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Elevated Plasma Level of Homocysteine is an Independent Risk Factor for Peripheral Neuropathy

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Authors' contributions

This work was carried out in collaboration between all authors. Author JJL designed and supervised the study, wrote the protocol, and wrote the first draft and critical revision of the manuscript. Authors KS and AN collected data and wrote critical revision of the manuscript. Author NJD involved in study concept, wrote the critical revision of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Introduction: Elevated plasma level of homocysteine (eHcy) is associated with increased prevalence of peripheral neuropathy (PN) in diabetic patients. However, it is not known whether eHcy is an independent risk factor for the development of PN.
Methods: We retrospectively reviewed clinic and laboratory data of patients with PN, and patients with headaches serving as controls. The study consisted of two separate cohorts in two different settings. Setting-A was designed to address whether the *isolated* eHcy is relevant to PN and setting-B to analyze various risk factors in patients with PN.
Results: Fifty seven and 217 subjects with PN and 42 and 252 individuals with headache were included in the setting-A and setting-B, respectively. A significantly elevated level of homocysteine was observed in the patients with PN in both setting-A and setting-B (11.3±7.1 and 13.4±14.6 μmol/L, mean±SD) than in the patients with headaches (8.6±2.8 and 8.1±2.5, *P*=.02 and *P*=.02, respectively). In addition, significantly increased frequency of eHcy was observed in PN (21% and 38% in setting-

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A and setting-B) than that in headache controls (4.5% and 4.8%; *P*= .05 and *P*= .002, respectively).
Conclusion: eHcy may potentially act as an independent risk factor for the development of PN.

Keywords: Homocysteine; neuropathy; vitamin deficiency; risk factor.

1. INTRODUCTION

According to the 2005 US Congress report, there are twenty million Americans suffering from peripheral neuropathy (PN) [1]. PN may be caused by numerous etiologies, such as metabolic, infectious, inflammatory, and toxic (including adverse effects of certain drugs and radiation), malnutritional, inherited, or autoimmune-mediated mechanisms). However, a large percentage (32-70%) of all neuropathies remains "idiopathic" after a routine clinical investigation. Patients who were diagnosed with idiopathic neuropathy may have a heretofore unidentified etiology, which is less than optimally investigated [2]. Recent clinical studies disclosed that elevated plasma level of homocysteine (eHcy) exaggerates the prevalence of PN in diabetics and exacerbates the preexisting diabetic neuropathy [3-5]. Since eHcy is a treatable condition, we performed a retrospective study to evaluate whether eHcy is an independent risk factor for PN.

2. METHODS

A retrospective review was conducted on clinical and laboratory data of patients with PN and patients with headaches as controls from October 2004 to October 2009. This study was approved by the Institutional Review Board of Temple University. The study included two separate cohorts in two different settings. In setting-A, straightforward analysis of the frequencies of "*isolated*" eHcy in patients with PN and patients with headaches were performed using the data from October 2004 to December 2007. Setting-A was performed to address whether the "*isolated*" eHcy, a condition that was without any identifiable etiology for PN and with normal findings of vitamin B12, folic acid, and other laboratory results, is relevant to the increased frequency of PN. Setting-B consisted of analysis of multiple risk factors for PN using the data from January 2008 to October 2009, to confirm if eHcy is a risk factor for PN. PN was defined as the clinical findings from history, clinical manifestation, and neurologic examination, such as presenting with numbness and tingling of sensory deficits with or without weakness in distal limbs, decrease or absence of at least two of the following three sensory modalities or reflexes of either foot: light touch or temperature sense, ankle reflex, and vibration sensation [6]. Continuous variables are presented as mean ± standard deviation (SD) and discrete variables, as ratio or percentage, and compared by two-tailed Student t or chi-squared (Fisher's exact) tests in univariate analysis, as appropriate. Alpha error level was set to 0.05.

In setting-A, subjects with a clinical diagnosis of PN or headache were initially identified through chart review. Individuals with a complete laboratory data set including plasma homocysteine (Hcy) with a normal level of vitamin B12 and folic acid were recorded. Measurement of Hcy was performed by a commercial laboratory (Quest Diagnostics, Inc., Philadelphia, PA, USA). eHcy was determined according to the laboratory provided reference (abnormal: >12.5 μmol/L). The frequency of eHcy in the cohort was analyzed. Inclusion criteria comprised any of the following: i) patients with a diagnosis of PN based on

a retrospective chart analysis at the neuromuscular clinic; ii) subjects with headaches, without any other neurological abnormality seen at the general neurology clinic (controls). Exclusion criteria were any of the following: i) absence of simultaneously measured fasting plasma levels of Hcy, vitamin B12 and folic acid.; ii) pregnancy; and iii) concomitance of headache and PN. For setting A, which analyzed the prevalence of isolated eHcy, additional exclusion criteria were: i) deficiency of B12 or folic acid; ii) presence of an identifiable etiology for PN, such as diabetes, renal or liver dysfunction, other metabolic, endocrinologic, infectious, inflammatory, and inherited disorders, neoplasms, and exposure to environment or medical neurotoxins, such as illicit substance use, chemotherapy, and radiotherapy. Fifty seven PN subjects (age: 54±12 years, male/female=26/31) and 42 headache subjects (age: 51±14 years; male/female =9/35) were recruited in setting-A.

In setting-B, successive patients with a clinical diagnosis of PN seen in the neuromuscular clinic and individuals with a clinical diagnosis of headache seen in the general neurology clinic were identified. Their past medical history including the concomitant medical conditions, and various laboratory data, including hematogram, creatinine, hemoglobulin A1c (HbA1c), cholesterol, triglycerides, vitamin B12, folate, methylmanolic acid, Hcy, copper, zinc, hepatitis panel, thyroid function tests, *erythrocyte sedimentation rate,* C-reactive protein, antinuclear antibody (ANA), *angiotensin-converting enzyme, and human immunodeficiency virus*, were collected and analyzed. In setting-B, 217 subjects with PN (age: 59±14 years; male/female: 91/126), and 252 with headaches (age: 47±15 years; male/female: 48/204) were included.

Two-tailed Chi-square and *t*-test analyses were employed for setting-A. One-way analysis of variance, two-tailed Chi-square and *t*-test was performed for setting-B. *P* values less than 0.05 were considered statistically significant.

3. RESULTS

In setting-A, the plasma Hcy level was significantly higher (11.3±7.1 μmol/L) in patients with PN as compared to that in headache controls (8.6±2.8; *P*= .02). The frequency of the "*isolated*" eHcy was 21% (12 of 57) in PN patients compared to 4.8% (2 of 42) in headaches, which was statistically significant (*P*= .05; Table 1). The levels of B12 and folate were all within normal limits and no significant difference between the PN and headache groups was observed.

Table 1. Measurements in patients with *isolated* PN and patients with *isolated* headache

Measures	PN	Headache	P
Hcy (μmol/L)	11.3±7.1	8.6±2.8	0.02
Vit B12 (pg/mL)	594±229	573±198	0.6
Folate (ng/mL)	15.2±7.9	14.6±5.6	0.7
eHcy	21.1% (12/57)	5% (2/42)	0.05

In setting-B, subjects with PN, when compared with headache controls, showed significantly elevated plasma levels of creatinine (*P*= .01), Hcy (*P*= .02), and a decreased level of vitamin B12 (*P*=.03) (Table 2). Interestingly, an increased level of high-density lipoprotein (HDL) (*P*=.05) and decreased ratio of low-density lipoprotein (LDL) to HDL (*P*=.01) were also noted. No statistically significant difference was seen in the remaining variables analyzed

(see the Supplimentary Table 1). Next, the frequencies or the ratios of the concomitant medical conditions were analyzed. Significantly increased frequencies of comorbidities were seen in the PN group, as compared to those in headaches, including diabetes mellitus (*P*=.01), chronic renal disease (*P*=.01), alcohol consumption (*P*=.001), B12 deficiency (*P*=.01), and eHcy (*P*= .002) (Table 3). However, no significant differences were seen in other conditions, including the ratio of LDL/HDL (see the Supplimentary Table 2 and Supplimentary Table 3). Some variables were not analyzed due to incomplete data, e.g. plasma levels of copper and zinc, frequency of sarcoidosis, gout, or lupus. No difference was seen in use of tobacco, or illicit drugs between the PN and headache groups (see the Supplimentary Table 2).

Table 2. Plasma levels of B12, creatinine and homocysteine in patients with PN or headache

	PN	Headache	P
B12	480±229 pg/mL	560±223 pg/mL	0.03
Creatinine	1.3±1.7 mg/Dl	0.9±0.4 mg/dL	0.01
Hcy	13.4±14.6 μmol/L	8.1±2.5 μmol/L	0.02

Table 3. Frequency of hyperhomocysteinemia and other comorbidities among patients with PN or with headache

	PN	Headache	P
Homocysteine > 12.5 μmol/L	38%	5%	0.002
Diabetes mellitus	31%	18%	0.01
End stage of renal disease	4%	0%	0.01
B12 < 400 pg/mL	46%	24%	0.01
Alcohol consumption	18%	7%	0.001

4. DISCUSSION

Elevated Hcy is an established risk factor for cardiovascular diseases [7,8]. However, to the best of our knowledge and after an extensive MedLine search, there is no reported clinical study on the relationship between the occurrence of the *isolated* eHcy and the development of PN in the literature. Although several studies showed that eHcy increased the prevalence of PN in diabetes and exacerbated the preexisting diabetic-neuropathy, only data from animal models and *in vitro* experiments have linked homocysteine directly to neuropathy [9,10].

In our initial setting-A study, we aimed directly to address if eHcy is related to the increased frequency of PN. Notably, the entries of the subjects with the measures of plasma Hcy in setting-A were in a restricted condition that precluded any identifiable clinical etiologies and laboratory abnormalities that may be linked with a cause of PN. The findings from the setting-A showed significantly increased level and frequency of eHcy, with normal levels of vitamin B12 and folic acid, in patients with PN when compared with controls (Table 1), indicating that the *isolated* eHcy may potentially act as a risk factor for the development of PN. Of note, a normal level of Hcy was observed in some patients in the PN group, which suggests that there could be additional, yet to be identified, etiologies causing PN.

Next, we analyzed multifactorial conditions that may be considered as risk factors relevant to PN. Our findings from the setting-B study confirmed the findings from the setting-A showing that eHcy was independently relevant to the increased frequency of PN. Additionally, our findings from the setting-B also confirmed the previously recognized risk factors for PN, such as chronic renal insufficiency, diabetes, alcohol consumption, and vitamin B12 deficiency (Tables 2 and 3). A population-based study on the relationship between known risk factors and PN was conducted by Hoogeveen and colleagues, who suggested eHcy is not a risk factor for distal somatic PN [6]. Notably, their data were analyzed based on the glucose tolerance stratified random samples in a general population of 50- to 75-years old, in which the relationship between eHcy and PN may have been diluted. Additionally, their study did not address the "*isolated*" eHcy condition but, instead five subjects with possibly isolated eHcy were excluded from the analysis [6]. Our setting-A study analyzed the *isolated* eHcy condition which eliminated the possible multi-factorial interaction related to PN, and the findings of setting-B were in agreement with the conclusion from studies on eHcy and DM that eHcy exaggerates the prevalence of PN in diabetics [3-5].

Interestingly, although an increased level of HDL with decreased ratio of LDL/HDL was initially noted in our multi-variable analysis, it failed to show a similar tendency in the frequency assay (see the Supplimentary Table 3). The discrepancy between the measurements of individual HDL values and the frequency of the ratios of LDL/HDL may be related to a statin administration in the clinic, with apparently therapeutic effects of increase in the level of HDL and decrease in LDL. However, our data was insufficient to suggest, nor to refute, that increased HDL with decreased ratio of LDL/HDL was relevant to PN, although statin-induced neuropathy has been reported in the literature [11-13].

It has previously been noted that age and gender may contribute to the development of PN [14,15]. Female dominant tendency in PN has been reported in the literature [3] and was consistent with our current observation. Headaches are more commonly seen in women [16] and elderly may suffer from more comorbidities than younger adults. Apart from gender and age factors, the subjects in PN and headache group were comparable in their general medical conditions in our study. No significant differences were observed in other laboratory findings, such as plasma levels of glucose, HbA1c, and ANA (see the Supplimentary Table 2 and Supplimentary Table 3). Lack of statistically significant differences in plasma levels of glucose and HbA1c between these two groups in setting-B suggested that diabetes, if presented, was well controlled. A previous study indicated that the duration of diabetes, not the level of HbA1c, is an important predictor for diabetic neuropathy [4,17,18]; and aggressive control of diabetes delays or prevents the development of diabetic neuropathy [19].

It is well known that eHcy can be caused by deficiency of either B12 or folate alone or in combination, while elevated methylmanolic acid is only caused by B12 deficiency [20]. Elevated methylmanolic acid is a sensitive biomarker for vitamin B12 deficiency; the latter is a well-known etiology for both central and peripheral nervous system dysfunction, as seen in sub-acute combined degeneration and PN [21,22]. Plasma Hcy level is determined by both genetic and environmental factors. Hcy is a demethylated derivative of the essential amino acid methionine which undergoes metabolism by two pathways: catabolism by trans-sulphuration and remethylation back to methionine. Three enzymes, each of which requires the presence of a vitamin, are involved in these two metabolic pathways; cystathionine β-synthase requires vitamin B6, methionine synthase requires folate and vitamin B12, and methylenetetrahydrofolate reductase (MTHFR) requires folate for its normal function.

Dysfunction of methylation, as a result of either deficiency of the vitamin cofactors in question or alteration in enzymatic activities, results in eHcy.

Individuals who were found to have eHcy with normal levels of B12 and folate may have a genetic variation of enzymes involved in homocysteine metabolism, for example, MTHFR polymorphisms. Though controversy remains, dietary and lifestyle conditions such as excessive coffee or alcohol consumption, cigarette smoking, and exercise may play a role in the development of eHcy [23]. However other unidentified mechanisms causing eHcy may exist. Clinical observations showed a high frequency of eHcy in patients with type-1 and type-2 diabetes [24,25]. There is an independent association between eHcy level and glucose utilization [26]. In a streptozotocin–treated rat model of diabetes, insulin has been shown to have a direct role in regulating the metabolism of Hcy. Administration of insulin in insulin-deficient diabetic rats resulted in an increased level of hepatic cystathionine β-synthase and its mRNA, a trans-methylation enzyme that catalyzes the conversion of Hcy to cystathione [27], and a reduced level of eHcy [28]. *In vitro* studies suggest that the pathogenesis of vascular diseases associated with eHcy is related to endothelial dysfunction, smooth muscle proliferation, and abnormalities of coagulation [7] which may potentially contribute to the development of some types of PN, such as non-arteritic anterior ischemic optic neuropathy [29]. In addition, increasing Hcy levels could enhance the vulnerability of neurons to excitotoxic [30,31] and oxidative injury [31-35] *in vitro* and *in vivo*; indicating that Hcy may serve as an excitatory amino acid [36,37]. Administration of a very high (200 times higher than physiologic) concentration of Hcy to cell cultures caused astroglial cell death [38]. Association of eHcy with exacerbation of preexisting diabetic neuropathy has been observed in humans [3-5]. It was estimated that an increase of 5 μmol/L in Hcy levels would increase the odds ratio to 2.6 for PN (95% confidence interval 1.07±6.33) in diabetic patients [4]. Heretofore the exact cellular mechanism of eHcy in causing PN remains to be elucidated [39].

There are limitations to our study. Firstly, the study is retrospective with a relatively small sample size. Secondly, the mean age of the subjects was younger in the headache group than that in the PN group, likely due to the fact that younger women suffer from headaches more often than men [16]. Thirdly, genetic evaluation, such as MTHFR polymorphism status, was not measured. Fourthly, the plasma level of vitamin B6, a cofactor of cystathionine β-synthase, was not measured. Finally, the dietary style of the subjects was not investigated.

5. CONCLUSION

Taken together, findings of the increased frequency of *isolated* eHcy seen in patients with PN suggest that eHcy may potentially be an independent risk factor for the development of PN. Our pilot finding suggests significant clinical implications because administration of vitamin B-complex with folate to reduce eHcy is an inexpensive and potentially effective regimen, though the efficacy in treating eHcy-related PN remains to be elucidated [36,37,39]. Large-scale and prospective studies may be needed to validate our finding and to explore the role of the *isolated* eHcy in the pathogenesis of PN.

CONSENT

Not applicable.

ETHICAL APPROVAL

This study was approved by the Institutional Review Board of Temple University.

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COMPETING INTERESTS

All authors have declared that no competing interests exist.

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