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AMINO ACIDS OF SERUM AND URINE IN FAMILIAL AMYLOIDOTIC POLYNEUROPATHY: STUDY ON THE NON-AFFECTED SUBJECTS IN THE FAMILIES

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Abstract

An anylysis of amino acids of serum and urine was performed in four non-affected subjects and four patients in the families with amyloidotic polyneuropathy.

1. The concentration of all free amino acids in both serum and urine of the non-affected subjects was similar to those of the patients (statistically not significant).
2. There were no unusual peaks in any analysis of urine or serum in both groups.
3. The amount of lysine in serum of the non-affected subjects was higher than the patients ($P < 0.01$). The amount of glycine in serum of the non-affected subjects was less than the patients ($P < 0.05$).
4. The marked decrease of total amount of amino acids in the urine was found only in one case with 5 years duration of the illness. This case also showed absent of methionine, leucine, and isoleucine in the urine.

INTRODUCTION

In 1952, Andarde[1] first clearly described a familial form of primary amyloidosis with polyneuropathy in Portugal. In 1956, Rukavina and his coworkers[2] described another large family in North America. Subsequently, there were several reports of similar families throughout the countries including Japan.[3]

In Japan, since 1966, Nakao et al.[4] and Araki et al[5] first discovered two pedigrees of familial amyloidotic polyneuropathy independently, several additional pedigrees have been reported from different areas of Japan. The families described by Araki et al.[5] and Kito et al[6] have many affected subjects. The clinical features and autosomal dominant pattern of inheritance were similar in almost all reported cases.

We have previously described the clinical manifestations, genetics, postmorted findings and laboratory data in the ten patients.[5][7] Although there were no remarkable laboratory abnormalities including serum

proteins, electrolytes and liver function tests, our previous analysis of amino acids of serum and urine in ten patients with amyloidotic polyneuropathy disclosed generalized hypoaminoacidemia ($0.1 < P < 0.2$), hypoaminoaciduria ($P < 0.001$), and no abnormality of a specific amino acid excretion.[8]

The purpose of this communication is to describe amino acids of serum and urine in the non-affected subjects in the families with amyloidotic polyneuropathy and compare with the results of the affected subjects in the families.

MATERIALS

Subjects: Four normal individual with the affected subjects in the families were selected at random for study (Table 1). Two individuals presented definite signs of polyneuropathy and gastro-intestinal symptoms, and two others showed early manifestations of symptoms of amyloidotic polyneuropathy. Although amyloidosis was not proved by biopsy, these four cases are thought to be affected by the same disorder (Table 2).

TABLE 1.
The normal subjects in the families with amyloidotic polyneuropathy.

Case No.	Age	Sex	Family name	No. of patients in a family
N-1	39	M	HI	2 (brother. sister)
N-2	28	F	HI	2 (brother, sister)
N-3	25	F	HI	2 (brother, sister)
N-4	50	M	NA	3 (3 brothers)

TABLE 2.
The patients with amyloidotic polyneuropathy

Case No.	Age (yr)	Sex	Family Name	No. of other patients in a family	Duration of illness (yr)	Polyneruopathy		Diarrheas and constipation
						Subject sign	Object sign	
P-1	28	F	IN	3 (father, 2 aunts)	1/2y	−	−	+
P-2	32	F	SH	2 (sister, brother)	1/2y	+	+	−
P-3	43	F	SH	2 (sister, brother)	4y	+	+	+
P-4	37	F	HA	3 (father, 2 sisters)	5y	+	+	+

Case P-1: The patient is a 28-year-old female who developed alternating diarrhea and constipation about 6 months prior to the examination. She also experienced fainting feeling on standing. Examination disclosed neither orthostatic hypotension nor sensory impairment in the extremities. The case was diagnosed to be early manifestation of amyloidosis (borderline case).

Case P-2: The patient is a 32-year-old female who developed numbness in the feet within 2 and a half year of the present study. Examination showed only subjective involvement of feet (paresthesia) and loss of ankle jerk. There was no gastro-intestinal symptom. The case was diagnosed to have early manifestation of amyloidotic polyneuropathy (borderline case).

Case P-3: The patient is a 43-year-old female who developed numbness in the feet at age 32. The numbness ascended to the level of knees by age 39. At about same year, she developed alternating diarrhea and constipation. By age 40, pain and temperature sensations became absent in the feet. At age 43, the examination disclosed sensory type of polyneuropathy in the lower extremities and pitting form of edema in the legs. Ankle jerks were absent. Arrythmia and orthostatic hypotension were negative. The case was diagnosed to have amyloidotic polyneuropathy and early sign of cardiac decompensation.

Case P-4: The patient is a 37-year-old female who developed numbness in feet by age 32. Numbness slowly ascended to the lower legs, and at age 36, about one year to the present study, she was operated upon ovarian tube tumor. Since then, symptoms increased, and she developed alternating diarrhea and constipation, fainting episode on standing, urinary incontinence, and numbness below the knees within 6 months of the present examination. Examination revealed sensory deficits, especially pain and temperature sensations in the lower extremities, and vibration and position sensations were within normal limits (sensory dissociation). The distal part of the feet was cold. Orthostatic hypotension was positive. The case was diagnosed to have amyloidctic polyneuropathy.

METHODS

Ten ml of venous blood was obtained from 4 normal subjects and 4 patients, after they had fasted overnight. Diet and activity were not controlled. Serums were separated immediately and transported on solidified carbon dioxide (−60°C). Serums were stored at −20°C until analyzed. The urine was collected for 12 hrs during fasted overnight

(from 8 PM to 8 AM). A few drops of toluene were added to the collecting bottles. A 50 ml aliquot of each urine was stored at −20°C until analyzed.

Free amino acid analysis of the serum and urine samples wer performed by the similar method of Spackman et al[8] and Araki et al[9], using an automatic amino acid analyzer (Yanagimoto: LC-5). The sample was separated through a 70 cm column of resin (Aminex A-4) at 54°C with pH 3.25 to 4.25. 0.2 N Citrate buffer was used and the buffer change was done at 73 minutes. Urinary creatinine was measured by the method of Folin.[10]

RESULTS

The total amount of identified amino acids in serum, excluding threonine and serine, was generally similar in the normal subjects as well as in the patients (Table 3). The total value of two groups was statistically not significant. Each amount of threonine and serine was difficult to calculate, but their peaks were almost similar. The amount

TABLE 3.

Amino acids in serum of normal subjects and patients with familial amyloidotic polyneuropathy (Amino acids: μ mol/liter)

	N-1	N-2	N-3	N-4	P-1	P-2	P-3	P-4
Lys	277	231	242	278	156	198	158	162
His	83	65	75	106	54	81	48	152
Arg	58	79	84	62	49	51	46	62
Asp	35	31	32	37	34	31	30	41
Glu	115	95	92	147	89	93	122	145
Pro	157	141	138	129	157	171	145	210
Gly	288	231	282	224	285	461	375	516
Ala	480	348	380	351	335	511	389	775
2/1Cys	69	38	64	t	30	41	32	42
Val	270	187	182	202	190	223	155	256
Met	27	17	25	19	20	23	20	24
IsoLeu	67	50	57	62	52	57	41	63
Leu	144	109	112	133	115	118	90	144
Tyr	79	48	52	59	52	60	49	75
Phe	83	60	62	75	83	65	68	97
Total*	2232	1730	1879	1884	1701	2184	1768	2764

* Thr. and Serine: excluded. t: trace

of lysine in serum was lower in the patients than those of the normal subjects ($P < 0.01$). Also, the amount of glycine in serum was higher in the patients than in the normal subjects ($P < 0.05$).

There were no unusual peaks in any analysis of urine and serum of either the normal subjects or the patients.

The total amount of urinary excretion of each amino acids by the normal subjects and the patients (P-1, 2, 3) was about similar. Significant decrease of total amino acids in the urine, and of urinary amino acids per creatinine were found only in a patient (Case P-4) with a long duration of the illness (Table 4). In the urine of the patient (Case P-4), fouramino acids were abnormally low in compared to those of the normal subjects and 3 other patients, (Table 5) and follows: methionine, leucine, and isoleucine were absent, and valine was trace.

TABLE 4.

Total free amino acids in urine of the normal subjects and the patients in the families with amyloidotic polyneruopathy

	Total amino acids (mg/liter)	Total amino acids (mg/creatinine)
N-1	281.2	230.4
N-2	219.8	301.0
N-3	596.4	380.2
N-4	303.1	393.8
P-1	489.0	440.6
P-2	416.1	378.4
P-3	437.8	373.8
P-4	110.6	120.0

N: normal subjects P: patients

DISCUSSION

Amyloidosis is a rare disorder of unknown etiology and regarded as a metabolic disorder, since it is associated with hypergammaglobulinemia, abnormalities of the reticuloendothelial system, and chronic immunologic stimulation leading to production or deposition of antibody and plasma cell abnormalities.[11]

Before 1970, there were no prior study of amino acids in familial amyloidotic polyneurophaty. Araki et al[8] first attempted to clarify the

TABLE 5.

Specific amino acids in urine of the patients with amyloidotic polyneuropathy and the normal subjects in the families.
(Amino acids: μ mol/liter)

	N-1	N-2	N-3	N-4	P-1	P-2	P-3	P-4
Valine	41.8	23.9	56.8	21.8	46.4	40.9	37.1	t
Methis.	30.5	22.6	23.7	t	18.8	t	t	-
Leucine	50.1	39.0	77.2	71.6	40.6	44.5	50.2	-
Isoleucine	46.5	16.5	27.2	40.6	15.5	30.4	24.6	-

amino acids abnormality in this disorder, and described that the concentration of all free amino acids in both serum and urine of the patients was less than the controls (the normal subjects of unrelated families). Araki et al also reported that the decrease and absence of amino acids in the serum and urine of the patients was found to be relatated to the duration of the disease (7 to 12 years course of the illness). They have not proved as yet the mechanism of the decrease of amino acids in both serum and urine.

The present study attempted to clarify to see if there are any abnormalities found in the non-affected subjects in the families with amyloidotic polyneuropathy. The study disclosed no abnormal descrease of amino acids in both serum and urine of the normal subjects. Even in the patient's group, one case with a long duration of the illness showed only significant decrease of the amino acids in the urine.

The significant decrease of serum lysine and increase of serum glycine in the patients should await further study.

In 1974, Itoga et al.[12] reported amino acid analysis in other familial amyloid polyneruopathy found in Nagano prefecture, Japan. The study was made on the 21 patients (male 15, female 6) with variable duration of the illness (from 1 year to 17 years), and on the 7 normal controls. Total amount of amino acids, and amounts of each amino acids: proline, glycine, alanine, glutaminic acid and lysine, were higher in the urine of the patient's group than those of the normal controls. The result by Itoga et al was different from those of ours, and this could be due to the genetic-biochemical difference.

Although it is much too early to draw conclusions from the present number of test subjects, the above mentioned values seem to be worthy reported.

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