Research Article

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Clinical significance of serum homocysteine as a biomarker for early diagnosis of diabetic nephropathy in type 2 diabetes mellitus patients

<https://doi.org/10.1515/pteridines-2020-0025>

received December 19, 2020; accepted February 24, 2021

Abstract

Objective – The aim of this study was to investigate the clinical significance of serum homocysteine (Hcy) as a biomarker for early diagnosis of diabetic nephropathy (DN) in type 2 diabetes mellitus (T2DM) patients.

Methods – Fifty-five T2DM patients with DN and 51 T2DM patients without DN were prospectively recruited from January 2016 to May 2020 in our hospital. The serum Hcy was tested by electrochemiluminescence assay in DN and T2DM groups and compared. The diagnostic efficacy of serum Hcy as a biomarker for early diagnosis of DN was evaluated by calculating the diagnostic sensitivity, specificity and area under the ROC curve (AUC).

Results – The serum levels of Hcy were 15.49 ± 5.40 and 9.23 ± 3.15 $\mu\text{mol/L}$ for DN and T2DM patients, respectively, with statistical difference ($t = 7.21$, $P < 0.001$). In the DN group, the serum Hcy levels for patients with hyperfiltration, intermittent proteinuria, microalbuminuria, macroalbuminuria and uremic were 10.99 ± 2.57 , 13.90 ± 2.86 , 15.38 ± 4.77 , 18.98 ± 4.36 and 23.31 ± 5.22 $\mu\text{mol/L}$, respectively, which indicated that serum Hcy levels in DN were higher than those of T2DM patients and correlated with patient's renal damage. Using the serum Hcy level as the reference, the diagnostic sensitivity, specificity and AUC were 84.31 (71.41–92.98)%, 74.55 (61.00–85.33)% and 0.85 (0.78–0.92)%, respectively, with the cutoff value of 12.08 between DN and T2DM. The serum Hcy also had relatively good differential diagnostic efficacy

between different DN stages with high sensitivity, specificity and AUC.

Conclusion – Serum Hcy was obviously elevated in DN compared to T2MD and correlated with the renal damage severity, which can be applied as a potential serological marker for early diagnosis of DN.

Keywords: homocysteine, diabetic nephropathy, type 2 diabetes mellitus, biomarker

1 Introduction

Diabetes mellitus (DM) is one of the most frequent metabolic diseases and the prevalence of DM is rising [1,2]. Diabetic nephropathy (DN), mostly characterized by proteinuria, is one of the most common microvascular complications of type 2 diabetes mellitus (T2DM) [3,4]. For diagnosis of DN, serum creatinine and urinary albumin were generally measured [5–7]. Hypertension, edema, proteinuria, systemic microvascular disease and even renal failure were often identified in the uremia stage of DN patients [8]. Therefore, early identification of DN and proper intervention had important clinical significance in the aspects of delaying the development of renal failure [9–11].

Hcy is a kind of sulfur-containing amino acid, which is an intermediate product in the process of methionine metabolism [12]. It metabolizes through methylation and trans-sulfurization, maintaining the two major abilities of human body: methylation and antioxidation. Folic acid and vitamin B12 play an important role in the Hcy metabolic cycle [13–15]. Hcy was first isolated from bladder stones in 1931 [16]. Subsequent studies found that the increased serum Hcy level was an independent risk factor for cardiovascular and cerebrovascular diseases [17,18]. In recent years, it has been reported that serum Hcy level was correlated with degree of renal function damage [19]. With the development of renal damage, serum Hcy was also increased correspondingly [20].

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Therefore, we performed a prospective study in order to investigate the clinical significance of serum Hcy as a biomarker for early diagnosis of DN in T2DM patients. In this study, we selected T2MD patients with DN with different clinical stages and T2MD cases without DN as the study objects to explore whether the serum Hcy level is different in different stages of DN and T2DM.

2 Materials and methods

2.1 Patients

Fifty-five T2DM patients with DN and 51 T2DM patients without DN were prospectively recruited from January 2016 to May 2020 in the Sixth Affiliated Hospital of Wenzhou Medical University. The inclusion criteria were as follows: (1) the patients were older than 18 years; (2) all the patients were diagnosed with T2MD according to the WHO diagnostic criteria for DM in 1999; (3) the patients also met the diagnostic criteria for DN designated by the American Diabetes Association in 1977; (4) the patients did not take any drugs affecting renal function within 4 weeks before enrollment; and (5) no folic acid and VB12 intake within 3 months. The exclusion criteria were as follows: (1) patients with type 1 diabetes mellitus; (2) patients with malignant tumor; (3) patients with other primary kidney diseases such as IgA nephropathy and glomerulonephritis; (4) cases of acute infection; (5) patients with acute cerebral tissue or myocardial infarction; and (6) pregnant or lactating women.

In the DN group, there were 55 cases with 28 males and 27 cases, aged 41–78 years (58.6 ± 12.8). Of the 55 patients, 14 cases had hyperfiltration, 16 cases had intermittent proteinuria, 8 cases had microalbuminuria, 10 cases had massive proteinuria and 7 cases had uremia. There were 25 males and 26 females with a mean age of (54.3 ± 14.1) years (range, 33–79 years) in the T2MD group (Table 1). The gender distribution and mean age were not statistically different between DN and T2MD groups.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance with the tenets of the Helsinki Declaration and has been approved by ethical committee of the Sixth Affiliated Hospital of Wenzhou Medical University.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Table 1: Basic data of the DN and T2DM groups

Group	DN (<i>n</i> = 55)	T2DM (<i>n</i> = 51)
Gender (<i>n</i>)		
Male	28	25
Female	27	26
Age (years)		
Range	41–78	33–79
Mean $\pm$ SD	58.6 ± 12.8	54.3 ± 14.1
Hyperfiltration (<i>n</i>)	14	0
Intermittent proteinuria (<i>n</i>)	16	0
Microalbuminuria (<i>n</i>)	8	0
Massive proteinuria (<i>n</i>)	10	0
Uremia (<i>n</i>)	7	0

2.2 Serum Hcy examination

After fasting for 12 h, 5 mL of the peripheral venous blood was collected from the included cases. The peripheral venous blood was centrifuged for 10 min under 3,000 rpm and then the upper serum was collected for Hcy detection through electrochemiluminescence assay.

2.3 Statistical analysis

All data were analyzed through the statistical software STATA11.0 (<http://www.stata.com>). Serum Hcy was expressed by $\bar{x} \pm s$ and measured by Student's *t*-test between DN and T2DM groups. For early diagnosis of Hcy as a biomarker for DN, the diagnosis sensitivity, specificity and area under the ROC curve (AUC) were calculated according to Bayes' theorem. Two tailed $P < 0.05$ was deemed to be statistical difference significant.

3 Results

3.1 Serum Hcy in DN was higher than that of T2DM patients and correlated with patient's renal damage

The serum levels of Hcy were 15.49 ± 5.40 and 9.23 ± 3.15 $\mu\text{mol/L}$ for DN and T2DM patients, respectively, with statistical difference ($t = 7.21$, $P < 0.001$) (Figure 1a). In the DN group, the serum Hcy levels for patients with hyperfiltration, intermittent proteinuria, microalbuminuria, macroalbuminuria and uremic were 10.99 ± 2.57 , 13.90 ± 2.86 , 15.38 ± 4.77 , 18.98 ± 4.36 and 23.31 ± 5.22 $\mu\text{mol/L}$,

Figure 1: Scatter plot of serum Hcy in DN and T2DM patients and correlated with patient's renal damage. (a) Serum Hcy level between DN and T2DM patients. (b) Serum level of Hcy of DN patients with different renal damage.

respectively, which indicated that serum Hcy in DN was higher than that of T2DM patients and correlated with patient's renal damage (Figure 1b).

3.2 Serum Hcy had high efficacy for early diagnosis of DN in T2DM patients

Using serum Hcy as the reference, the diagnostic sensitivity, specificity and AUC were 84.31 (71.41–92.98)%, 74.55 (61.00–85.33)% and 0.85 (0.78–0.92)%, respectively, with the cutoff value of 12.08 between DN and T2DM groups (Figure 2a). The serum Hcy also had relatively good differential diagnostic efficacy between different DN stages with high sensitivity, specificity and AUC (Table 2 and Figure 2b–k).

4 Discussion

In the present work, we found that the serum level of Hcy was obviously elevated in DN cases compared to T2DM patients and correlated with patient's renal damage severity. Serum Hcy can also be used as a serological marker for early diagnosis of DN with relatively high sensitivity and specificity. Wang et al. evaluated the association between plasma Hcy and progression of early nephropathy in T2DM patients and found that plasma Hcy was significantly associated with eGFR decline after controlling for other progression parameters. The results demonstrated that plasma Hcy was an independent risk

factor as well as an early predictor for DN progression in T2DM patients [21]. Deebukkhum et al. [22] also found that Hcy was correlated with nephropathy development and could be used as a potential biomarker for early diagnosis of DN. All of the aforementioned relevant studies and our present work demonstrated that serum Hcy may be a potential biomarker for early diagnosis of DN. Furthermore, compared with the previously relevant study, our work also presents the diagnostic sensitivity, specificity and AUC for early diagnosis of DN, which was more useful for clinical practice. DN is a common chronic microvascular complication in T2DM patients [23]. The main cause of DN is the kidney damage caused by persistent hyperglycemia, which can injure the whole kidney, including glomerulus, renal interstitium and renal vessels. In addition, if the T2DM cases also smoke, have hypertension, hyperlipidemia and other risk factors, the patients would be more likely to develop DN [24–26]. In clinical practice, there are several parameters, including serum creatinine, urea nitrogen, serum cystatin C and serum Hcy, which can be used as reference for clinical diagnosis of DN.

Although serum creatinine and urea nitrogen levels can reflect the degree of renal damage in patients with DN, in clinical application, it is found that serum creatinine level was easy to be affected by body mass index, drug influence, metabolic difference, protein intake and other factors in patients with DN [27,28]. Usually, the level of serum creatinine will increase when the renal function damage is more than 50% [29]. Urea nitrogen is also easily affected by heart function and other factors, and the sensitivity of early diagnosis of DN is relatively low [30].

Figure 2: ROC curve of serum Hcy as a biomarker for early diagnosis of DN in T2DM patients. (a) DN vs T2DM; (b) hyperfiltration vs intermittent proteinuria; (c) hyperfiltration vs microalbuminuria; (d) hyperfiltration vs macroalbuminuria; (e) hyperfiltration vs uremic; (f) intermittent proteinuria vs microalbuminuria; (g) intermittent proteinuria vs macroalbuminuria; (h) intermittent proteinuria vs uremic; (i) microalbuminuria vs macroalbuminuria; (j) microalbuminuria vs uremic; and (k) macroalbuminuria vs uremic.

Hcy is the intermediate product of cysteine and methionine [31,32]. Relevant studies have shown that serum Hcy had positive correlation with proteinuria in

DN patients, which can reflect the degree of renal function damage in DN patients [33]. At the same time, compared with serum creatinine, urea nitrogen and other

Table 2: Diagnostic efficacy of serum Hcy as a biomarker for early identification of DN in T2DM patients

Renal damage	Sensitivity (%)	Specificity (%)	AUC	Cutoff
DN vs T2DM	84.31 (71.41–92.98)	74.55 (61.00–85.33)	0.85 (0.78–0.92)	12.08
Hyperfiltration vs intermittent proteinuria	81.25 (54.35–95.95)	71.43 (41.90–91.61)	0.79 (0.63–0.95)	12.50
Hyperfiltration vs microalbuminuria	75.00 (34.91–96.81)	78.57 (49.20–95.34)	0.79 (0.57–1.00)	12.93
Hyperfiltration vs microalbuminuria	90.00 (55.50–99.75)	85.71 (57.19–98.22)	0.94 (0.84–1.00)	13.56
Hyperfiltration vs uremic	100 (59.04–100)	85.71 (57.19–98.22)	0.98 (0.92–1.00)	13.81
Intermittent proteinuria vs microalbuminuria	62.50 (24.49–91.48)	62.50 (35.43–84.80)	0.62 (0.35–0.90)	14.68
Intermittent proteinuria vs macroalbuminuria	80.00 (44.39–97.48)	81.25 (54.35–95.95)	0.84 (0.66–1.00)	16.66
Intermittent proteinuria vs uremic	85.71 (42.13–99.64)	93.75 (69.77–99.84)	0.95 (0.84–1.00)	18.05
Microalbuminuria vs macroalbuminuria	80.00 (44.39–97.48)	62.50 (24.49–91.48)	0.73 (0.49–0.96)	16.93
Microalbuminuria vs uremic	85.71 (42.13–99.64)	87.50 (47.35–99.68)	0.89 (0.71–1.00)	19.43
Macroalbuminuria vs uremic	71.43 (29.04–96.33)	80.00 (44.39–97.48)	0.76 (0.51–1.00)	22.59

indicators, serum Hcy is not easily affected by the patient's age, gender, inflammation and other factors. Xu et al. [19] evaluated serum Hcy and cystatin C as biomarkers for progression of DN. They found that serum levels of Hcy and cys-C in DN patients were elevated compared to those of simple T2DM cases, making them potential biomarkers for early diagnosis of DN in T2DM patients. In our present work, we further divided the DN cases into five subgroups: hyperfiltration, intermittent proteinuria, microalbuminuria, massive proteinuria and uremia and evaluated the differential diagnosis ability of serum Hcy for the four subgroup cases. We found that serum Hcy was obviously elevated in DN compared to T2DM and correlated with the renal damage severity, which can be applied as a potential serological marker for early diagnosis of DN. However, many factors may affect Hcy levels in DM such as 5-methylenetetrahydrofolate reductase, cystathionine- β -synthase, insulin, fasting, non-fasting states and so on [34]. Serum Hcy is also easily affected by folate [35] and VB12 levels *in vivo*. Therefore, if the patients take folic acid and VB12 during Hcy detection [36], Hcy cannot reflect the renal function damage of DN patients.

In conclusion, Hcy was increased in serum of DN cases and correlated with the severity of renal damage in T2DM cases. Combined detection of serum Hcy and other parameters such as serum creatinine and urinary albumin may improve the early DN diagnosis efficacy and patient prognosis. However, the work also had several limitations. First, the sample size is relatively small with 55 DN and 51 T2DM cases. Second, all of the cases were from a single medical center, and the patients' selection bias seems to be ineluctable. Third, due to short-term follow-up, the correlation between serum Hcy and DN patient prognosis was not investigated.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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