Association of Serum Homocysteine with Controlled and Uncontrolled Type2 Diabetes Mellitus in Sulaimani City

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Article Info:

Received Sep 2021
Accepted May 2022
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DOI:

Abstract:

Background: Diabetes mellitus is a serious metabolic disorder of multiple etiologies manifested by chronic hyperglycemia. The type 2 diabetes mellitus is characterized as multifactorial genetic syndrome, induced by mutations of different genes and environmental factors. Circulating homocysteine, a non- essential amino acid containing sulfur, is a broad biochemical marker for health/disease status diagnostics. The aim of our study is to evaluate circulating serum homocysteine levels in type 2 diabetes mellitus patients.
Method: A cohort of 197 individuals randomly identified for this study. Of those, 148 individuals were diagnosed by consultants as type 2 diabetes mellitus and the rest 49 volunteer were normal controls group. In clinical chemistry laboratory, serum samples were analyzed for serum homocysteine, fasting blood sugar, glycated hemoglobin, serum creatinine, blood urea, blood urea nitrogen and lipid profile.
Results: There was a significant difference in the HbA1c and sugar level between type 2 diabetes mellitus patients and control group. There was non-significant (P-value=0.32), the serum level of homocysteine was (13.6 ± 4.8, 12.5 ± 5.0 and 12.7 ± 6.2) in T2DM glycemic (controlled, poorly controlled and un controlled), respectively and (11.4 ± 4.8) in control group.
Conclusions: Serum level of homocysteine was not differed significantly in type 2 diabetes mellitus when compared with control group. According to ages of T2DM and control group, there was no significant difference in serum level of homocysteine. There was significant difference between male and female in the level of serum homocysteine in glycemic uncontrolled group.

Key words: glycemic status, homocysteine, T2DM.

أرتباط مستوى الهوموسيستين في مصل الدم لمرضى السكري النوع الثاني المسيطر والغير مسيطر في مدينة السليمانية

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الخلاصة:

الخلفية: داء السكري هو اضطراب في التمثيل الغذائي، يسبب الارتفاع المزمن لسكر في الدم. النوع الثاني من داء السكري هو متلازمة وراثية شائعة متعددة العوامل، و تحددها العديد من الجينات والعوامل البيئية المختلفة. الهوموسيستين هو حامض أميني غير اساسي يحتوي على الكبريت، وينتج من خلال نزع مجموعة المثيل من الحامض الاميني (الميثيونين) بعد المرور بعدة مراحل.

الهدف من الدراسة: الهدف من هذه الدراسة هو قياس مستوى الهوموسيستين في الدم لدى المرضى المصابين بداء السكري من النوع الثاني.

طريقة العمل: تمت تجميع نماذج هذه الدراسة من مركز السليمانية لمرض السكري وأمراض الغدد الصماء للفترة ما بين شهر حزيران الى شهر تشرين الثاني 2019. يبلغ عدد المشاركين في الدراسة (197) شخص تم انتقائهم بشكل عشوائي، من بين هؤلاء تم تشخيص (148) حالة من قبل استشاريين كمصابين بمرض السكري من النوع الثاني و(49) حالة طبيعية تمثل مجموعة السيطرة. تم تحليل مصل الدم واجراء الفحوصات التالية: الهوموسيستتين، سكر الدم (الصيام) ، السكر التراكمي (HbA1c)، الكرياتينين، يوريا، نيتروجين اليوريا في الدم (BUN) و مستوى الدهون والذي يتضمن: الكولسترول، الدهون الثلاثية، البروتين الدهني عالى الكثافة، البروتين الدهني منخفض الكثافة و البروتين الدهني منخفض الكثافة العالي.

النتائج:اظهرت النتائج أن: مرض السكري من النوع الثاني اكثر شيوعاً في الاعمار الاكثر من 50 عام ونسبة النساء اكثر من الرجال. هناك اختلاف معنوي كبير في مستوى السكر في الدم والسكر التراكمي لمرضى السكري النوع الثاني مقارنتاً بمجموعة السيطرة. لم يكن هناك فرق معنوي في: الجنس، كتلة الجسم، التدخين وضغط الدم بين مرضى السكري النوع الثاني ومجموعة السيطرة. مستوى الهوموسيستين في مصل مرضى السكري النوع الثاني (التحكم الجيد، التحكم الضعيف والتحكم السيء) كان (13.6 ± 4.8 ، 12.5 ± 5.0 و 12.7 ± 6.2) على التوالي وبينما كان مستوى الهوموسيستتين في مصل مجموعة السيطرة (11.4 ± 4.8) ، ولم يكن هناك فرق معنوي في مستوى الهوموسيستين(P-value= 0.32) . علاوة على ذلك، لم يكن هناك فرق معنوي في مستوى الهوموسيستين مع اختلاف الاعمار والجنس لمرضى السكري النوع الثاني مقارنتاً بمجموعة السيطرة الا في مجموعة التحكم الضعيف لمرضى السكري النوع الثاني حيث كان هناك اختلاف كبير في مستوى الهوموسيستين في الاناث و الذكور مقارنتاً بمجموعة السيطرة. لم يكن هناك علاقة بين مستوى الهوموسستين مع العمر، الجنس، ضغط الدم، عمر المرض، التدخين، سكر الدم، مستوى الدهون، يوريا الدم ، نيتروجين اليوريا في الدم (BUN)و الكرياتنيين لمرضى السكر النوع الثاني.

الاستنتاجات: مستوى الهوموسستين كان ثابتا ولا يوجد فرق كبير في مستوى الهوموسيستين في مصل مرض السكري النوع الثاني مقارنة مع مجموعة السيطرة. لم يكن هناك فرق معنوي في مستوى هوموسيستين في الفئات العمرية المختلفة لمرضى السكري النوع الثاني. ولم يكن هناك فرق معنوي بين مستوى الهوموسيستين في مصل الدم والجنس في مرضى السكري النوع الثاني (التحكم الجيد، التحكم الضعيف)، بينما كان هناك فرق معنوي كبير بين مستوى الهوموسيستين والجنس في مجموعة التحكم السيء لمرضى.

لم يكن هناك عامل ارتباط بين مستوى الهوموسيستين و: العمر، الجنس، ضغط الدم، عمر المرض، التدخين، السكري النوع الثاني والكرياتنين لمرضى السكر النوع الثاني(BUN) سكر الدم,مستوى الدهون،يوريا الدم،نيتروجين اليوريا في الدم.

الكلمات المفتاحية: الحالة السكرية في الدم، الحامض الاميني هوموسيستين، داء السكري النوع الثاني .

Introduction

Diabetes mellitus is a metabolic disorder of multiple etiologies displayed by chronic hyperglycemia with disorder of carbohydrate, lipid and protein homeostasis resulting from abnormal insulin secretion, insulin action, or both. The defects of insulin secretion result from insufficient functioning of pancreatic β cells (type 1 diabetes), whereas those acting by insulin action are generally

related to the resistance of peripheral tissues to insulin (type 2 diabetes). In all cases, the end result is defective insulin action abrogating intracellular insulin signaling on target cells(1) .

The type 2 diabetes mellitus (Type 2 DM) is a multi-genetic variation, which is determined by various genes and environmental factors. This type of diabetes mellitus accounts for about 90% of all cases of diabetes mellitus. Unlike type 1 diabetes mellitus (Type 1 DM), type 2 is not usually caused by autoimmune destruction of pancreatic cells but is characterized by multiple defects in insulin action and insulin signaling. Many patients with type 2 diabetes have basal hyperinsulinemia, also reduce stimulation of insulin secretion with elevated blood glucose levels(2) . Type 2 DM is characterized by three pathophysiologic abnormalities: genetically impaired insulin secretion, peripheral insulin resistance (receptor cause) and excessive hepatic glucose production (as a sum of liver glycogenolysis and gluconeogenesis)(3) . Type 2 diabetes is usually diagnosed using the fasting plasma glucose ≥ 126 mg\dl (7.0 mmol \L), Glycated hemoglobin (HbA1C) ≥ 6.5%, Random plasma glucose ≥ 200 mg\dl (11.1 mmol\L), two hours after a 75gram oral glucose load as in a glucose tolerance test, plasma glucose ≥ 200 mg\dL (11.1 mmol⁄ L)(4) .

Homocysteine (Hcy) is a non - proteinogenic α-amino acid containing sulfur, resulting from the demethylation of methionine by a multistep process. Approximately 70% of the plasma Hcy is bound to albumin and the remaining 30% is in the form of free sulfides. Total plasma Homocysteine (tHcy) refers to the sum of all Hcy species present in the plasma/serum, including the free form and the protein bound form. The normal plasma concentrations of Hcy range from 5 to 15 µmol/L. A hyperhomocysteinemia corresponds to a high level of Hcy in plasma, generally greater than 15 µmol/L (5). A strong association between elevated plasma Homocysteine levels and early cardiovascular events was reported in Type 2 DM. Plasma levels of Hcy, has been associated with vascular complications of diabetes. Several studies have shown that Hcy level predicted the risk of death or coronary events patients with Type 2 DM (6).

Homocysteine (Hcy) has adverse effects on vascular endothelium and smooth muscle cells, resulting in changes in the structure and function of subclinical arteries. These actions include increased proliferation of vascular smooth muscle cells, endothelial dysfunction and damage, accelerates thrombin formation, inhibits native thrombolysis, promotes lipid peroxidation through free radical formation, and induces vascular smooth muscle proliferation and monocyte chemotaxis (7). Hyperhomocysteinemia also increases the risk of venous thrombosis by increasing platelet adhesion to endothelial cells, β-thromboglobulin, and tissue plasminogen activator (8, 9).

In this case-control study, we estimated serum homocysteine in a patient with type2 DM (glycemic controlled, poorly glycemic controlled and glycemic uncontrolled) and control group.

Material and Methods

The inclusion criteria for 197 (74 males and 123 females) was one hundred and forty-eight are known as cases of type 2 Diabetes Mellitus diagnosed by specialists in the Sulaimani Center for Diabetes and Endocrine diseases, and forty-nine are normal individuals with no chronic diseases as healthy control group, who collected from the Central Laboratory of Directory of Heath. The ages of the participants were between 20-75 years old. The exclusion criteria for this study are type 1 Diabetes Mellitus and pregnant women.

Using disposable syringes, 5 ml of venous blood was drawn from each patient and healthy controls in the study. The 2 ml of blood collected in EDTA tubes for HbA1c

analysis by SIEMENS Dimension. Then 3 ml of drawn blood has been collected in a serum gel separator tube and left in room temperature for 10 minutes to clot. After centrifugation for 15 min. at 3000 rpm (revolution per minutes), the serum was immediately analyzed for serum glucose, lipid profile, blood urea, blood urea nitrogen, and serum creatinine by Cobas c311 instrument (Abbott), while serum homocysteine by SIEMENS IMMULITE 2000 (Siemens AG, Germany).

The study granted approval by the Ethics committee in the College of Medicine / Sulaimani University Samples were collected from June to November 2019.

Statistical Analysis

SPSS version 24 software (IBM SPSS Statistical Package for the Social Sciences) was used for statistical analysis in this study. The data presented in tabular forms showing the frequency and relative frequency distribution of different variables of all groups and quantitative continuous variables were described by mean and SD (standard deviation). We used ANOVA test to get difference in mean and standard deviation in results of all tests that done for glycemic controlled, poorly glycemic controlled, and glycemic uncontrolled with control group except in blood sugar and HbA1c that we used t- test to get the difference between result mean of them between T2DM patients and control group. P values of 0.05 or $P<0.05$ were used as a cut off point for significance of statistical tests. Chi-square test used to find association between different qualitative variables as between type of HbA1c groups and each of: gender, age groups, smoking status, duration, and hypertension status and body mass index groups.

Results

In the results, T2DM patients (148) were separated in three groups: glycemic controlled (33), poorly glycemic controlled (73), and glycemic uncontrolled (42) cases. In table 1 showed that, there was a significant difference (P- value < 0.001) of age among T2DM cases, and there was non-significant difference (P- value > 0.30) of BMI among T2DM cases, but it had been noticed that obesity has a highest percentage among T2DM cases. Regarding gender, smoking status, blood pressure and duration among T2DM cases, there were no significant differences.

Table (1): Age, gender, body mass index, smoking statues, blood pressure and duration in T2DM patients.

		Number (Percentage)			P- value
		T2DM, glycemic controlled (HbA1c ≤6.5)	**T2DM, poorly glycemic controlled (HbA1c 6.5-8)**	**T2DM, glycemic uncontrolled (HbA1c >8)**	
Age (Years)	**20 – 35**	0 (0.0%)	1 (1.4%)	0(0.0%)	< 0.001
	36 – 50	5 (15.2%)	18 (24.7%)	17(40.5%)	
	> 50	28 (84.8%)	54 (74.0%)	25 (59.5 %)	
Gender	**Male**	15 (45.5%)	30 (41.1%)	13 (31.0%)	0.47
	Female	18 (54.5%)	43 (58.9%)	29 (69.0%)	

BMI	**Normal**	15 (45.5 %)	10 (13.7%)	10 (23.8%)	0.42
	Overweight	9 (27.3%)	29 (39.7%)	11 (26.2%)	
	Obese	9 (27.3%)	34 (46.6%)	21 (50.0%)	
Smoking Status	**Smoker**	2 (6.1%)	11 (15.1%)	5 (11.9%)	0.42
	Non-smoker	31 (93.9%)	62 (84.9%)	37 (88.1%)	
Blood Pressure	**Normotensive**	28 (84.8%)	57 (78.1%)	36 (85.7%)	0.52
	Hypertensive	5 (15.2%)	16 (21.9%)	6 (14.3%)	
Duration (Months)	**3-12**	5 (15.2%)	2 (2.7%)	7 (16.7%)	0.095
	13 – 24	3 (9.1%)	10 (13.7%)	4 (9.5%)	
	> 24	25 (75.8%)	61 (83.6%)	31 (73.8%)	

A $P<0.05$ was considered significant.

The results in Table 2 showed that there was no significant difference among different age groups in glycemic controlled, poorly glycemic and glycemic uncontrolled groups. In addition, the table showed that, there was no significant difference between male and female in glycemic controlled and poorly glycemic, while in glycemic uncontrolled there was significant difference in the level of serum homocysteine.

Table (2): Distribution of serum homocysteine in different age and gender in type 2 diabetes mellitus patients and control group.

		Mean ± Standard Deviation of S. Homocysteine			
		T2DM, glycemic controlled (HbA1c ≤6.5)	**T2DM, poorly glycemic controlled (HbA1c 6.5-8)**	**T2DM, glycemic uncontrolled (HbA1c >8)**	**control group**
Age (Years)	**20-35**	0	19.4 ±0.0	0	11.13 ± 4.9
	36-50	12.97± 3.81	12.01±5.47	12.58 ± 6.0	11.91 ± 5.24
	>50	13.91± 4.97	12.54±4.83	12.74± 6.53	12.64 ± 1.91
P-value		0.70	0.36	0.94	0.80
Gender	**Male**	15.25±4.52	13.28±5.15	17.25 ± 8.6	12.72 ± 3.9
	Female	12.20±4.70	11.97±4.87	10.63±3.13	10.82±5.16
P-value		0.07	0.27	<0.001	0.20

The table 3 showed the result of the analyzed HbA1c and blood sugar after 12 h fasting. The data indicated significant difference (P-value < 0.001) in the HbA1c and Blood Sugar level between T2DM patients and control group.

Table (3): Mean, stander deviation and P-value for blood sugar and HbA1c in type 2 diabetes mellitus patients and control group.

Variable	**Mean± Std. Deviation**		**P- value**
	T2DM	**Control group**	
B. sugar mg/dl	173.20 ± 61.38	92.49 ± 10.14	< 0.001
HbA1c	7.60 ± 1.44	5.01 ± 0.41	< 0.001

A P<0.05 was considered significant.

In another set of sera analysis, according to HbA1c Cut-off values: P- value was< 0.001 indicated significant difference in the HbA1c and blood sugar level among T2DM patients (Table 3).

Table (4): Classification of type 2 diabetes mellitus according to blood sugar and HbA1c results.

Blood Sugar	T2DM, glycemic Controlled (HbA1c ≤6.5)	T2DM, poorly glycemic control (HbA1c6.5-8)	T2DM, glycemic uncontrolled (HbA1c >8)	P- value
Normal 60-120 mg/dl	9 (27.3%)	70 (95.9%)	42 (100%)	< 0.001
High > 120 mg/dl	24 (72.7%)	3 (4.1%)	0 (0.0%)	

A P<0.05 was considered significant.

We examined in table 5, the lipid profile which include: Serum Total Cholesterol (S.TC), Serum Triglycerides (S.TG), Serum High Density Lipoprotein (S.HDL), Serum Low Density Lipoprotein (S.LDL), and Serum Very Low-Density Lipoprotein (S. VLDL) for 148 T2DM and 49 healthy individuals. P- value showed that there was no significant difference for all variables of lipid profile among all T2DM patients and control groups (Table5). According to the findings in table 4, there was no significant difference in B. urea and BUN among T2DM and control groups (P- value = 0.17), while there was significant difference in s. creatinine (P- value = 0.03)

Table (5): Concentration of serum lipid profile in type 2 diabetes mellitus patients and control group.

Variable (normal range)	Mean ± Std. Deviation				P- value
	T2DM, glycemic controlled (HbA1c ≤6.5)	T2DM, poorly glycemic controlled (HbA1c 6.5-8)	T2DM, glycemic uncontrolled (HbA1c >8)	Control group	
Cholesterol (<200mg/dl)	160.4±40.8	170.1±38.4	167.6±39.7	159.5±13.9	0.31
TG(<150mg/dl)	141.9±135.3	138.9±63.6	139.1±61.1	127.0±24.0	0.78
HDL(<45mg/dl)	40.8 ± 13.7	41.4 ± 8.7	38.8 ± 8.8	41.6 ± 3.2	0.43
LDL (<100 mg/dl)	91.2 ± 28.6	101.0±33.7	101.0±32.9	92.4 ± 11.4	0.19
VLDL (0-30 mg/dl)	28.4 ± 27.1	27.8 ± 12.7	27.8 ± 12.4	25.4 ± 4.8	0.78
B.urea (18-42 mg/dl)	32.4±12.6	29.2 ±8.4	28.0 ± 8.2	28.9± 6.1	0.17
BUN (6.5-20 mg/dl)	15.1 ± 5.9	13.6 ±3.9	13.1 ± 3.8	13.5 ± 2.8	0.17
S. creatinine(0.3-1.2 mg/dl)	0.85±0.45	0.7±0.3	0.70 ± 0.24	0.69± 0.16	0.03

A P<0.05 was considered significant throughout all the study.

The results showed that the mean and standard deviation for concentration of serum homocysteine was (13.6 ± 4.8), (12.5± 5.0), (12.7 ± 6.2) and (11.4 ± 4.8) in glycemic controlled, poorly glycemic controlled, glycemic uncontrolled and control group respectively. These results indicated no significant differences (P-value = 0.32) of serum homocysteine among T2DM and control groups as shown in table 6

Table (6): Concentration of serum homocysteine in type 2 diabetes mellitus patients and control groups.

Variables (normal range)	Mean ± Std. Deviation				P-value
	T2D, glycemic control (HbA1c ≤6.5)	T2DM, poorly glycemic controlled (HbA1c 6.5-8)	T2DM, glycemic un controlled (HbA1c >8)	Control group	
S. Homocysteie (5-15 µmol\L)	13.6± 4.8	12.5±5.0	12.7 ± 6.2	11.4±4.8	0.32

A P<0.05 was considered significant.

Discussion

Table 1 showed age distribution of T2DM, there was a statistically significant difference and high percentage was in age >50 years, this finding is in agreement with other article who showed that, age was one of the most important risk factors for type 2 diabetes and the burden of the disease is very high in older age groups(10). In current study, there was statistically non-significant difference in gender between T2DM cases and there were more females than males. The high percentage of females in this study could prove that women are concerning about their health more than men. In addition, women may have more free time than men as most probably number was housewives. This is in agreement with other studies (11). Body mass index (BMI) was measured according to the following equation: dividing the weight in kilograms by the height in squared meters (kg/m2)(12) . BMI was categorized according to the European Society of Human Reproduction and Embryology into underweight (≤18.0 kg/m2), normal weight (18.0-24.9 kg/m2), overweight (25-29.9 kg/m2) and Obesity (≥ 30 kg/m2) (13). In the present study as shown in (Table 1), there was no significant difference of BMI, and it had been noticed that overweight and obesity had a highest percentage among T2DM cases. Nevertheless, Ganz et al., in United States showed that, the BMI was strongly and independently associated with the risk of being diagnosed with T2DM overweight and obesity was statistically significantly associated with being diagnostic T2DM(14). Other study was conducted in Southeast Asian population by Papier et al., wherein lower BMI cut-points is necessary for defining T2DM risk and lowering obesity prevalence would reduce T2DM incidence (14). Increasing weight of the body could be signaling for non-healthy body due to increasing in total fats content. Oxidation of these fats resulted in an oxidative stress that leads to free radical production: a process is currently suggested as one of the mechanism underlining diabetes mellitus, which affect carbohydrate, lipid, and protein metabolism. The process as a whole can generate a redox imbalance inside the cell, especially in liver(15). Current study showed that a statistically non-significant difference in smoking states between T2DM cases. Numerous studies have shown that smoking can increase the risk

of T2DM, and some recommend cessation of smoking to prevent development of T2DM. However, cessation of smoking had not always been associated with reduction in the risk of T2DM(16, 17). As for blood pressure, the study showed that there was non-significant difference in blood pressure between T2DM cases. According to UK Prospective Diabetes study management of blood pressure in T2DM, showed that all antihypertensive drugs are effective and safe in the management of blood pressure in the patient that have T2DM(18) .

The term HbA1c refers to glycated hemoglobin, HbA1c test does not require fasting because it provides a time-averaged estimate of blood glucose over the preceding 3 - 12 weeks(19) In this study the results showed statistically significant difference with high mean levels of HbA1c in T2DM group compared with control group (Table 3 and 4). This finding is in agreement with previous study(20), the authors found that people with Type 2 diabetes have a probability in increasing of HbA1c levels. Also showed in the study by Sulistyo et al., that glycated hemoglobin concentration was elevated than its normal range in diabetic patients and these levels decreased below normal cut-off value with glycemic control participate(21) Regarding blood glucose level there was significant difference between type 2 diabetes mellitus cases and control group (Table3 and 4). Similar observation was seen in a study conducted on China population by C. Liu et al., wherein an increased range of blood glucose level was significantly associated with T2DM (22).

Dyslipidemia is one of the major risk factors for cardiovascular disease in diabetes mellitus(23) . This study examined serum concentrations of lipids including serum total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG), and very low density lipoprotein (VLDL). In this study lipid profile result showed no significant difference between T2DM groups as compared with control group (Table 5). Nevertheless, this observation shows contrast with other studies, our study showed increase in lipid profiles levels in diabetic patients compared with normal individuals, in spite of that they received antihyperlipidemic drugs, they need dietary and lifestyle modifications to improve their lipid level results (24, 25). In the diabetes center in Sulaymaniyah city, most patients with T2DM received treatments to reduce fats and cholesterol in blood, which caused most of the lipid profile results close to normal levels.

Diabetes mellitus (DM) is characterized by some specific complications including diabetic nephropathy, urea and creatinine are useful parameters to diagnose the renal function in both diabetic and non-diabetic subjects(26). Elevating blood glucose level it can put too much stress on kidneys causing serious damage to the blood vessels, leading to renal complication. On the other hand, the high creatinine levels observed in diabetic patients may be as a result of impaired function of the nephrons, plasma creatinine is a more sensitive index of kidney function compared to plasma urea level because urea formation is influenced by a number of factors such as liver function, protein intake and rate of protein catabolism, if levels of creatinine rise then kidneys may be malfunctioning(27, 28) . According to results in this study as shown in Table 5 there was statistically non- significant difference in B- urea and BUN in T2DM cases when compared with control group. These results are disagreement with Singh et al. study(29) which showed that there was an increase of blood urea level in T2DM patients than in control group and was statistically significant difference between them . Serum creatinine level in this study showed statically significant difference between T2DM cases when compared to control group. This result is in agreement with a study conducted in Iraq population by J.H. Murtadha1 et al., who

reported that there was a significant difference between s.creatinine level and T2DM compared with control group(30) , as was accord with Aldler et al. report submitted that plasma creatinine significantly increased in T2DM and raised plasma creatinine in diabetic patient(28).

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Homocysteine is an intermediate product formed during the breakdown of the amino acid methionine, and may undergo remethylation to methionine or trans-sulphuration to cystathione and cysteine. Elevated serum levels of Hcy, which called Hyperhomocysteinemia have adverse effects on the cardiovascular system and considered to be an independent risk factor for CVD. Homocysteine play an important role in vascular endothelium injuries and could be induces diabetic disease(31). Many published studies dealt with plasma Hcy levels in type 2 diabetes and yielded multiplicity in results; the present study showed that homocysteine level in type 2 diabetic patients was not statically significant increased as compared with its level in control group (Table 6). This result agrees with Diakoumopoulou et al., study(32) reported that plasma tHcy levels were not different between diabetic and control group. Furthermore, our study is disagreement with Huang et al.,(33) which they showed higher values of tHcy in diabetic patients as compared with control group, which were statistically significant. Homocysteine level within different age groups was no significant difference between T2DM and control group (Table 2), this finding agree with Domingue et al.,(34) who reported that aging was accompanied by elevated tHcy opposite to our finding in younger subjects with type 2 diabetes mellitus. In this study, there was no significant difference in tHcy level in glycemic controlled and poorly glycemic controlled T2DM groups between the different sexes however in glycemic uncontrolled T2DM group was significantly different (Table 2); moreover the present study shows higher level of tHcy in male than female. Other article, as in Masudaet al., (35) reported that there are some differences in the factors associated with tHcy between diabetics and non-diabetics, and between males and females, glycemic status in DM was more strongly associated with tHcy in males than in females .They suggest that sex difference was possibly caused by sex hormones as estrogen inhibits the progression of atherosclerosis, shortage of estrogen in males may lead to elevation of tHcy which promotes thrombogenesis. Many articles reported that, there were several factors impact homocysteine circulation: Metformin, as a cornerstone in the treatment of type 2 diabetes for several years and vitamin B12 or folate deficiency, as its side effects have raised some questions about its effect on Hcy level (36) Wulffele et al., suggested that decreased folic acid and vitamin B12 after metformin consumption could result in Hcy elevation (37). Moreover, some other studies reported no changes in Hcy concentration after metformin treatment (38). Insulin treatment caused a reduction of the enzyme cystathionine b-synthase and increasing methylene tetrahydro folate reductase activity leading to elevated tHcy, this may suggest that not only chronic hyperinsulinemia but also acute hyperinsulinemia by insulin injection which increases plasma level of homocysteine(39). Status of nutritional vitamin B12, B6 and folic acid together with lifestyle factors are known determinants of plasma homocysteine concentration in the general population(40). Therefore, more comprehensive adjustment was needed in the future.

Conclusion

Serum level of homocysteine statically was not differed significantly in T2DM compared with healthy group. There was no significant difference between the level of serum homocysteine and age group in T2DM and no significant difference in the level of serum homocysteine in different

gender except in glycemic uncontrolled there was significant difference.

Acknowledgements

A great appreciation for all staffs of Central Medical Laboratory and all administrative and laboratory staffs in Endocrine and Diabetic Center in Sulaimani for their great assistance.

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