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DETERMINATION OF SERUM C-PEPTIDE (CPR*) AND URINARY EXCRETION OF CPR AS AN INDICATOR FOR INTRODUCING INTENSIVE INSULIN TREATMENT IN PATIENTS WITH DIABETES MELLITUS

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Diabetic complications could be prevented or blunted by better control of blood glucose with intensive insulin therapy. However, we need to have a reliable indicator to determine the timing when one patient should be introduced into the insulin therapy. In the present study, residual ß-cell function in the pancreas was evaluated by measuring serum C-peptide (CPR) levels and 24 hr urinary excretions of CPR in 43 non-obese patients with diabetes mellitus (DM). All patients were initially treated with three-time daily (t.i.d.) injections of regular insulin for one or two weeks, during which period those patients whose fasting blood glucose levels being ≦180 mg/dl were classified into the class 1, and those being > 180 mg/dl were into the class 2. In the following study period, class 1 patients (n=18) were maintained t.i.d. regular insulin alone, while class 2 patients (n=25) received at 9:00 p.m. a single injection of neutral protamine insulin suspension in addition to the t.i.d. injections of regular insulin. Both basal serum CPR levels and urinary excretions of CPR were revealed to be much lower in the class 2 patients than in the class 1 patients (mean ± SD; serum CPR, 1.1±0.4 ng/ml for class 1 vs. 0.6±0.4 ng/ml for class 2, $P<0.01$; urinary CPR excretion, 40±14 μg/day for class 1 vs. 20±16 μg/day for class 2, $P<0.01$). Moreover, there was a significant correlation between basal serum CPR levels and the urinary CPR excretions (r=0.64, $P<0.01$). These findings suggest that basal serum CPR levels as well as the urinary CPR excretions are useful indicators for determining the proper timing to introduce the intensive insulin therapy into non-obese DM patients.

Key words: C-peptide, intensive insulin therapy, diabetes mellitus

INTRODUCTION

It has been clearly shown that a risk of the development or progress of diabetic microvascular complications is much lowered in those patients whose blood glucose levels are well controlled by intensive insulin therapy (1-3). It is also important to assess a residual ß-cell function when insulin therapy is introduced (4,5). In the present study, we report that basal serum C-peptide (CPR) levels and urinary excretions of CPR are useful guides to introduce intensive insulin therapy in non-obese patients with diabetes mellitus (DM).

SUBJECTS AND METHODS

Patients

We investigated 43 patients with DM who were admitted for a short period to our division of Shimane Medical University Hospital in order to obtain better control of blood glucose. They consisted of 14 females and 29 males, aged from 22 to 85 yr with the mean (±SD) value of 56±16 yr. Patients with severe diabetic complication including chronic renal failure were not included in the present study. The purpose of the study was well informed to all the participants and their consent was obtained.

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* CPR: a conventional abbreviation for " Connecting Peptide immunoReactivity "

All patients were initially treated with a combination of diet and exercise for several days, followed by intensive insulin therapy. Practically, regular insulin was injected sc three times per day (t.i.d.), 30 min before each meal for one or two weeks. Then, the regular insulin was continued when fasting blood glucose (FBS) levels were lower than 180 mg/dl; these patients were classified into class 1. Single bolus sc injection of neutral protamine insulin (NPH) at 9:00 PM was added to those patients whose FBS levels remained above 180 mg/dl despite the regular insulin therapy; these patients were classified into class 2. Clinical characteristics of these patients are shown in Table 1.

CPR assay

On admission, blood samples were obtained from all the patients before breakfast. Serum samples were separated and stored at -20°C until assayed. Urine samples were collected for 24 hr and urine specimens were stored at 4°C until assayed. Serum CPR levels and urine excretions of CPR were measured by two-site immunoenzymometric assay on TOSOH AIA system analyzer (TOSOH Med. Inc., South San Francisco, CA, USA) in the Central Laboratory, Shimane Medical University Hospital. The minimal detectable concentration was 0.2 ng/ml.

Statistical analysis

Statistical differences between two groups were evaluated by unpaired Student's t-test. Regression analysis was performed by the least square method. A probability of $P<0.05$ was considered statistically significant.

RESULTS

As shown in Table 1, FBS and hemoglobin A_1c (HbA_1c) were considerably elevated in both classes 1 and 2 before starting the present study. There was no statistical difference in age, sex, body mass index (BMI) and HbA_1c levels between the two groups before starting the present study. Both values of urinary excretion of CPR and serum basal CPR were considerably lower in class 2 than in class 1 (mean ±SD: 0.6±0.4 vs 1.1±0.4 ng/ml, $P<0.01$; 20±16 vs 40±14 μg/day, $P<0.01$, respectively).

As shown in Fig.1, there was a significant correlation between urinary excretions of CPR and serum basal CPR levels (r=0.64, $P<0.01$). Urinary excretions of CPR or serum basal CPR levels were not related to body mass index (BMI) in these patients (data not shown).

Treatment with insulin was effective in both classes. FBS and HbA1c levels were much lower after the treatment than those before the treatment in both classes, although they were not normalized in most patients during the observation period (Table 1). Diabetic complications were not exaggerated in these patients.

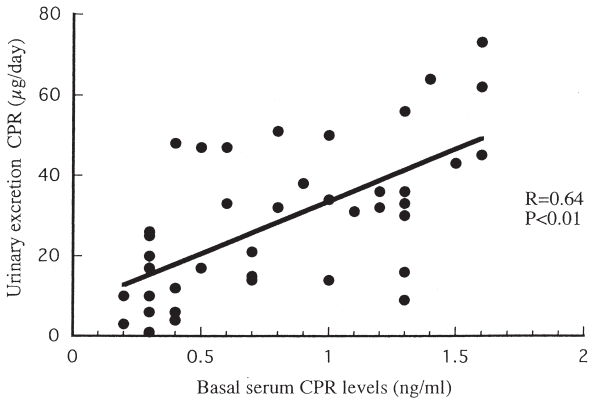

Fig. 1. Correlation between urinary excretions of CPR and basal serum CPR levels in 43 patients with non-obese diabetes mellitus. (R=0.64, $P<0.01$).

DISCUSSION

CPR is secreted from pancreatic ß-cells in equimolar concentrations with endogenous insulin. Recent development of specific immunoassay for CPR has enabled us to monitor residual ß-cell function in patients with insulin-requiring DM even if there existed circulating insulin antibodies or exogenously administered insulin (6-8). It has been reported that 24 hr urinary excretion of CPR is a useful parameter in determining insulin dependency (8).

In the present study, basal serum CPR levels were well correlated with urinary excretion of CPR in non-obese diabetic patients. Although we did not examine in detail, it should be noted that endogenously secreted proinsulin could affect the CPR assay since most CPR antisera react more or less with human proinsulin (5, 9).

Table 1. Clinical Characteristics of the Patients Studied

					Before treatment						After treatment				Dose of insulin (U)				Diabetic complications		
	Case No.	M/F	Age(yr)	BH(m)	BW(kg)	BMI (kg/m²)	FBS(mg/dl)	HbA1c(%)	sCPR(ng/ml)	uCPR(μg/day)	BW(kg)	BMI(kg/m²)	FBS(mg/dl)	HbA1c(%)	R1	R2	R3	N	Neuropathy	Retinopathy	Nephropathy
Class 1	1	M	66	1.62	52	20	297	10.7	1	50	53	20	113	9.1	8	4	8	0	+	-	-
	2	F	68	1.48	42	19	208	10.4	1.3	33	43	20	112	9	6	6	6	0	-	+	-
	3	M	71	1.48	40	18	189	7.7	1.5	43	41	19	92	6.9	8	6	8	0	+	+	+
	4	F	76	1.48	39	18	249	11.7	1.3	56	41	19	149	8.5	4	4	2	0	+	-	-
	5	M	64	1.65	59	22	259	10.3	1.3	30	59	22	146	7.9	10	4	6	0	+	+	+
	6	F	43	1.52	43	19	191	7.8	1	34	44	19	130	7.2	6	2	6	0	-	-	-
	7	M	28	1.72	58	20	175	14.9	1.1	31	58	20	91	12.1	2	2	2	0	-	-	-
	8	M	71	1.71	67	23	154	8.4	1.4	64	66	22	117	7.1	4	2	2	0	+	+	+
	9	F	70	1.52	43	19	547	10.7	1.2	36	42	18	121	8.9	6	2	6	0	+	+	+
	10	M	59	1.39	33.5	17	210	8.6	0.3	20	33	17	99	8.5	8	4	6	0	+	-	-
	11	M	52	1.68	63	22	178	8.2	1.6	45	61	21	95	7.8	4	3	5	0	+	+	-
	12	M	43	1.67	53	19	399	9.7	1.6	73	54	19	127	8.7	4	4	4	0	-	-	-
	13	M	59	1.63	52	20	196	7.9	1.2	32	52	20	126	6.1	4	6	4	0	+	+	-
	14	M	50	1.68	50	18	324	10.8	0.7	14	52	18	131	8.4	12	10	10	0	+	+	-
	15	M	25	1.61	50	19	296	8.5	0.8	51	50	19	94	7	4	2	4	0	-	-	-
	16	F	73	1.58	42	17	193	8	1.3	36	41	16	159	7.3	8	2	6	0	+	+	+
	17	M	53	1.55	43	18	349	11.7	0.6	47	43	18	119	10.2	2	2	2	0	+	-	-
	18	M	66	1.56	55	23	286	13	0.8	32	54	22	120	11	6	6	4	0	+	+	+
	mean		58	1.58	49	19	261	9.9	1.1	40	49	19	118	8.4	6	4	5	0			
	SD		15	0.09	9	1.8	99	2	0.4	14	9	1.7	20	1.5	3	2	2	0			
Class 2	19	F	78	1.34	34	19	142	8.5	0.6	33	35	19	83	7.4	6	4	6	4	+	+	+
	20	M	52	1.67	56	20	157	7.4	0.2	10	57	20	118	6.8	7	7	7	6	+	+	+
	21	M	34	1.65	61	22	312	16.3	0.3	17	61	22	120	9.9	7	6	6	6	-	-	-
	22	M	72	1.6	58	22	354	7.2	0.2	3	57	22	136	7	5	8	8	10	+	+	-
	23	M	46	1.66	67	24	171	8.4	1.3	9	65	24	140	8	6	8	8	6	+	+	+
	24	M	22	1.68	55	19	213	11.1	0.3	25	55	19	139	9.1	10	6	8	6	-	-	-
	25	M	58	1.6	45	18	198	8.8	0.4	4	46	18	116	8	6	4	4	4	+	+	+
	26	F	28	1.58	37	18	281	13.1	0.7	21	38	15	111	8.3	12	9	13	8	-	-	-
	27	M	24	1.7	52	15	214	9.8	1.6	62	53	18	92	9.4	8	4	4	4	-	-	-
	28	M	54	1.59	55	22	224	9.2	0.3	1	54	21	139	8.8	8	6	8	18	+	+	-
	29	M	66	1.58	49	18	490	14.5	0.3	6	50	20	152	9.3	8	8	8	8	+	+	-
	30	M	54	1.63	55	22	210	12.7	0.4	48	56	21	114	10.6	6	4	4	6	+	-	-
	31	M	52	1.63	55	20	196	8.7	0.3	26	54	20	124	8	8	6	8	16	+	+	+
	32	F	54	1.56	34	21	238	8.5	0.4	6	35	14	107	7.9	4	2	4	6	-	-	-
	33	M	54	1.6	55	21	192	8.2	0.8	32	55	21	101	8	6	4	6	4	+	+	+
	34	F	38	1.5	52	14	161	9.6	1.3	16	51	23	102	7.3	6	4	4	6	-	-	-
	35	F	71	1.64	52	21	220	9.9	0.5	47	52	19	147	9.6	6	4	6	6	+	+	+
	36	F	49	1.51	39	23	374	11.1	0.4	12	40	18	170	9.8	5	6	8	6	-	-	-
	37	F	85	1.48	38	19	267	7.1	0.3	10	39	18	84	7.1	8	4	6	6	+	+	+
	38	F	63	1.5	38	17	263	11.1	0.3	10	38	17	185	10.6	6	6	6	5	+	-	-
	39	M	76	1.6	57	17	256	11.7	0.4	12	57	22	145	11	8	4	4	8	+	+	+
	40	F	81	1.5	43	17	240	12.6	0.7	15	44	20	132	8.6	6	4	4	4	+	+	+
	41	M	54	1.68	54	22	257	10	0.9	38	53	19	121	9.4	10	6	6	6	+	+	-
	42	M	54	1.65	59	19	402	10.1	1	14	57	21	108	9.8	8	4	6	6	+	+	-
	43	M	48	1.7	60	19	380	11	0.5	17	58	20	141	10.6	6	4	6	12	-	-	-
	mean		55	1.59	50	20	256	10.3	0.6	20	50	20	125	8.8	7	5	6	7			
	SD		17	0.08	9	2.5	86	2.3	0.4	16	8	2.2	25	1.2	2	2	2	4			
P value	(1 vs 2)		0.55	0.7	0.72	0.15	0.86	0.55	<0.01	<0.01	0.7	0.11	0.33	0.34	0.2	0.1	0.1				

BW: Body Weight, BMI: Body Mass Index, FBS: Fasting Blood Glucose, HbA1c: Hemoglobin A1c, s CPR: serum Connecting Peptide immunoReactivity, u CPR: urinary CPR. R1: Regular insulin at 7: 00 am, R2: Regular insulin at 12: 00 noon, R3: Regular insulin at 5: 00 pm, N: NPH insulin

It was previously reported that serum CPR response to glucagon stimulation as well as 24 hr urinary excretions of CPR might reflect insulin dependency in well-controlled patients with DM (5). In the present study, basal serum CPR levels were lower in class 2 patients treated by a single bolus injection of neutral insulin plus t.i.d. injections of regular insulin than in class 1 patients treated by t.i.d. injections of regular insulin. On the other hand, FBS and HbA_1c were not remarkably elevated in class 2 compared to class 1. Therefore, basal serum CPR levels as well as 24 hr urinary excretions of CPR are reliable indicators for starting a single injection of NPH in addition to the three t.i.d. of regular insulin. Although 24 hr urinary excretions of CPR reflect the total daily amount of insulin secretion from ß-cells, these values may be underestimated in such conditions as urinary tract infection, renal dysfunction and extremely poor diabetic control (10).

In conclusion, basal serum CPR level as well as 24 hr urinary excretions of CPR are useful indicators for introduction to the intensive insulin therapy in patients with DM.

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