

AMINO ACID DYNAMICS IN URINE OF *S. haematobium* PATIENTS IN ISHIELU LOCAL GOVERNMENT AREA OF EBONYI STATE, NIGERIA

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ABSTRACT

The Amino acid dynamics in urine samples of Schistosoma haematobium patients were studied. The study was to evaluate the possibility and validity of using amino acid patterns recorded in highly, lightly and uninfected urines as diagnostic tool for rapid screening of Schistosomiasis. Paper chromatography was used to separate the different amino acids in the urine samples. The chromatographic method used in this study revealed the existence of 9 essential and 7 non-essential amino acids in the urine samples. It equally showed that histidine, glutamine, serine and proline were absent in all the urine samples. Furthermore the presence of two marker amino acids can be used to identify individuals with heavy infection (cystein) and no infection (methionine).

Keywords: Amino acids, Rapid diagnosis, Schistosomiasis, *S. haematobium*, Ebonyi State

INTRODUCTION

Studies on amino acid pattern in different diseases and in different areas can be found in literature. In a similar study, the amino acid pattern in plasma and urine of Bilharzial Egyptian patients with different degrees of complications were investigated. The result obtained showed that in mansoniiasis, accumulation of amino acids in the circulation was due to derangement in liver function which retards the utilization of amino acids in protein synthesis particularly in advanced stage of the disease (EL-Shobacki *et al.*, 1980). The amino acid profile of adult infected with *Brugia malayi* from 2 different endemic areas (Dushan and Libo) showed that both endemic areas contained 17 amino acids. The Dushan area contained serine but not tryosine and the Libo area lacked serine but contained tryosine (Cui *et al.*, 1996; Barus *et al.*, (1995) analyzed amino acid spectra for crude protein (CP) of *Lingula intestinalis*, *Rutilus rutilus*, *Abramis brama* and *Blica bjoerkna* from 5 localities in South Moravia, Czech Republic. There was considerable similarity in the quantitative rankings of both essential amino acid (EAA) and non essential amino acids (NEAA). Amino acid profiles of *Anguillicola crassus* and *Philometra ovata* were reported consisting of seventeen amino acids (11 – essential and 6 non-essential) (Barus *et al.*, 1998a). Quantitative differences between the 2 species were statistically significant for 9 essential and 4 non essential amino acids (Barus *et al.*, 1998b).

MATERIALS AND METHODS

Urine samples were collected from secondary schools students in Ishielu Local Government Area of Ebonyi State. After the preliminary examination of the urine samples for *S. haematobium* egg using centrifuge filtration technique, 6 urine samples were used for amino acid pattern analysis, 2 each from highly infected individuals (>50 eggs/10ml urine), lightly

infected individuals (<50 eggs/ 10ml urine) and uninfected individuals (0 egg/10ml urine) respectively.

In the analysis, the method of Harbone (1973) was used. Using micro-pipette, 0.25ml of each urine sample was spotted on the chromatography paper and allowed to dry. The papers were ran twice to ensure better separation in a tank containing two solvent system (Phenol-water; 30/g of phenol and 10/ml of water). The chromatograms were brought out and allowed to dry in fume cupboard after each run.

The standards were prepared by dissolving 0.1/g each of standard amino acid and made up to 100/ml with water. They were spotted on prepared chromatography papers and allowed to dry and ran in the solvent after which they were dried in the fume cupboard. The whole chromatograms were sprayed with Ninhydrin and allowed for colour development in the oven for 5 minutes at 110°C. The distances moved by the solvent and that moved by amino acids in each sample and each standard amino acid were noted. Their R_f values were found and by comparing the R_f values of the standard amino acids and that of each sample were noted. Each spot was excised and eluted with 5/ml of n-propanol water (7:10) solvent in test tube by continuous shaking for 5 minutes. The extract was filtered through whatman No.1 filter paper and absorbance of both the different amino acids in the samples as well as those of the standard amino acids were read in the spectrophotometer at 530/nm. Calculation of the concentration of each amino acid present in each sample was done using Beer Lambert Law as described by Plummer (1979). The differences in the common amino acids between males and females in highly, lightly and uninfected urine samples were tested for significance using student's t-test; difference was accepted at the probability level of P<0.05.

Table 1: Amino Acid Patterns and Concentrations of the Various Amino Acids in Urine of People with Different Grades of Infection with *S. haematobium*

Samples	Sex	Amino acid present	R _f value	Absorbance	Concentration mg/100/ml
1 Highly Infected	M	Aspartic acid	0.21	0.04	40.00
		Asparagine	0.37	0.015	3.33
		Glutamate	0.42	0.04	32.00
		Threonine	0.54	0.025	1.67
		Valine	0.71	0.03	5.00
		Tryptophan	0.78	0.015	23.08
		Tryosine	0.63	0.02	23.53
		Glycine	0.48	0.015	7.69
		Cystein	0.32	0.12	120.00
2 Highly Infected	F	Asparagine	0.37	0.115	25.56
		Aspartic acid	0.22	0.035	35.00
		Cystein	0.27	0.001	10.00
		Glycine	0.47	0.125	64.10
		Lysine	0.51	0.07	11.11
		Tryptophan	0.77	0.005	7.69
		Leusine	0.84	0.09	40.91
		Isoleucine	0.89	0.015	21.43
		Valine	0.74	0.03	5.00
		Arginine	0.70	0.045	75.00
		Phenylalanine	0.80	0.015	4.17
		Aspartic acid	0.73	0.05	50
		Alanine	0.57	0.025	13.89
3 Lightly Infected	M	Valine	0.73	0.02	3.33
		Tryptophan	0.76	0.1	15.38
		Leusine	0.86	0.03	13.64
		Asparagine	0.38	0.055	12.22
		Glycine	0.45	0.14	71.79
		Lysine	0.50	0.01	1.59
		Tryosine	0.63	0.03	35.29
		Arginine	0.69	0.01	16.67
		Aspartic acid	0.23	0.05	50
4 Lightly Infected	F	Glutamate	0.42	0.06	48
		Threonine	0.56	0.04	2.67
		Asparagine	0.37	0.06	13.33
		Tryosine	0.62	0.08	94.12
		Alanine	0.59	0.03	16.66
		Phenylalanine	0.82	0.017	4.72
		Leusine	0.85	0.04	18.18
		Aspartic acid	0.26	0.05	50.00
		Tryptophan	0.77	0.02	30.77
5 Uninfected	M	Asparagine	0.37	0.05	11.11
		Glutamate	0.41	0.015	12.00
		Tryosine	0.62	0.04	47.00
		Arginine	0.69	0.02	33.33
		Valine	0.74	0.04	6.67
		Methionine	0.90	0.015	4.84
		Aspartic acid	0.22	0.038	3.8
		Threonine	0.55	0.04	26.67
		Glycine	0.44	0.065	33.33
6 Uninfected	F	Alanine	0.57	0.004	2.22
		Tryptophan	0.78	0.01	15.38
		Phenylalanine	0.83	0.005	1.39
		methionine	0.92	0.025	8.06

RESULTS

The chromatographic method used in this investigation revealed the existence of 9 essential and 7 non-essential amino acids in urine. The 9 essential amino acids were arginine, tryptophan, leusine, valine, isoleucine, threonine, methionine, lysine and phenylalanine. The 7 non-essential amino acids were aspartic acid, asparagine, cystein, glutamate, glycine, tryosine and alanine (Table 1).

The different amino acids in the urine samples of highly, lightly and uninfected (male & female) separated in the different chromatography papers (Figures 1 – 6).

In highly infected urine samples, the male and female had aspartic acid, asparagine, valine, tryptophan, glycine and cystein in common but in different concentrations except for valine where they had the same concentration (5/mg/100/ml) (Table 1).

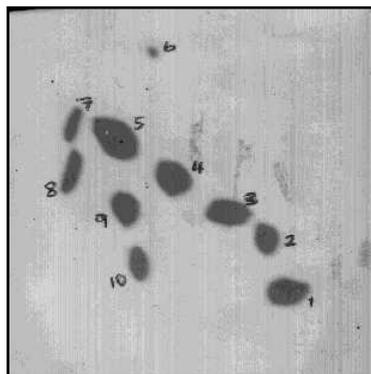


Figure 1: Amino acid profile of highly infected urine samples (male).

Key: 1 = Aspartic acid, 2 = Asparagine, 3 = glutatamate, 4 = threonine, 5 = Glycine, 6 = ----, 7 = Tryptophan, 8 = Tyrosine, 9 = glycine, 10 = Cystein



Figure 2: Amino acid profile of highly infected urine samples (female).

Key: 2 = Asparagine, 3 = Aspartic acid, 4 = Cystein, 5 = Glycine, 6 = Lysine, 7 = Tryptophan, 8 = Leusine, 9 = Isoleucine, 10 = Valine, 11 = Arginine and 12 = Phenylalanine

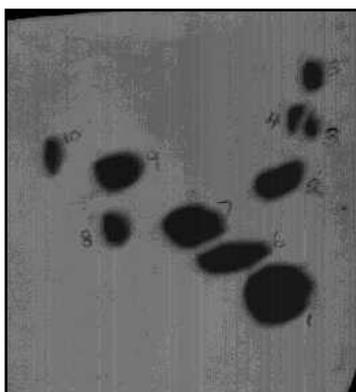


Figure 3: Amino acid profile of lightly infected urine samples (male).

Key: 1 = Aspartic acid, 2 = Alanine, 3 = Valine, 4 = Tryptophan, 5 = Leusine, 6 = Asparagine, 7 = Glycine, 8 = Tyrosine, 9 = glycine, 10 = Arginine

Glutamate, threonine and tryosine were seen in the male but not in the female urine while lysine, leusine, isoleucine, arginine and phenylalanine were seen in the female but not in the male urine (Table 1). In lightly infected urine samples, the common amino acids for both male and female were aspartic acid,

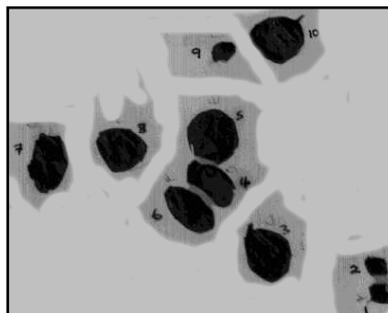


Figure 4: Amino acid profile of lightly infected urine samples (female)

Key: 1 = ----, 2 = ----, 3 = Aspartic acid, 4 = Glutamate, 5 = Threonine, 6 = Asparagine, 7 = Tryosine, 8 = Alanine, 9 = Phenylalanine, 10 = Leusine

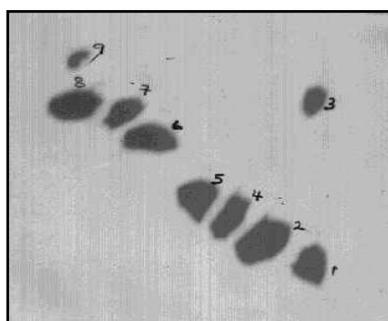


Figure 5: Amino acid pattern of uninfected urine samples (male)

Key: 1 = ----, 2 = Aspartic acid, 3 = Tryptophan, 4 = Asparagine, 5 = Glutamate, 6 = Tryosine, 7 = Arginine, 8 = Valline, 9 = Methionine

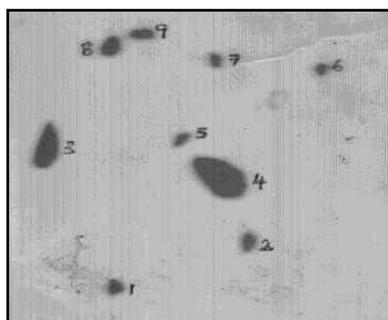


Figure 6: Amino acid pattern of uninfected urine samples (female)

Key: 1 = ----, 2 = Aspartic acid, 3 = Threonine, 4 = Glycine, 5 = Alanine, 6 = Tryptophan, 7 = Phenylalanine, 8 = Methionine. 9 = ----.

alanine, leusine, asparagine and tryosine in different concentrations except for aspartic acid where a concentration of 50/mg/100/ml was recorded in both male and female. Valine, tryptophan, glycine, lysine and arginine were seen in the male but not in the female urine while glutamate, threonine and phenylalanine were seen in the female but not in the male urine (Table 1). In uninfected urine samples, aspartic acid, tryptophan and methionine were common to both male and female. Asparagine,

glutamate, tryosine, arginine and valine were seen in the male but not in the female while threonine, glycine, alanine and phenylalanine were seen in the female but not in the male urine (Table 1) T-test showed that there was no significant difference in the common amino acids between males and females in highly infected, lightly infected and uninfected urine samples ($P > 0.05$).

Qualitatively, aspartic acid, arginine, glutamate, glycine, tryptophan, tryosine, valine, threonine, phenylalanine and asparagine were present in highly infected, lightly infected and uninfected urine samples. Lysine and leusine were only seen in highly and lightly infected urine samples (Table 1) Alanine was only seen in lightly and uninfected urine samples. Histidine, glutamine, serine and proline were absent in all the urine samples. The amino acids that can be said to be the marker amino acids for highly infected and uninfected individuals are cystein and methionine respectively being only present in highly infected and uninfected urine samples respectively.

DISCUSSION

Four of the 20 amino acids, histidine, glutamate, serine and proline were absent in the urines with different grades of infection and also in uninfected urine samples. Their absence is not well understood but being essential amino acids, may have been reabsorbed by the kidney. A striking difference between infected and uninfected urine samples was the presence of methionine in uninfected urine samples and its absence in both highly and lightly infected urine samples. This may mean that the parasite, *S. haematobium* thrives on it so leading to its depletion and complete absence in both highly and lightly infected urine samples.

The presence of cystein in only highly infected urine samples may be that in high infection, *S. haematobium* promotes excess secretion of cystein and inhibits its reabsorption at the kidney level. The difference in the concentration of the amino acids in highly, lightly and uninfected urine samples which is not statistically significant may be that the dietary protein intake of the students in the endemic area is similar as most of them are from poor families.

Using this study, it can be safely said that two amino acids can be used as marker amino acids to quickly identify people with heavy infection (cystein) and no infection (methionine).

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