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Coefficient of variation of R–R intervals in electrocardiogram is a sensitive marker of anemia induced by autonomic neuropathy in type 1 diabetes

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Abstract

The present study investigated the relationship between hemoglobin (Hb) levels and autonomic failure using a sensitive marker, coefficient of variation of R–R intervals in electrocardiogram (CVR-R) in order to clarify a cause of normocytic normochromic anemia in type 1 diabetic patients without overt nephropathy. We recruited 46 patients with type 1 diabetes and measured creatinine clearance (Ccr), HbA1c, albuminuria, Hb levels and CVR-R of all patients. In addition, the status of diabetic retinopathy and neuropathy were also evaluated. Serum erythropoietin (EPO), Fe, total iron binding capacity, lactate dehydrogenase, total bilirubin levels and number of reticulocytes and mean corpuscular volume were also measured to distinguish types of anemia. To survey the statistical correlation existing between Hb and body mass index (BMI), Ccr, HbA1c, albuminuria or retinopathy, multiple regression analysis was performed. Serum EPO, Fe, TIBC, LDH and TB levels and number of reticulocytes and MCV were within normal limits. Multiple regression analysis disclosed that HbA1c, nephropathy evaluated by albuminuria and Ccr, and retinopathy has no concern with Hb level. There is only significant relationship between Hb levels and CVR-R. Similar results were obtained even if we analyzed a group of male and female separately. We conclude that CVR-R has the strong relationship on anemia without overt nephropathy in type 1 diabetes, indicating that autonomic failure contributes on the progression of anemia via a poor response of EPO to anemia.

Keywords: Anemia; CVR-R; Autonomic failure; Diabetic nephropathy; Type 1 diabetes

1. Introduction

Concomitant with progression of renal failure, normocytic normochromic anemia also deteriorates. Renal anemia is considered to initiate gradually when creatinine clearance (Ccr) decreases down to about 30 or 40 ml/min [1,2]. Impairment of erythropoietin (EPO) production to anemia is considered to be a main cause, and treatment with human recombinant EPO is very effective [3–6]. It is commonly known that replacement therapy with EPO not only delays progression of renal failure but also improves clinical symptoms [6–8].

Patients with type 1 diabetes without renal failure often show normocytic normochromic anemia for no clear reason [9]. It has been argued that anemia is induced by a poor response of EPO to low hemoglobin (Hb) level and may be related to a severity of autonomic neuropathy; however, the clear reason for anemia remains to be elucidated [10–14].

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In order to address the causal factors related to anemia, we conducted a cross-sectional study to examine the relationship between anemia and autonomic neuropathy among a group of patients with type 1 diabetes. This study was done by comparing Hb levels and coefficient of variation of R–R intervals in electrocardiogram (CVR-R), an established sensitive and quantitative method for estimating autonomic neuropathy [15,16].

2. Research design and method

2.1. Patients

Patients were recruited from the Division of Diabetes and Endocrinology, the Jikei University hospital. All patients started insulin treatment within several months of the onset of diabetes, and since their urinary excretion of C-peptide was less than 20 μg/day (normal value: 20.5–198), they were diagnosed to have type 1 diabetes.

Inclusion criteria were age <70 years, creatinine clearance >40 ml/min (normal value: 70–130) and serum creatinine <120 μmol/l. Subjects with Hb <13 g/dl in males and <12 g/dl in females were defined as anemic [17].

Patients taking medicines that may have had an influence on the Hb level or nervous system were excluded. Patients who had normocytic normochromic anemia that could be related to other causes, such as hemolysis, previous history of gastrectomy, chronic infectious disease or gastrointestinal bleeding, were excluded. Based on medical and neurological evaluations, patients with conditions such as familial, alcoholism and nutritional neuropathy were excluded. Additionally, subjects with complications of orthopedic disease, immunological neuropathy, arteriosclerosis obliterans, or neuropathy associated with collagen disease were also excluded.

2.2. Methods

Serum EPO levels were measured by a radioimmunoassay using the double-antibody sandwich method (Recombigen RIA Kit, Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). Routine laboratory tests and hematological values were provided by our clinical laboratory. Ccr was calculated from 24 h urinary creatinine excretion and serum creatinine concentration, and adjusted for both height and body weight.

If present, the condition of diabetic retinopathy evaluated by ophthalmologists was classified into two stages comprising no detectable change and simple/proliferative retinopathy. Diabetic nephropathy was assessed by albuminuria, serum creatinine level and Ccr. Albuminuria was divided into two groups consisting of none detectable and micro/macroalbuminuria. Albuminuria and non-albuminuria were also defined as ≥30 mg/day and <30 mg/day, respectively. Vibration test by the on–off method was conducted using a tuning fork applied to the bilateral malleolar surface of talus. The patient was asked to report the perception of both start of the vibration sensation and the cessation of vibration. We diagnosed that vibration sensation has fallen off significantly, in the case if it was 10 or fewer seconds. Gastroparesis was judged from the subjective symptom such as nausea, vomiting or unpleasant feeling of upper abdomen. Leg numbness also was judged from the subjective symptom such as plantar and/or toe numbness. Orthostatic hypotension was defined as a decrease of 20 mmHg or more in systolic blood pressure confirmed by an inadequate response to standing after 10 min supine. Deep tendon reflex (DTR) was evaluated using hammer at Achilles tendon. CVR-R at rest is measured as R–R intervals of 100 samples on electrocardiogram. CVR-R is calculated as follows: CVR-R = S.D./average of R–R intervals × 100%. CVR-R reflects autonomic nerve function; therefore, it is used to evaluate diabetic neuropathy [18]. CVR-R measured in each subject is adjusted to make it possible to compare each result between groups of different ages. At first, we measured

Table 1
Patient's profiles in type 1 diabetes

	Anemic group		Non-anemic group	
	Male	Female	Male	Female
n	7	13	13	13
Age (years)	53.3 ± 10.6	48.2 ± 13.6	46.1 ± 15.1	39.7 ± 13.2
Duration of diabetes (years)	14.3 ± 7.6	18.5 ± 9.7	5.7 ± 8.9	14.8 ± 9.8
HbA1c (%)	9.0 ± 2.3	9.2 ± 3.7	10.2 ± 2.6	9.1 ± 1.4
BMI (kg/m^2)	19.5 ± 2.8	22.2 ± 3.2	20.8 ± 2.7	22.1 ± 4.3
Hb (g/dl)	11.7 ± 1.3***	10.7 ± 1.3***	14.8 ± 1.5	13.5 ± 0.9
Retinopathy (yes/no)	5/2†††	10/3†††	1/12	5/8
Albuminuria (yes/no)	5/2†††	9/4†††	1/12	2/11
Ccr (ml/min)	69.7 ± 18.9*	83.5 ± 16.3*	102.9 ± 27.1	112.4 ± 29.3
CVR-R (%)	1.49 ± 0.60***	1.45 ± 0.44*	4.87 ± 2.34	3.66 ± 2.61
Adjusted CVR-R (%)	0.46 ± 0.19***	0.39 ± 0.12***	1.37 ± 0.61	0.82 ± 0.39
Erythropoietin (mU/ml)	25.0 ± 5.4	20.4 ± 5.9	–	–

Data were analyzed between same sexuality and shown as mean ± S.D. or number; *t*-test $^{*}P < 0.05$ or $^{***}P < 0.001$. Fisher's exact probability test $^{\dagger\dagger\dagger}P < 0.001$.