients. Whereas, less significant association (p=0.02) was found in non-diabetic individuals. We also found a positive higher significant correlation either between serum Homocysteine and FPG (r=0.811) or serum Homocysteine and HbA1c (r=0.324).

Similar to that,

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