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Variation in Free Plasma Amino Acid due to Parasitic Infection in the Blood of Rats

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Abstract: Parasitic infection and biochemical analysis have been done on rats with and with out parasitic infections found in Sindh province. Free plasma amino acids were analyzed by paper chromatography, using two different solvent systems for avoiding over lapping etc. Infection was established after opening the rats, identifying the responsible parasites and counting the parasites. As compared with non-infected rats, free plasma amino acids showed variation in blood of parasitically infected rats {(Trypanosome and sporozoan) protozoan}. Intensity of infection especially Trypanosome and Sporozoan were also responsible for variation of free plasma amino acids.

Key words: Rats, endoparasite, plasma amino acid , parasite, amino acids, parasitic infections, trypanosome, sporozoan

Introduction

Rats are most widely distributed rodents of world and have always followed human civilization as like associated with residential colonies, buildings and store houses. The involvement of rats is responsible for contact between rats and man leading to the transmission of rats-borne diseases, where they act as a reservoir host for various parasitic infections, (Mushtaq-ul-Hassan, 1997).

Keeping in view the importance of rats. Several surveys and ecological studies on parasites have been carried out in various parts of the world (Tenora *et al*., 1973; Jawadat Mahmoud, 1980; Haq *et al*.,1985). Little record in the literature is available about the endoparasites of house rats of different regions of Pakistan (Bilqees and siddique, 1981; Khera and Wadhawan, 1983; Al Bawari *et al*.,1987; Fatima, 1991; Noor-un-Nisa and Ghazi, 1993)

Blood is the chief circulatory medium of animal, by virtue of its circulation through every organ also blood participates in every major functional activity of the body (Guyton *et al*., 1996). Study of blood is important from parasitological point of view. It is known that parasitic infection affect the blood picture and its composition (Chappell, 1980).

It is most abundant organic compounds in the animals. It is required for growth, maintenance and repair of body tissues. They act as a regulator of physiological relationship when present in the form of hormones. The supply of amino acids is also essential to meet the several nutritional problems in animals and synthesis of proteins in organism (Ahmed *et al*., 1987; Ahmed, 1991). These organic acids exist naturally in Zwitterion State where the carboxylic acid moiety is ionized and basic group is protonated. Amino acids are the principal building blocks of proteins and enzymes. Arginine is often used at active sites of enzymes. Histidine is responsible for histamine biosynthesis. Isoleucine is hydrophobic amino acid almost exclusively in protein and enzyme construction. Lysine helps to form collagen (which makes up bone cartilage & connective tissues). Methionine is essential amino acid, which helps to initiate protein synthesis. Phenylalanine plays a key role in the biosynthesis of other amino acids and some neurotransmitters. Proline is important for the proper functioning of joints and tendons; also helps to maintain and strengthen heart muscles. Threonine helps to prevent fat build-up in the liver; helps the digestive and intestinal tracts function more smoothly; assists metabolism and assimilation. Tryptophen is a natural relaxant, helps alleviate insomnia by inducing normal sleep; reduces anxiety & depression; helps in the treatment of migraine headaches; helps the immune system; to reduce the risk of artery & heart spasms; works with lysine in reducing cholesterol levels. Tyrosine transmits nerve impulses to brain; helps to overcome depression; improves memory; increases mental alertness; promotes the healthy functioning of thyroid, adrenal and pituitary glands. Valine promotes mental vigor, muscle co-ordination and calm emotions (Horton, 1996; Munro, 1980; Sabelli, 1986).

Each animal species and even each type of body tissue is distinct being characterized by its specific type of protein, the species specificity of proteins is shown by the production of hypersensitivity or allergy to foreign proteins. The normal plasma protein level in the rat is 6.0 ± 0.2 g 100 ml^{-1} and free amino acid nitrogen in the blood is 8.9 mg/100 ml (Long, 1971).

The level is somewhat lower in children. In starvation the plasma amino acid levels are slightly higher than in the usual post absorption period. This probably represents a more active break down of tissue protein. However in chronic protein deficiency the plasma amino level remains nearly normal changes in the plasma amino acid concentration in disease are on the whole of little diagnostic value.

The studies conducted in Sindh region are mostly centered upon the morphotaxanomy of endoparasites of rats (Bilqees, 1985, Farooq and Yousuf, 1986; in Karachi. Noor-un-Nisa and Ghazi, 1993). Memon (2001) also observed variation occurred in bacterial infections in human blood.

However the influence of the parasitic infection on the biochemical indices in the blood of the rats has not yet been shown which is supported by the fact that there is no published information on this aspect from the rats of this region more over the effect of parasitic infection on amino acid variation has not been studied at any level.

Therefore it was planned to record the variations that occur in the amino acid content of the blood in the rats, naturally infected with endoparasites, The information which will be gathered during this investigation will not only be important from the academic point of view but will also provide us a current status of parasitic infection in rats.

Materials and Methods

Rats were collected from different areas of Sindh province. They were kept in cages (4 rats / cage) and placed in Parasitology research lab and fed with vegetables, fruits, bread and water.

Dissection for sampling

Blood, for haematological and biochemical analysis. Parasites, from different tissues of the body for microscopic study.

Preparation of blood slide: By taking a fresh drop of blood from heart on a grease free clean slide at a distance of about half

an inch from the right end, hold another slide at an angle of 45 degrees in contact with the drop of blood, then slide is lowered to an angle of 30 degrees and push gently to the left, till the blood is exhausted, the film begins to form "tails" which should end near about the center of the slide. The film was allowed to dry (Wintrobe, 1951).
The film was fixed with pure Methyl alcohol (CH_3OH) for 3 to 5 min. and allowed to dry.
Giemsa's stain was diluted by adding 1 drop to each 2 ml of distilled water in a coupling jar. The diluted stain was poured over the film and kept for 45 min. The slide was then flushed in a gentle flow of tap water, after it was placed in an upright position with the film slide inwards to drain water and dry it.
The stained blood film was examined for blood parasites under microscope in low power, then with 100 power objective using in oil immersion.

Qualitative identification of free amino acids in plasma of the rat: Two solvent systems for avoiding the over lapping was used which are
Solvent No.1 n-butanol : acetic acid : water (4:1:5)
Solvent No.2 n-butanol : pyridine : water (1:1:1).
Solution of 0.001mg/1ml was prepared and placed in screw lid test tubes as standard amino acid solution.
A 0.2% solution of ninhydrin was prepared in acetone by dissolving 200 mg of ninhydrine in 100 ml of acetone, as spray reagent.
What man's filter paper no. 1 was used, for the preparation of chromatograms.

Development of chromatograms : Unidimentional ascending technique was used for developing chromatograms.

Measure solute and solvent fronts: The R_f value was calculated by this formula.
Rf = Distance travelled by the solute
Distance travelled by the solvent

Identification of amino acids: By R_f value, colour and standard amino acids were identified (Stock and Rice, 1974).

Results

The percentage of infected rats is divided into 9 groups according to the parasitic infections in blood with and without involvement of liver or/and intestine in the rats found in different regions of the Sindh province (Table 1).
Fig. 1. shows group wise percentage of rats infected with endoparasites.
Variations data in different plasma amino acids in 9 groups of infections.
Table 2, Group 1. Blood (sporozoan infection).
Table 3, Group 2. Blood (trypanosome infection).
Table 4, Group 3. Blood with double parasitic infection (sporozoan and trypanosome).
Table 5, Group 4. Blood (sporozoan infection) and liver (cyst infection).
Table 6, Group 5. Blood (trypanosome infection) and liver (cyst infection).
Table 7, Group 6. Blood (sporozoan and trypanosome infections) and liver (cyst infection).
Table 8, Group 7. Blood (sporozoan infection) liver (cyst infection) and intestinal (cestode infection).
Table 9, Group 8. Blood (trypanosome infection), liver (cyst infection) and intestine (cestode infection).
Table 10, Group 9. Blood (sporozoan infection) and intestine (cyst infection).

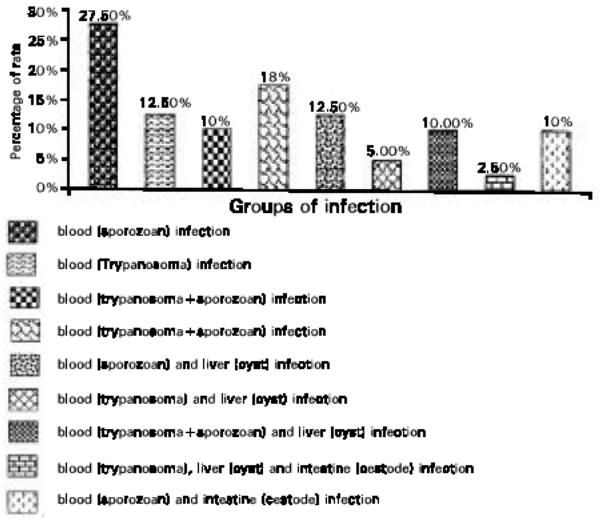

Fig. 1: Percentage of rats infected with endoparasites

Aliphatic essential amino acid:
Arginine is missing in, group 6 blood (sporozoan + trypanosome infections) + liver (1-2 cyst infection).
Group 8 blood (trypanosome infection) + liver (1 cyst infection) and intestine (2 cestode infection).
Isoleucine was found to be absent in all groups of infections.
Leucine was present only in, group 4 blood (sporozoan infection) and liver (1-4 cyst infection), group 7 blood (sporozoan infection) liver (1-7cyst infection) and intestinal (cestode infection), while it is missing in all other plasma chromatograms of infected rats.
Lysine was missing only in, group 6 blood (sporozoan + trypanosome infections) + liver (1-2 cyst infection).
Methionine was present in, group 6 blood having double parasitic infection (sporozoan and trypanosome).
Group 9 blood (sporozoan infection) and intestine (cyst infection).
Threonine was missing only in, group 6 blood (sporozoan and trypanosome infections) + liver (1-2 cyst infection).

Aromatic essential amino acids:
Phenylalanine was found missing in, group 5 blood (trypanosome infection) + liver (1-4 cyst infection), group 6 blood (sporozoan + trypanosome infections) + liver (1-2 cyst infection), group 8 blood (Trypanosome infection) + liver (1 cyst infection) and intestine (2 cestode infection).
Tryptophine was not present in plasma chromatograms such as, group 6 blood (sporozoan + trypanosome infections) + liver (1-2 cyst infection), group 8 blood (trypanosome infection) + liver (1 cyst infection) intestine (2 cestode infection).

Aromatic non-essential amino acids:
Tyrosine was absent in, group 6 blood (sporozoan + trypanosome infection) + liver (1-2 cyst infection), group 8 blood (trypanosome infection) + liver (1 cyst infection) intestine (2 cestode infection).

Hetrocyclic non-essential amino acid:
Proline was missing in, group 6 blood (sporozoan + trypanosome infections) + liver (1-2 cyst infection), group 8 blood (trypanosome infection) + liver (1 cyst infection) intestine (2 cestode infection).

Table 1: Showing %age of infection in different groups and %age of rats having amino acids in each groups

No. of group	Infected tissues	Parasites	% of inf:rats	% of rat have arg	% of rat have his	% of rat have lys	% of rat have met	% of rat have phe	% of rat have pro	% of rat have thr	% of rat have try	% of rat have tyr	% of rat have val
1	Blood	Sporozoan	27.50	54.54	81.81	54.54	0	18.18	54.54	18.18	18.18	9.09	0
2	Blood	Trypanosome	12.50	100	50	100	0	50	100	100	100	100	50
3	Blood	Trypanosome Sporozoan	10	50	100	75	0	25	100	50	25	50	0
4	Blood and Liver	Sporozoan 1-4 cyst.	18	71.42	57.14	42.85	28.57	42.85	42.85	57.14	14.28	28.57	14.28
5	Blood and Liver	trypanosome 1-4 cyst.	12.50	20	100	40	0	0	60	40	20	20	0
6	Blood and Liver	Sporozoan trypanosome 1-2 cyst	5.00	0	100	0	0	0	0	0	0	0	0
7	Blood Liver Intestine	sporozoan 1-7 cyst 3-13 cest	10.00	50	75	50	25	75	75	50	75	75	0
8	Blood Liver, Intestine	Trypanosome 1cyst 2 cest.	2.50	0	100	100	0	0	0	100	0	0	100
9	Blood and Intestine	Sporozoan 1-4 cyst	10	50	100	75	25	75	50	50	75	75	0

Table 2: Plasma amino acids in blood (sporozoan) infection)

Infection	Sex	Weight (gm)	S.No.	Arg	His	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
(+sporo)	f	100	J_1			M	M	M	M	M	M	M	M	M	M
(+sporo)	f	90	J_{13}			M	M	M	M	M	M	M	M	M	M
(+sporo)	f	25	J_{16}	M		M	M	M	M	M		M	M	M	M
(+sporo)	f	24	J_{17}	M	M	M	M	M	M	M			M		M
(+sporo)	f	40	S_{16}		M	M	M		M	M	M	M	M	M	M
(+sporo)	f	80	S_{12}			M	M		M					M	M
(+sporo)	f	95	S_{13}			M	M	M	M	M		M		M	M
(+sporo)	f	30	S_{18}	M		M	M		M	M	M	M	M	M	M
(+sporo)	f	68	L_{13}			M	M		M			M	M	M	M
(+sporo)	f	70	L_2	M		M	M		M	M	M				M
(+sporo)	f	92	H_1	M		M	M		M	M	M	M	M	M	M

Table 3: Plasma amino acids in blood (Trypanosoma) infection

Infection	Sex	Weight (gm)	S.No.	Arg	His	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
(+ tryp)	f	45	J_3			M	M		M	M					M
(++tryp)	f	95	S_8		M	M	M		M						

Table 4: Plasma amino acids in blood (trypanosoma+sporozoan) infection

Infection	Sex	Weight (gm)	S.No.	Arg	His	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
(+tryp/+sporo)	m	160	J_{14}			M	M		M	M			M	M	M
(+tryp/+sporo)	f	30	L_{23}	M		M	M		M			M			M
(++tryp/+sporo)	f	68	L_{15}	M		M	M		M	M		M	M	M	M
(++tryp/+sporo)	f	75	H_3			M	M	M	M	M			M		M

Table 5: Plasma amino acids in blood (trypanosoma+sporozoan) infection

Infection	Sex	Weight (gm)	S.No.	Arg	His	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
2cyst/+sporo	m	40	S_3	M		M		M		M		M	M	M	M
1cyst/+sporo	f	70	S_{10}		M	M	M	M	M		M		M		
2cyst/+sporo	f	60	S_7		M	M	M		M				M	M	M
1cyst/+sporo	f	75	L_3			M	M		M		M				M
1cyst/+sporo	f	80	L_{12}	M		M	M	M	M	M	M	M	M	M	M
3cyst/+sporo	f	60	L_{25}			M	M		M	M	M		M	M	M
8cyst/+sporo	f	95	J_{12}			M	M	M		M		M	M	M	M

Table 6: Plasma amino acids in blood (trypanosome) and liver (Cyst) infection

Infection	Sex	Weight (gm)	S.No.	Arg	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
1cyst/+Tryp	f	44	J_{10}		M	M		M	M			M	M	M
1cyst/+Tryp	f	95	L_5	M	M	M	M	M	M			M	M	M
4cyst/+Tryp	f	100	L_4	M	M	M	M	M	M		M			M
4cyst/+Tryp	f	61	L_8	M	M	M	M	M	M	M	M	M	M	M
1cyst/+Tryp	m	50	L_{10}	M	M	M	M	M	M	M	M	M	M	M

Table 7: Plasma amino acids in blood (trypanosoma+sporozoan) and liver (Cyst) infection

Infection	Sex	Weight (gm)	S.No.	Arg	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
2cyst/++Tryp/spo	f	85	S_{17}	M	M	M	M	M	M	M	M	M	M	M
1cyst/++Tryp/spo	m	76	L_{19}	M	M	M	M	M	M	M	M	M	M	M

Table 8: Plasma amino acids in blood (sporozoan), liver (Cyst) and intestine (cestode) infection

Infection	Sex	Weight (gm)	S.No.	Arg	His	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
1cyst/13ces/spo	f	96	S_{20}			M						M			M
5cyst/6ces/spo	f	70	S_4		M	M	M	M	M						M
1cyst/6ces/spo	m	100	L_{21}	M		M	M		M		M	M			M
7cyst/3ces/spo	f	90	S_{11}	M		M	M	M	M	M			M	M	M

Table 9: Plasma amino acids in blood (Trypanosome), liver (cyst) and intestine (cestode) infection

Infection	Sex	Weight	S.No.	Arg	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
1cyst/2ces/+try	f	80(gm)	S_6	M	M	M		M	M	M		M	M	

Table 10: Plasma amino acids in blood (sporozaon) and intestine (cestode) infection

Infection	Sex	Weight (gm)	S.No.	Arg	Iso	Leu	Lys	Met	Phe	Pro	Thr	Try	Tyr	Val
1ces/+spo	m	150	J_{11}	M	M	M	M	M		M	M			M
4ces/+spo	f	127	J_5	M	M	M				M		M	M	M
2ces/+spo	f	70	S_{14}		M	M		M			M			M
1ces/+spo	m	45	H_5		M	M		M	M					M

Arg = Arginine, His = Histidine, Iso = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Phe = Phenylalanine, Pro = Proline, Thr = Threonine, Try = Tryptophine, Tyr = Tyrosine, Val = Valine, Tryp = Trypanosome, Sporo=Sporozoan, Ces = Cestode, Cy = Cyst, m = male, f = female, (J = Jamshoro, S = Sanghar, H = Hyderbad, L = Larkana) S. H. L. J. = Location of the Sindh Province and Number = indicates serial no: of the rats of total area. M = Missing

Hetrocyclic essential amino acid:

Histidine was present in all group's chromatograms of the infected rats.

Valine was missing in the following six types of infections i.e., group 1, 3, 5, 6, 7, 9.

From the above discussion it is clear that in different parasitic infection different amino acids are absent or missing from chromatogram of plasma of infected rats but these amino acids normally present in the blood of rats.

Discussion

No work has been reported in the literature. After this investigation another work was done on Bacteria and results for amino acid variation in human serum and Urine has been shown which is totally different (Memon, 2001). The percentage of infected rats is divided into 9 groups according to the parasitic infections in blood with and without involvement of the liver and intestine in different region of the Sindh province. Table 2 shows that the percentage of rats in single sporozoan infection is higher (27.50%) than other group of infections while less % of rats has been found in blood (trypanosome), liver (cyst) and intestinal (cestode) infections. The percentage of rats from all localities in group 1 infection i.e. sporozoan infection in blood. 81.81% rats have histidine in their plasma chromatograms. Isoleucine, leucine, methionine and valine have not been found in infected rats as compared to normal rats of the same location. 54.54% rats have arginine, lysine and proline in their plasma. 18.81% plasma chromatograms of the rats have phenylalanine, tryptophen and threonine (Table 2).

So variation or absence of amino acids depend upon the severity of the infection. The type 2 infection in blood. The percentage of trypanosome infected rats have been found to be only 12.50% of the total infected rats investigated (Table 3).100% plasma chromatograms of the infected rats have arginine, lysine, proline, threonine tryptophine and tyrosine. 50% infected rats have histidine, phenylalanine and valine while other amino acids are missing from the plasma of the infected rats as compered to normal chromatograms of the plasma of rats.

The plasma free amino acids, variation in blood having double (sporozoans and trypanosome) parasitic infections. This type of infection is found in 10% rats only (Table 4). Histidine, and proline are present in 100% of the infected rats. Lysine is present in 75% rats. Arginine, threonine and tyrosine are present in 50% plasma chromatograms of the infected rats. 25% rats have phenylalanine and tryptophine. Isoleucine, leucine, methionine and valine is totally absent in infected rats as compared to normal values. The plasma amino acids in infected blood (sporozoan) along with liver (1-4 cyst) infection, 18% rats were involved in this type of double infection. Arginine is present in 71.42% infected rats (Table 5).

57.14% rat's plasma contains histidine and threonine.

42.85% plasma chromatograms in this type of infection show the presence of lysine, phenylalanine and proline. Methionine and tyrosine are present in 28.57% of infected rats. Plasma chromatograms of the 14.28% infected rats have leucine, tryptophane and valine. Isoleucine was found is missing in plasma of the infected rats. The plasma amino acids in blood (trypanosome) with liver (1-4 cyst) infection. 12.50% rats have type 5 infections (Table 6). Arginine, tryptophane and tyrosine are present in 20%-infected rats.100% rats have histidine and 40% rats have lysine and threonine. Proline is present in 60% rats and Phenylalanine and valine are absent in all. The plasma amino acids in double blood (trypanosome + sporozoan) with liver (1-2 cyst) infections which is present in 5% rats (Table 7).

Only histidine is present in all rats, having both the infections i.e., sporozoan and trypanosome + liver infection while all others amino acids are absents in the plasma of the infected rats in the infections. It is investigated that double infection in blood along with liver infection gave more variations than all other groups of infection. The type 7 group of infection i.e. blood (sporozoan) with liver (cysts) and intestinal (cestode) infections. 10% rats are involved this type of infection. The liver cyst ranged from 1-7 cyst, cestode (intestine) ranged were 3-13 cestode (Table 8). 75% plasma chromatograms of the rats have histidine phenylalanine, proline, tryptophan and tyrosine. Arginine, lysine, threonine are present in 50% of the rats. 25% plasma chromatograms of the infected rats contain leucine and methionine. Isoleucine and valine, which have infection in three tissues, are totally absent.

Type 8 Infection in blood (trypanosome) liver (1 cyst) and intestine (2 cestode) found only in one rat, only histidine, lysine threonine and valine are present in the plasma of the infected rat while other amino acid are totally absent (Table 9). The type 9 group of infections showed that 10% rats are involved in blood (sporozoan) and intestine (1-4 cestode) infection. Histidine is present in 100% plasma chromatograms of the rats. Colour and R_f values showed that lysine, phenylalanine, tryptophan and tyrosine are present in 75% rats. Plasma chromatograms contain arginine, proline and threonine in 50% rats, methionine is present in 25% plasma chromatograms while others plasma amino acids are absent (Table 10).

The protocol of the above infections shows that in all the cases amino acids are utilized for synthesis of different proteins of parasites. Hence the different infections showed lack of different amino acids. Every organ has different requirement for it, hence these amino acid are utilized during these infections. From this one can conclude that different parasitic infection can be identified from the chromatographic separation of amino acids of plasma of infected rats, infected with different parasites of the different organs, sites of the body.

Leucine, methionine and valine were mostly utilized by the endoparasites, hence they were not present in the blood of parasitical infected rats. Histidine was not taken up by the parasites, hence this amino acid was present in almost all parasitically infected rats. Similarly isoleucine was also utilized by the parasites of rats, its absent shows that this amino acid is utilized by the parasites studied during the work.

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