The Relation between Plasma Homocysteine Levels and Diabetic Foot Ulcer in Egyptian Patients with Type 2 Diabetes

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Abstract

Background: Diabetic foot ulcers and lower-limb amputation account for morbidity, mortality and healthcare expenditures among diabetic patients. Ulceration is the most common precursor to amputation. Diabetic foot ulceration development involves several mechanisms, such as neuropathy, increased biochemical stress, external trauma and peripheral artery disease. Most of the studies showed that hypercystinemia is related with microvascular and macrovascular complications in diabetic patients. Hyperhomocysteine is related to poor ulcer healing.

Aim of the Work: This study was designed is to evaluate plasma homocysteine levels in patients with D.M. and the potential role of homocysteine in occurrence of diabetic foot ulcer in patients attending the outpatient clinic of National Institute of Diabetes & Endocrinology (NIDE).

Subjects and Methods: This study was conducted on a total number of 90 subjects which were subdivided as follows: Group I: included 35 type 2 diabetic subjects without foot ulcer. Group II: included 35 type 2 diabetic subjects with foot ulcer. All patients are selected from the outpatient clinic of National Institute of Diabetes and Endocrinology (NIDE) Group III: included 20 normal healthy subjects (as controls). In addition to the routine investigations C-reactive protein, and serum Homocysteine were measured.

Results: When we compared group I and group II we found that there was a significant statistical difference between them as regard serum homocysteine, p-value <0.025. When we compared group I and group III, we found that there were a significant statistical differences between them as regard serum homocysteine, p-value <0.025. When we compared group II and group III there were a significant statistical differences between them as regard, serum homocysteine, p-value <0.025.

Conclusion: It can be concluded that serum hyperhomocysteineinemia was associated with diabetic patients and carry risk for development of diabetic foot ulcers. However, this finding can be used in the, prevention of the development of diabetic foot ulcer especially if further studies will be done on large scale of diabetic Egyptian patients.

Key Words: Plasma homocysteine – Diabetic foot ulcer – Type 2 diabetes.

Introduction

DIABETES Mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of kidneys, nerves, blood vessels resulting in foot ulcers [1]. Diabetic foot ulcers and lower-limb amputation account for morbidity, mortality and healthcare expenditures among diabetic patients and will occur in 5-10% of diabetic population and, up to 3% will have a lower extremity amputation [2]. Ulceration is the most common precursor to amputation and is identified in more than two-thirds of lower limb amputations [3]. Diabetic foot ulceration development involves several mechanisms, such as neuropathy, increased biochemical stress, external trauma and peripheral artery disease [4]. Homocysteine (Hcy) is an amino acid, is a homologue of the amino acid cysteine, differing by an additional methylene (-CH₂-) group. It is biosynthesized from methionine by the removal of its terminal methyl group and can be recycled into methionine or converted into cysteine with the aid of B-vitamins [5]. Most of the studies showed that hypercystinemia is related with microvascular and macrovascular complications in diabetic patients. Also hyperhomocysteine is related to poor ulcer healing [6]. The objective of this study is to evaluate plasma homocysteine levels in patients with D.M. and the potential role of homocysteine in occurrence of diabetic foot ulcer.
Subjects and Methods

This study was conducted on a total number of 90 subjects which were subdivided as follows:

Group (I): Included 35 type 2 diabetic subjects without foot ulcer.

Group (II): Included 35 type 2 diabetic subjects without foot ulcer.

Group (III): Included 20 normal healthy subjects (as controls).

All patients were selected from the outpatient clinics of National Institute of Diabetes and Endocrinology (NIDE), between September 2014 and October 2015. T2DM was diagnosed according to the report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [7]. The normal control subjects were clinically free from any recognizable disease. They were not receiving any medications. Approval had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes. An informed consent was obtained from all patients and normal control subjects that described the aim of the study and the procedures that would be required from them.

Inclusion criteria:

Type 2 diabetes mellitus with & without foot ulcers.

Exclusion criteria:
- Type 1 diabetes mellitus.
- Other chronic diseases (e.g. CVD, Liver, Renal, Malignancy, Thyroid disorders, or Diseases with acute inflammation).
- Medications: Metformin, and/or vitamin supplements (vitamin B6,12 and folic acid) so that homocysteine values could be influenced.

Laboratory investigations:

Ten ml of venous blood were withdrawn from each patient in dry sterile vacutainers after overnight fasting. First part of collected blood was taken on EDTA tubes for determination of HbA1c level Second part of collected blood was left to clot. Serum was rapidly separated by centrifugation. It was tested for: Fasting Blood Glucose, Lipid Profile (Total cholesterol, Triglycerides, HDL, LDL) and C-reactive protein levels by using ARCHITECT 8000 chemistry analyzer (USA, supplied by Abbott, Al kamal company Cairo, Egypt). While Serum Homocysteine levels were measured using Enzyme Linked Immunosorbant Assay (ELISA) technique according to manufacturer’s instructions.

Statistical analyses:

Statistical analysis was performed using the statistical package for social sciences (SPSS, USA). Data are expressed as means ± standard deviation. Statistical differences between groups were evaluated by using the student’s t-test.

Results

The study comprised 70 Egyptian type 2 diabetics and 20 normal healthy control. Patients and controls were divided into three groups. Group (I): 35 type 2 diabetic without foot ulcer & comprised 14 males and 21 females, their mean age was 55 ±9. Group (II): 35 type 2 diabetic with foot ulcer & comprised 15 males & 20 females, their mean age was 56±7. Group (III): 20 healthy control subjects & comprised 10 males & 10 females, their mean age was 39±10 (Table 1). Homocysteine levels were 23.8±21.8 nmol/L in diabetic patients without foot ulcer, 42±29.2 nmol/L in diabetic group with foot ulcer. 7.8±4.9 nmol/L in control group. When we compared group I and group II as regard Fasting blood glucose, HbA1c, Lipid profile (Cholesterol, Triglycerides, HDL, LDL), Serum C-reactive protein and serum Homocysteine we found that there were insignificant statistical differences between group I and group II as regard Fasting blood glucose, HbA1c, Lipid profile (Cholesterol, Triglycerides, HDL, LDL), Serum C-reactive protein while there was a significant statistical difference between them as regard serum homocysteine, p-value <0.025 (Table 2). When we compared group I and group III as regard Fasting blood glucose, HbA1c, Lipid profile (Cholesterol, Triglycerides, HDL, LDL), Serum C-reactive protein and serum Homocysteine we found that there were insignificant statistical differences between group I and group III as regard (Cholesterol, HDL, LDL), Serum C-reactive protein while there were a significant statistical differences between them as regard Fasting blood glucose, HbA1c, Triglycerides, serum homocysteine, p-value <0.025 (Table 3). When we compared group II and group III as regard Fasting blood glucose, HbA 1c, Lipid profile (Cholesterol, Triglycerides, HDL, LDL), Serum C-reactive protein and serum Homocysteine we found that there were insignificant statistical differences between group II and group III as regard (Cholesterol, HDL, LDL), Serum C-reactive protein while there were a significant statistical differences between them as regard Fasting blood glucose, HbA1c, Triglycerides, serum homocysteine, p-value <0.025.
Discussion

Diabetic foot ulceration is one of the serious complications of diabetes causing major extremity amputation with considerable economic and public health implications [8]. Hyperhomocysteinemia is not uncommon in diabetic patients. Increased homocysteine levels were found to be associated with arteriosclerotic outcomes and risk of stroke in elderly individuals, and are considered as an independent risk marker for cardiovascular diseases [9], and it can aggravate it. The mechanism of this increased hyperhomocysteinemia prevalence is vague but it is suggested that insulin plays a role in the regulation of plasma homocysteine and insulin resistance causing hyperhomocysteinemia [10]. Besides, the mechanism that cause peripheral and/or autonomic neuropathy are complex and are not fully understood. Hypothetically, homocystein can also contribute to the neuropathy development through neurovascular disruption or through direct toxic effect. In some studies in this field it has been shown that there could be a link between hyperhomocysteinemia and autonomic or peripheral neuropathy [11]. The relation between hypercysteinemia and cardiovascular diseases in patients with type 2 diabetes is 1.6 times than in non-diabetic patients [10]. Another important factor is the link between nitric oxide (NO) and homocysteine. Nitric oxide is a free radical gas which has a critical role in wound healing. Homocysteine hinders the NO production in various ways, such as the inhibition of the destruction of NO inhibitor, asymmetric dimethyl arginine (ADMA), and pro-oxidant behaviours [12]. We can say that, all the above mentioned mechanisms, combined with the presence of hyperhomocysteinemia, cause the development of foot ulcer in diabetic patients. We designed this study to investigate the relationship between homocysteine levels and diabetic foot ulcer. In our study the homocysteine levels were showed significant differences between group I without foot ulcer & group III (control group) similarly to the study of [13]. Also in our study the homocysteine levels were showed significant difference between group I & group II which means that we found a relation between the concentration of homocysteine and the incidence of chronic complication in the form of diabetic foot ulcers. While [14] found significant difference in the homocysteine levels between control group and the other two groups (diabetic group without foot ulcer and diabetic group with foot ulcer but they found no difference in homocysteine levels between diabetic foot ulcer group and diabetic group without foot ulcer. Also Mehmet, et al., found elevated homocysteine levels in the diabetic patient without foot ulcers and diabetic foot ulcer group compared to control, similarly our study the significance was also observed in diabetic foot ulcer patients in which chronic complications are seen more frequently -value was less than 0.05.

**Discussion**

Diabetic foot ulceration is one of the serious complications of diabetes causing major extremity amputation with considerable economic and public health implications [8]. Hyperhomocysteinemia is not uncommon in diabetic patients. Increased homocysteine levels were found to be associated with arteriosclerotic outcomes and risk of stroke in elderly individuals, and are considered as an independent risk marker for cardiovascular diseases [9], and it can aggravate it. The mechanism of this increased hyperhomocysteinemia prevalence is vague but it is suggested that insulin plays a role in the regulation of plasma homocysteine and insulin resistance causing hyperhomocysteinemia [10]. Besides, the mechanism that cause peripheral and/or autonomic neuropathy are complex and are not fully understood. Hypothetically, homocysteine can also contribute to the neuropathy development through neurovascular disruption or through direct toxic effect. In some studies in this field it has been shown that there could be a link between hyperhomocysteinemia and autonomic or peripheral neuropathy [11]. The relation between hypercysteinemia and cardiovascular diseases in patients with type 2 diabetes is 1.6 times than in non-diabetic patients [10]. Another important factor is the link between nitric oxide (NO) and homocysteine. Nitric oxide is a free radical gas which has a critical role in wound healing. Homocysteine hinders the NO production in various ways, such as the inhibition of the destruction of NO inhibitor, asymmetric dimethyl arginine (ADMA), and pro-oxidant behaviours [12]. We can say that, all the above mentioned mechanisms, combined with the presence of hyperhomocysteinemia, cause the development of foot ulcer in diabetic patients. We designed this study to investigate the relationship between homocysteine levels and diabetic foot ulcer. In our study the homocysteine levels were showed significant differences between group I without foot ulcer & group III (control group) similarly to the study of [13]. Also in our study the homocysteine levels were showed significant difference between group I & group II which means that we found a relation between the concentration of homocysteine and the incidence of chronic complication in the form of diabetic foot ulcers. While [14] found significant difference in the homocysteine levels between control group and the other two groups (diabetic group without foot ulcer and diabetic group with foot ulcer but they found no difference in homocysteine levels between diabetic foot ulcer group and diabetic group without foot ulcer. Also Mehmet, et al., found elevated homocysteine levels in the diabetic patient without foot ulcers and diabetic foot ulcer group compared to control, similarly our study the significance was also observed in diabetic foot ulcer patients in which chronic complications are seen more frequently -value was less than 0.05.
HbA1c is one of the most important risk factors for the development of complication. In our study there is similarity between group I (type 2 diabetic subjects without foot ulcer) & Group II (type 2 diabetic subjects without foot ulcer) in HbA1c levels which may explained by that our diabetic patients with foot ulcer weren’t on intensive therapy during sampling for the research which may be due to delay seek for intensive therapy in our patients. This finding was in contrast with Mehmet et al who found difference between groups in HbA1c. He found that HbA1c levels in the non ulcer patients were statistically higher than the ulcer patients. Lower HbA1c levels in patients with foot ulcer were explained by intensive therapy in this group and this finding was regarded as a temporary characteristic of their study group. In our study similarity in HbA1c levels between group I & group II make homocysteine effect will have been far more meaningful. We recommend other studies using large scale.

**Conclusion:**

It can be concluded that serum hyperhomocysteinemia was associated with diabetic patients and carry risk for development of diabetic foot ulcers. It is safe and simple to measure homocysteine. A cheap treatment could lower the homocysteine levels in most patients, therefore, in practice homocysteine levels should be measured routinely before the development of diabetic foot ulcer and high levels should be given treatment to lower homocysteine levels which may be consider as prophylactic treatment to diabetic foot ulcer and it will be cost effective. However, this finding can be used in the, prevention of the development of diabetic foot ulcer especially if further studies will be done on large scale of diabetic Egyptian patients.

**References**

الملخص العربي

ترجح القسم السكري واحد من أهم الأساليب المؤدية إلى القلب القديم كما أن ترجح القسم والبتر من الأسباب التي تؤدي إلى الوفاة في مرضى السكر من النوع الثاني.

ترجح القسم السكري له طرق حديثة عدة منها زيادة الضغط الدوري والتخبط العصبي والختم للمضادات الخارجية وأمراض الشرايين الطرفية.

كثير من الدراسات وجدت زيادة في مستوى الهوموسيستاتين في مرضى السكر من النوع الثاني الذين يعانون من مضاعفات على مستوى الأوعية الدموية كما وجد ان ارتفاع مستوى الهوموسيستاتين له علاقة بالشفاء البطيء ترجح قسم السكري.

هدف البحث: تقييم العلاقة بين مستويات الهوموسيستاتين وترجحات القسم السكري في المصابين بالنوع الثاني من مرضى السكر.

تمت الدراسة على ثلاث مجموعات: المجموعة الأولى وتشمل 35 مريض بالسكر من النوع الثاني غير مصابين بترجح السكري والمجموعة الثانية وتشمل 25 مريض بالسكر من النوع الثاني ومصابين بترجح السكري والمجموعة الثالثة وتشمل 20 شخص لا يعانون من أي مرض.

تم عمل التحالل الريتاني للمجموعات الثلاث بالإضافة إلى مستوى سي او بب ومستوى الهوموسيستاتين.

بمقارنة نتائج المجموعة الأولى والثانية وجدت فروق احصائي ذات قيمة بينهم بالنسبة لمستوى الهوموسيستاتين كما وجدت فروق احصائي ذات قيمة عند مقارنة كلا من المجموعة الأولى والثانية والمجموعة الثانية بالنسبة لمستوى الهوموسيستاتين مما يجعلنا نستنتج صحة مستويات الهوموسيستاتين العالية ترجح القسم السكري في المصابين بالنوع الثاني من مرضى السكر.