Advanced Medical Journal, Vol.9, No.2, P.155-162, 2024 https://doi.org/10.56056/amj.2024.268

Evaluation of serum folic acid level in patients with type 2 diabetes mellitus

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Abstract

Background and objectives: Type-2 diabetes mellitus is a metabolic disorder. Diabetic patients with low folic acid, high homocysteine levels, have a higher chance of developing coronary heart disease based on previous studies. Therefore, this study aimed to investigate the serum levels of folic acid and homocysteine in type-2 diabetes mellitus patients and their association with insulin resistance.

Methods: This cross-sectional study has been conducted on 138 participants from February 2nd 2022 to August 3rd 2022, at Azadi teaching hospital/clinical biochemistry department- Duhok, Kurdistan region, Iraq. The participants were composed of two groups, 70 patients with type-2 diabetes mellitus, and 68 apparently healthy control. Both groups have been investigated for serum folic acid level, serum glucose, serum insulin, serum homocysteine, glycosylated hemoglobin, and Homeostatic Model Assessment for Insulin Resistance.

Results: The mean age for type 2 diabetic patients was (51.24 years) with males predominant (64.29%). Type 2 diabetic patients showed a low level of mean serum folic acid concentrations (2.73 ng/ml) in comparison with healthy subjects (5.91 ng/ml) ($p<0.001$), and the mean serum homocysteine concentration was (13.493 µmol/L) among type 2 diabetic patients compared to healthy subjects (10.041 µmol/L) ($p<0.001$). Significant correlation was found between serum folic acid and serum homocysteine levels, and glycosylated hemoglobin ($p<0.05$ and $p<0.001$, respectively).

Conclusion: Patients with type-2 diabetes mellitus were associated with low mean serum folic acid levels and high mean serum homocysteine levels.

Keywords: Folic acid, Homocysteine, Insulin resistance, Type 2 diabetes mellitus

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Introduction

Diabetes mellitus is a prevalent endocrine disorder that affects more than 537 million of all adults aged (20-79) globally (10.5% of the populations).[1]More than 95% of cases of diabetes worldwide are type 2 diabetes mellitus (T2DM), which is more prevalent,[2] and it is a complicated disease with significant heterogeneity,[3] which is characterized by insulin resistance and/or aberrant insulin secretion-induced hyperglycemia.Type 2 diabetes often develops in patients after age 40, but it can also affect younger ones. Type 2 diabetes mellitus in children and adolescents is an increasing, significant issue,[4] and is regarded as a risk factor for atherosclerosis formation and cardiovascular development. Obesity was associated with insulin resistance with and without diabetes mellitus and has emerged as the primary factor driving increases in insulin resistance and its complications.[4] Insulin resistance is characterized as a reduced physiologic or biological response to normal levels of circulating insulin. Recent studies have suggested that insulin resistance etiology may be significantly influenced by epigenetic changes, notably DNA methylation.[5] Insulin resistance is associated with hyperhomocysteinemia in type 2 diabetic patients.[6-7]By causing reversible beta cell damage and inhibiting insulin release, hyperhomocysteinemia worsens T2DM.[7-8] Folate, a water-soluble vitamin B (vitamin B9), is a necessary vitamin that may be found in many fruits and vegetables. The word "folic acid" refers to a more stable which is manufactured supplement form, whereas the name "folate" alludes to its natural form, which may be found in food and body fluids.[9] Folate is the most important dietary substrate and cofactor involved in one-carbon metabolism, where together with methionine, it serves as a dietary methyl donor. By supplying methyl groups for DNA methylation, one-carbon metabolism is crucial for epigenetic changes. The nutritional deficiency of such vitamin-important cofactors of homocysteine metabolism is commonly related to high circulating levels of homocysteine.[10] Patients with T2DM have been shown to have more elevated risk of coronary heart disease when their homocysteine levels are high.[11-12] We aimed to estimate serum folic acid level and serum homocysteine level among type 2 diabetic patients, as well as ascertain the relation of serum folic acid level and serum homocysteine level with glycemic control and insulin resistance in patients with type diabetes mellitus compared to healthy control.

Patients and methods

This research was performed in Duhok Azadi general teaching hospital/clinical biochemistry department from February 2nd 2022 to August 3rd 2022. Seventy patients with T2DM (aged 19-68 years) and 68 apparently healthy subjects (aged 21-50 years) were incorporated into the research. The exclusion criteria of participants were patients having chronic diseases such as chronic liver disease, chronic kidney disease, Cushing syndrome, and Malignancy. Subjects using drugs affect the metabolism of folate like Steroids, Non-steroidal anti-inflammatory drugs (NSAIDs), and chemotherapy. Pregnancy, Smoking, and Alcohol intake. All participants underwent physical examination after history taking and blood were taken after an overnight fast for estimation of serum glucose level, serum Folic acid level, serum insulin, glycosylated hemoglobin percent (HbA1c%), and serum Homocysteine level. Ethical permission was obtained from the Research Committee of Ministry of Health and The Medical Ethics Committee of the Duhok - General Health Directorate, Duhok, Kurdistan region-Iraq with the study protocol (06072022-5-5 R1). For each participant she/he was given the right to know the contents of research and tests to be done and

written permission was collected from each one and had the right to withdraw at any time. The weight in kilograms of all participants was measured by a standard weight scale, the height in meters was obtained when the subject is standing, and the body mass index (BMI) was estimated using the special equation as weight in kg divided by square of height (kg/ m^2). Subjects with a body mass index < 25 kg/ m^2 were considered normal, while those with body mass index between 25-29.9 kg/m^2 were considered overweight and those with a body mass index of ≥30 kg/ m^2 were considered obese.[13] Blood samples were drawn from all participants in the morning after an overnight fast of about 8 hours into a tube with EDTA for measurement of HbA1c, and gel tube for biochemical parameters. The serum was separated by centrifuge at 3200rpm for 10-15 minutes. All parameters measured by (Cobas 6000, Roche Hitachi), depending on different principles: Insulin and folic acid levels were analyzed using the electrochemiluminescence immunoassay technique (normal range 2.6-24.9 mU/mL for insulin, and 5-20 ng/mL for folic acid). Serum homocysteine was measured by the immune-chemiluminescence method (normal range 5-15 μmol/L). Serum glucose levels were determined using an enzymatic colorimetric method. HbA1c is evaluated using a turbidimetric assay technique. Insulin resistance was measured using Homeostatic Model Assessment for Insulin Resistance formula (HOMA-IR = fasting insulin [μU/ml] × fasting glucose [mg/dL]/405), patients with a HOMAIR score ≥2.7 were classified to have insulin resistance (IR).[14] Using IBM SPSS Statistic 20, all data were analyzed. Results were given as the mean and standard deviation. Statistical hypotheses were examined using an independent T-test between two groups. The p-value less than 0.05 is statistically considered significant.

Results

Anthropometric and biochemical parameters of T2DM patients compared to healthy individuals was shown in table 1. The mean age of type 2 diabetic patients (51.24) with males predominant (64.29%). Patients with T2DM had significantly higher mean values of body mass index, serum glucose, serum insulin, serum homocysteine, blood HbA1c%, and HOMA-IR, except the mean serum folic acid level was lower in patients with T2DM compared to healthy individuals.

Table (1): General and biochemical characteristics of participants

Characteristics	Diabetic patients (n=70) Mean ± SD	Healthy (n=68) Mean ± SD
Age (years)	51.24±9.27	29.57±7.2
Gender Female Male	25(35.71%) 45(64.29%)	45(66.18%) 23(33.82%)
BMI (kg/m2)	31.01±4.7	23.4±2.77
Glucose (mg/dl)	224.1±50.5	90.96±5.05
Insulin (μU/mL)	12.6±3.8	6.57±2.46

HOMA-IR	6.82±2.0	1.47±0.56
HbA1c (%)	9.70±1.17	5.07±0.22
Folic acid ng/ml	2.73±0.822	5.91±3.14
Homocysteine (µmol/L)	13.493±4.8	10.041±2.2

Association of serum folic acid level with demographic and biochemical characteristics in participants shown in (table 2). According to Pearson correlation coefficient(r), a significant association of serum folic acid level with body mass index, serum glucose, serum insulin, serum homocysteine, blood HbA1c%, and HOMA-IR have founded with ($p<0.05$) in both groups.

Table (2): Correlation of serum folic acid level with general and biochemical variables, in the studied population.

Variables	Study population (n=138)	
	R	p value
BMI (kg/m^2)	0.436**	<0.001
Glucose (mg/dl)	0.638**	<0.001
Insulin (µU/ml)	0.295*	<0.001
HOMA-IR	0.528**	<0.001
HbA1c (%)	0.562**	<0.001
Homocysteine(µmol/L)	0.069	<0.05

**. Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

Table (3): Shown that Serum folic acid level and serum homocysteine level correlated significantly with HbA1c% (poor glycemic control)

Variables	T2DM patients (n=28) HbA1c <6.5% Mean ± SD	T2DM patients (n=42) HbA1c ≥6.5% Mean ± SD	p value (2-tailed)
Folic acid (ng/ml)	6.245±2.146	2.249±0.872	<0.001
Homocysteine (µmol/L)	11.253±3.41	13.568±5.38	<0.05
Independent t-test			

In (table 3) patients with type 2 diabetes mellitus divided into two groups, T2DM with HbA1c <6.5% and HbA1c ≥6.5%. Subject with HbA1c ≥6.5% have lower mean value of folic acid and higher mean value of Homocysteine.

Table (4) shows patients with T2DM showed higher mean values of demographic and biochemical parameters with low serum folic acid concentration compared to those with normal serum folic acid concentration.

Table (4): distribution of the variables between two groups of T2DM according to the level of folic acid

Variables	)M patients mal folic acid (n=38)	)M patients folic acid (n=32)	alue
Age (years)	50.39±10.02	52.25±8.34	< 0.05
BMI (kg/m^2)	30.51±4.24	32.20±5.60	< 0.05
Glucose (mg/dl)	210.44±47.9	235.6±50.3	< 0.05
Insulin (µU/mL)	12.58±3.27	12.65±4.36	< 0.05
HOMA-IR	6.41±1.79	7.16±2.13	< 0.05
HbA1c (%)	9.487±1.037	9.887±1.27	> 0.05
Homocysteine (µmol/L)	12.55±5.19	13.91±4.7	>0.05
Independent t-test			

Discussion

Folic acid has great interest among patients with T2DM as its deficiency prone those patients to impaired endothelial function.[15] Moreover, serum folic acid deficiency was associated with increased serum homocysteine levels which could be a contributing factor to cardiovascular disease development, as hyperhomocysteinemia associated with arterial stiffness one of the pathological mechanism of cardiovascular disease.[16] In the present study, a considerable reduction in serum folic acid levels of type 2 diabetic patients was noticed compared to healthy individuals. The current study corroborated those of Ebesunun et al, who documented that low levels of folic acid in type 2 diabetics were found in comparison to healthy subjects[15]. Change in serum folic acid concentrations has an adverse effect on the integrity and stability of DNA as its deficiency disrupts the physiological system of type 2 diabetic patients with DNA damage and a negative effect on methylation of different genes.[10] In accordance with current findings, the levels of plasma homocysteine were significantly higher in T2DM patients than in healthy controls. Similar results were found by Gargari et al. who reported that patients with T2DM have higher levels of circulating homocysteine than healthy individuals.[17] In the current study data shows a no significant correlation between the folic acid level and glycemic control, However, similar findings have been reported by Asbaghi et al, as showing folic acid supplementation improves glycemic control.[18] Moreover, another study shows significant correlation of folic acid level to

glycemic control reported by Jankar and Keerti, who demonstrated that folic acid supplementation did not significantly affect the HbA1c.[19] In the current investigation, a significant positive connection between homocysteine levels and HbA1c was discovered. Similar findings were reported by Huang et al, who documented that the correlation between homocysteine and HbA1c was positively significant.[20] However, Khan A, et al, showed that the correlation of homocysteine with HbA1c was not significant.[21]

In accordance with our findings, insulin resistance was significantly higher in diabetic patients in comparison to healthy individuals, as well as, a strong association between insulin resistance and levels of folic acid was observed.[22] Prior studies have demonstrated that the serum insulin levels and HOMA-IR were dramatically reduced with improved glycemic control after receiving folic acid supplementation.[17-23-24] Homocysteine has the potential to block insulin-stimulated tyrosine phosphorylation of the insulin receptor beta subunit and its substrates, which would limit the formation of glycogen,[25-26] leading to hyperglycemia and insulin resistance as a result. Our findings revealed a significant inverse relationship between folic acid levels and BMI. Similarly, other authors documented those obese diabetic patients had lower serum folic acid levels, compared to normal-weight diabetic patients.[27] Moreover, another study revealed that increase in body mass index led to a significant decrease in serum folic acid level.[28] This can be explained by changing the diet quality of obese patients as they take a smaller number of vegetables and fruit which are regarded as the main source of folate.[29]

Conclusions:

Patients with type 2 diabetes mellitus have low mean serum folic acid and high mean serum homocysteine levels compared to healthy individuals. In correlation with glycemic control, the serum level of folic acid found a significant negative correlation as decreasing serum folic acid level associated with poor glycemic control, while increasing serum homocysteine level significantly showed a positive correlation with poor glycemic control.

Conflict of interest: None.

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