

Evaluation of Relationship between Serum Levels of Homocysteine and Folic Acid with Glycemic Control and Insulin Resistance among Individuals with Type 2 Diabetes Mellitus

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Abstract

Background: In individuals who have type 2 diabetes, hyperhomocysteinemia is linked to insulin resistance (IR). Hyperhomocysteinemia exacerbates type 2 diabetes by impairing the release of insulin and resulting in irreversible beta cell destruction. The most significant dietary base and coenzyme in one-carbon metabolism is folate, which also acts as a dietary methyl contributor in conjunction with methionine. **Aims:** Our objectives were to measure the levels of serum homocysteine as well as folic acid in type 2 diabetes mellitus (T2DM) patients and to determine the relationship between these levels and glycemic management and IR among individuals with T2DM in comparison to a healthy control group. **Materials and Methods:** The study included 136 participants who appeared to be in good health (aged 21–50) and 140 T2DM patients (aged 19–68). Blood was drawn for the measurement of serum insulin, glycosylated hemoglobin percent (HbA1c%), serum glucose, serum folic acid, and serum homocysteine levels following an overnight fast. The electrochemiluminescence immunoassay technique was used to measure the levels of folic acid and insulin (normal range: 5–20 ng/mL for folic acid and 2.6–24.9 mU/mL for insulin). Using the immunological chemiluminescence method, serum homocysteine was analyzed (normal range: 5–15 μ mol/L). **Results:** Mean serum folic acid level in diabetic patients (2.84 ± 0.933 ng/mL) was lesser as compared to healthy subjects (5.82 ± 2.25 ng/mL). Mean values of homeostatic model assessment-IR, HbA1c, and homocysteine were greater in diabetic subjects as compared to healthy subjects. It was observed that mean folic acid levels were greater in T2DM patients with HbA1c <6.5% (6.245 ± 2.146 ng/mL) as compared to T2DM patients with HbA1c $\geq 6.5\%$ (2.249 ± 0.872 ng/mL). On the contrary, mean homocysteine was lesser in T2DM patients with HbA1c <6.5% (11.253 ± 3.41 μ mol/L) as compared to T2DM patients with HbA1c $\geq 6.5\%$ (13.568 ± 5.38 μ mol/L). It was inferred that poor glycemic control was significantly correlated with low serum folic acid and higher serum homocysteine level. **Conclusion:** Compared to healthy people, patients suffering have higher mean serum homocysteine concentrations and lower mean serum folic acid concentrations.

Keywords: Folic acid, Homocysteine, Serum, Type 2 diabetes mellitus.

INTRODUCTION

Over 537 million persons in the world between the ages of 20 and 79 have diabetes mellitus, an endocrine illness that impacts 10.5% of the population. Type 2 diabetes mellitus (T2DM) is the most common form of the disease, accounting for more than 95% of cases globally.^[1-3] It is a complex condition with considerable variability, marked by insulin resistance (IR) and/or abnormal insulin production that results in hyperglycemia. Patients with type 2 diabetes may acquire it at any age, although it usually strikes those over 40 first. A growing and important problem in young people and adolescents T2DM is thought to be a precursor for the occurrence of atherosclerotic and cardiovascular disease.^[4-6]

Obesity is now recognized as the main factor promoting elevated IR and related repercussions. Obesity has been linked to IR regardless of the presence of diabetes mellitus. A decreased physiological or biological reaction to normal amounts of insulin in circulation is known as IR.^[7-10] Recent research has indicated that epigenetic modifications, particularly DNA

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methylation, may have a major impact on the etiology of IR. In individuals who have type 2 diabetes, hyperhomocysteinemia is linked to IR.^[11-13] Hyperhomocysteinemia exacerbates type 2 diabetes by impairing the release of insulin and resulting in irreversible beta-cell destruction. Folate is a water-soluble form of vitamin B (also known as vitamin B9) that is essential and present in a wide variety of vegetables and fruits.^[14-16]

While “folate” refers to the naturally occurring form of the vitamin that can be detected in food and bodily fluids, “folic acid” pertains to a form with greater stability that is made as a supplement. The most significant dietary base and coenzyme in one-carbon metabolism is folate, which also acts as a dietary methyl contributor in conjunction with methionine.^[17-19] One-carbon metabolism is essential for epigenetic modifications because it provides methyl groups that are necessary for DNA methylation. Elevated homocysteine levels in the blood are frequently linked to the nutritional shortage of these vital vitamins that participate in the metabolism of homocysteine.^[20-22] It has been demonstrated that patients with type 2 diabetes who have high homocysteine levels are at an increased risk of developing coronary heart disease.^[7,23,24]

Our objectives were to measure the levels of serum homocysteine as well as folic acid in T2DM patients and to determine the relationship between these levels and glycemic management and IR among individuals with T2DM in comparison to a healthy control group.

MATERIALS AND METHODS

The study included 136 participants who appeared to be in good health (aged 21–50) and 140 T2DM patients (aged 19–68). Patients with chronic illnesses, such as cancer, Cushing syndrome, chronic renal disease, and chronic liver disease, were excluded from the study. Patients on medications such as steroids, non-steroidal anti-inflammatory medicines, and chemotherapy have altered folate metabolism. Alcohol use, smoking and pregnancy also affect folate metabolism. Following the collection of medical histories, all participants underwent physical examinations. Blood was drawn for the measurement of serum insulin, glycosylated hemoglobin percent (HbA1c%), serum glucose, serum folic acid, and serum homocysteine levels following an overnight fast.

All participants' weights were measured in kilograms using a standard weight scale; each subject's height was measured in meters while they were standing; and the body mass index (BMI) was calculated using a special calculation that divided each participant's weight in kilograms by their height in square meters (kg/m^2). Individuals who had a BMI of $<25 \text{ kg}/\text{m}^2$ were classified as normal, those who had an index of $25\text{--}29.9 \text{ kg}/\text{m}^2$ as overweight, and those who had an index of $30 \text{ kg}/\text{m}^2$ or higher as obese.^[13] After an approximately 8-h overnight fast, blood samples were taken from each participant in the morning and placed in a gel tube for biochemical parameters and an ethylenediaminetetraacetic acid tube for measuring HbA1c.

Centrifuging the serum for 10 to 15 min at 3200 rpm separated it. Every parameter assessed using the Roche Hitachi cobas 6000 is based on various concepts. The electrochemiluminescence immunoassay technique was used to measure the levels of folic acid and insulin (normal range: 5–20 ng/mL for folic acid and 2.6–24.9 mU/mL for insulin). Using the immunological chemiluminescence method, serum homocysteine was analyzed (normal range: 5–15 $\mu\text{mol}/\text{L}$). One technique used to measure serum glucose levels was enzymatic colorimetric. Using a turbidimetric test approach, HbA1c is assessed. Patients with a homeostatic model assessment-IR (HOMA-IR) score of ≥ 2.7 were defined as having IR. IR was evaluated using the HOMA for IR formula ($\text{HOMA-IR} = \text{fasting insulin } [\mu\text{U}/\text{mL}] \times \text{fasting glucose } [\text{mg}/\text{dL}] / 405$).

Statistical analysis

Every dataset was examined using IBM Statistical Packages for the Social Sciences Statistic 20. The standard deviation and mean of the results were provided. An independent *t*-test was utilized to investigate statistical hypotheses between two groups. A $P < 0.05$ is deemed statistically significant.

RESULTS

The mean age was greater in the category of diabetic patients (52.35 ± 8.38) years as compared to healthy subjects (30.68 ± 8.3) years. Proportion of males was greater in diabetic subjects (65.71%) as compared to healthy subjects (34.55%). Proportion of females was lesser in diabetic subjects (34.29%) as compared to healthy subjects (65.45%). BMI, glucose, and serum insulin were greater in diabetic subjects as compared to healthy subjects (Table 1).

Mean serum folic acid level in diabetic patients ($2.84 \pm 0.933 \text{ ng}/\text{mL}$) was lesser as compared to healthy subjects ($5.82 \pm 2.25 \text{ ng}/\text{mL}$). Mean values of HOMA-IR, HbA1c, and homocysteine were greater in diabetic subjects as compared to healthy subjects (Table 2).

The values of Pearson correlation coefficient(*r*) showed a statistically significant correlation between serum folic acid and BMI, insulin, HOMA-IR, HbA1c, and homocysteine (Table 3). The diabetic subjects were divided into two groups. T2DM patients with HbA1c are $<6.5\%$ ($n = 56$) and T2DM patients with HbA1c are $\geq 6.5\%$ ($n = 84$). It was observed that mean folic acid levels were greater in T2DM patients with HbA1c $<6.5\%$ ($6.245 \pm 2.146 \text{ ng}/\text{mL}$) as compared to T2DM patients with HbA1c $\geq 6.5\%$ ($2.249 \pm 0.872 \text{ ng}/\text{mL}$). On the contrary, mean homocysteine was lesser in T2DM patients with HbA1c $<6.5\%$ ($11.253 \pm 3.41 \mu\text{mol}/\text{L}$) as compared to T2DM patients with HbA1c $\geq 6.5\%$ ($13.568 \pm 5.38 \mu\text{mol}/\text{L}$). It was inferred that poor glycemic control was significantly correlated with low serum folic acid and higher serum homocysteine levels (Table 4).

The diabetic subjects were divided according to serum folic acid as T2DM patients with normal folic acid ($n = 76$) and T2DM patients with low folic acid ($n = 64$). Mean values

Table 1: General and biochemical information of study participants

Groups	Age (years) mean±SD	Gender		BMI (kg/m ²)	Glucose mg/dL	Insulin (μU/mL)
		Male n (%)	Female n (%)			
Diabetic patients (n=140)	52.35±8.38	92 (65.71)	48 (34.29)	32.12±5.8	235.2±42.7	13.7±4.9
Healthy subjects (136)	30.68±8.3	47 (34.55)	89 (65.45)	24.5±3.88	91.85±6.12	7.68±3.57

BMI: Body mass index

Table 2: Serum level of folic acid in study participants

Groups	HOMA-IR mean±SD	HbA1c (%) mean±SD	Folic acid ng/mL mean±SD	Homocysteine (μmol/L) mean±SD
Diabetic patients (n=140)	6.93±1.1	9.81±1.28	2.84±0.933	14.504±5.9
Healthy subjects (136)	1.58±0.56	5.18±0.33	5.82±2.25	10.152±1.0

SD: Standard deviation, HOMA-IR: Homeostatic model assessment-insulin resistance, HbA1c (%): Hemoglobin percent

Table 3: Correlation of serum folic acid level with general and biochemical variables, in the study participants

Correlation parameters	BMI (kg/m ²)	Glucose (mg/dL)	Insulin (μU/mL)	HOMA-IR	HbA1c (%)	Homocysteine (μmol/L)
<i>r</i>	0.447	0.649	0.306	0.539	0.573	0.070
<i>P</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05

BMI: Body mass index, HOMA-IR: Homeostatic model assessment-insulin resistance, HbA1c (%): Hemoglobin percent

Table 4: Correlation between serum folic acid level and serum homocysteine level with HbA1c% (glycemic control)

Groups	n	Folic acid (ng/mL) mean±SD	Homocysteine (μmol/L) mean±SD
T2DM patients (HbA1c<6.5%)	56	6.245±2.146	11.253±3.41
T2DM patients (HbA1c≥6.5%)	84	2.249±0.872	13.568±5.38
<i>P</i> -value (2-tailed)		<0.001	<0.05

SD: Standard deviation, HbA1c (%): Hemoglobin percent, T2DM: Type 2 diabetes mellitus

of age, BMI, glucose, insulin, and HOMA-IR were greater in T2DM patients with low folic acid level. The finding was significant statistically (Tables 5 and 6).

DISCUSSION

Folic acid is of particular interest to patients with type 2 diabetes because a deficiency in it can lead to compromised endothelial function.^[25,26] In addition, a deficiency in serum folic acid has been linked to elevated levels of homocysteine in the blood, which may play a role in the development of cardiovascular disease, since hyperhomocysteinemia is linked to arterial stiffness, which is one of the pathological processes of cardiovascular illness.^[27,28] In the current study, the levels of folic acid in the bloodstreams of T2DM were significantly lower than those of healthy individuals. This finding supported the findings of Ebesunun *et al.*, who reported that decreased levels of folic acid were found in type 2 diabetics when compared to healthy subjects.^[15]

Variations in plasma folic acid levels have a detrimental impact on the stability and integrity of DNA because a lack of it

impairs DNA damage and negatively affects the methylation of several genes, disrupting the physiological processes of people with type 2 diabetes.^[10] According to recent research, T2DM patients had plasma homocysteine levels that were noticeably greater than those of healthy controls.^[16] Similar findings were made by Gargari *et al.*, who discovered that homocysteine levels in the blood are higher in T2DM patients than in healthy people.^[17] The data from the current study indicate that there is no significant link between folic acid levels and glycemic control. Asbaghi *et al.* have published similar findings, indicating that glycemic control is improved by folic acid supplementation.^[18]

Furthermore, Jankar and Keerti's study, which showed that folic acid supplements had no discernible effect on HbA1c, demonstrates a strong link between folic acid levels and glycemic management.^[19] The present study found a statistically significant positive correlation between HbA1c and homocysteine levels. Huang *et al.* reported similar results, showing a positive and substantial connection between homocysteine and HbA1c.^[20] However, Khan *et al.* demonstrated that there was no significant association between homocysteine and HbA1c.^[21] Our results show that IR was considerably higher in diabetic patients than in healthy persons and that there was a clear correlation between IR and folic acid levels.^[22]

Previous research has shown that supplementing with folic acid significantly decreased levels of insulin in the blood and HOMA-IR while also improving glycemic management.^[7,17-23] To inhibit the production of glycogen and thus cause hyperglycemia and IR, homocysteine has the ability to prevent insulin-mediated tyrosine phosphorylation of the insulin receptor beta subunit and its substrates. Our research

Table 5: Distribution of the variables such as age, BMI, glucose, and insulin between two groups of T2DM according to the level of folic acid

Groups	n	Age (years) mean±SD	BMI (kg/m ²) mean±SD	Glucose (mg/dL) mean±SD	Insulin (μU/mL) mean±SD
T2DM patients with normal folic acid	76	51.40±11.13	31.62±5.35	211.55±48.0	13.69±4.38
T2DM patients with low folic acid	64	53.36±9.45	33.31±6.71	236.71±51.4	13.76±4.47
P-value (2-tailed)		<0.05	<0.05	<0.05	<0.05

BMI: Body mass index, SD: Standard deviation, T2DM: Type 2 diabetes mellitus

Table 6: Distribution of the variables such as HOMA-IR, HbA1c (%), glucose, and homocysteine (μmol/L) between two groups of T2DM according to the level of folic acid

Groups	n	HOMA-IR	HbA1c (%)	Homocysteine (μmol/L)
T2DM patients with normal folic acid	76	7.52±1.80	9.598±1.048	13.66±5.20
T2DM patients with low folic acid	64	8.27±1.24	9.594±1.381	14.02±3.2
P-value (two-tailed)		<0.05	>0.05	>0.05

HOMA-IR: Homeostatic model assessment-insulin resistance, HbA1c (%): Hemoglobin percent, T2DM: Type 2 diabetes mellitus

showed a strong adverse correlation between BMI and folic acid levels. In a similar vein, other investigators found that, in comparison to diabetic individuals of normal weight, obese patients had decreased serum folic acid levels.^[26] Furthermore, a different study found that a rise in BMI was associated with a considerable drop in serum folic acid levels.^[27] This can be attributed to altering the diets of obese individuals, as they consume fewer fruits and vegetables, which are considered the primary sources of folate.^[28]

CONCLUSION

Compared to healthy people, patients suffering have higher mean serum homocysteine concentrations and lower mean serum folic acid concentrations. Serum folic acid levels were found to have a strong negative correlation with glucose management, with lower levels being linked to poor glycemic control. Conversely, higher serum homocysteine levels were significantly correlated with poor glycemic control.

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