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Plasma Free Amino Acids Levels in Rats with Parasitically Infected Liver

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Abstract: Amino acid variation in different organs of rats *Rettus norvegicus* collected from different areas of Sindh for parasitic and biochemical analysis. Chromatographic separation of free amino acids, in normal and infected rats with endoparasites, using two different solvent system for avoiding overlapping have been used. The amino acid variation have been observed in liver (cyst) infection, liver (cyst) with intestine single (cestode) infection, liver (cyst) with intestine double (cestode + nematode) infection, liver (cyst) with blood single or both (sporozoan + trypanosome) infection, liver (cyst) with intestine (cestode) and blood (sporozoan or trypanosome) infection, liver (cyst) with intestine (nematode) infection. Isoleucine was totally absent in all types of infections. In liver (cyst) and blood (trypanosome and sporozoan) with double infection, only histidine was present in the plasma of the infected rats while all other amino acid which are normally present were totally absent. Leucine, methionine and valine were mostly absent except in some rats, infected with all type of endoparasitic infections.

Key words: Endoparasite, liver, plasma amino acid in rats, parasite, amino acids, parasitic infections

Introduction

Rats are definitive as well as reservoir host for various parasitic infections (Faiyaz-ul-Haque *et al.*,1990). The rat is a carrier of number of diseases transmissible to humans, such as plaque, murine typhus, leptospirosis, trichinosis, salmonellosis, lymphocytic choriomeningitis tape worm infections and rickettsial pox (Mushtaq-ul-Hassan *et al.*, 1997). The ancestral home of the house rat is the Southeast Asia and also present in Philippines, Indonesia and China upto the Yangtze river as well as along the slopes of Himalayas upto north Afghanistan and Central Asian countries east of the Caspium sea. From this range the rat spread across India, Pakistan and Middle East by moving from settlement to settlement along the ancient caravan routes (Mushtaq-ul-Hassan *et al.*, 1997). *Rattus rattus* and *Rattus norvegicus* are most common rats in Sindh province.

Rats are found indoors and therefore, confined to cities, towns and villages (Noor-un-Nisa and Ghazi, 1993). Several survey and ecological studies of parasites have been carried out in various parts of the world (Lim and Heyneman, 1965; Dun *et al.*, 1968; Tenora *et al.*, 1973; Jawadat *et al.*, 1980; Haq *et al.*, 1985). The studies conducted by Bilqees (1985), Khera and Wadhawan (1983), Al-Bawari *et al.* (1987), Fatima, (1991) and Noor-un-Nisa and Ghazi (1993) clearly revealed that rats harbour both protozoan and helminth parasites. The pathogenic mechanism during parasitic infection include mechanical injury, toxic effects, growth effects, effects on the host's reproductive system and various metabolic changes (Chappell, 1980).

In animals amino acids serve as building blocks of protein and as precursors of many other important biomolecules (hormones, purines, pyrimidines, prophyrines) and some vitamins. Amino acids are source of energy, particularly when they are ingested in excess of the amounts required to replace body proteins (Lehninger, 1993). Deficiency of amino acids can cause the tiredness, inability to concentrate irritability, retarded growth, hair loss, anemia, reproductive and nutritional problems.

Liver plays a very important role in the metabolism of different compounds. Variations occur in amino acids during different infections. Whether infection is in liver, intestine or blood, they will directly or indirectly be affecting liver cells or metabolic functions (Ahmed, 1987 and Taylar, 1999). Under some diseased conditions proteins disintegrate and amino acids are released in the body fluids. Such variations in the levels of the amino acids in the blood may affect the normal physiological functions of the body (Ahmed, 1991).

It has also been demonstrated that parasitic infection brings about biochemical and haematological changes in their hosts (Joshi, 1979; Kameswari *et al.*, 1975; Mohsin *et al.*, 1991; Kolb, *et al.*, 1993; Romaniuk, *et al*, 1993 and El-Naggar, 1994). Such variation also occur in bacterial infections (M. A. Memon, 2001).

The experiment were mostly centered upon the morphotaxonomy of the endoparasites of the rats (Noor-un-Nisa and Ghazi, 1993; Bilqees, 1985; Bilqees and Siddiqui 1981; Farooq, 1986).

The effect of the parasitism on the level of haematological indices and amino acid contents of the blood and urine 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.

In this work only the variations that occur in the amino acid contents of the body fluid (blood and urine), were investigated and the changes in the haematological parameters in the rats naturally infected with endoparasites also observed. And biochemical variation depending upon the severity of the infection and upon the part of the body or organ were studied, in which infection is there, amino acid variation have been observed.

It is investigated that infection in liver, in intestine or in blood gives variations in plasma amino acids in each of them cases of infection.

Materials and Methods

Rats *Rattus norvegicus* were collected from the different areas of the Sindh province i.e Sanghar, Larkana, Jamshoro, Hyderabad. Temperature range was 15-40°C. They were kept in cages (4 rat/cage) and fed *ad libitum*. Rats were anaesthetized and dissected for collecting samples.

Blood for haematological parameters and parasitic analysis was collected from the heart with the syringe and immediately transferred to vial contains mixture of ammonium and potassium oxalate, which was used as anticoagulant. Plasma for amino acid chromatography was obtained after performing PCV from the upper portion of the Wintrobe tube. Amino acids were analyzed by unidimentional ascending paper chromatography (Stahl, 1969 and Stock, 1974) using two different solvent systems:

Solvent No.1 n-butanol : acetic acid : water in the ratio of

4:1:5 and
Solvent No. 2; n-butanol : pyridine : water in the ratio of 1:1:1. These solvent systems enabled us to trace out more no. of amino acids from the plasma which was not possible with single solvent system because of over lapping of amino acids. Chromatograms were developed, after keeping them in oven at 105 °C for 10 minute. R_f values were calculated and colours developed were matched (Long, 1971 and Lehninger, 1993) to identify the amino acid and also they were compared with chromatograms of the standard amino acids.
For the identifications of parasites dissecting and high power microscopes were used. The parasites were identified in liver, alimentary canal, kidneys, heart, gall bladder, urinary bladder and lungs (Zelmer, 1998).

Endoparasites of the rats: In liver, larval forms/ bladder worm of cestode was found. *Hymenolepis diminuta, Hymenolepis nana* (Cestode) and *Aspicularis* sp. (Nematode) were identified in intestine. (Protozoan) *Trypanosoma lewisi* and Sporozoans (in RBC) were demonstrated in blood of rats.

Results and Discussion

Group of parasitic infection in liver (with and without intestine and blood), percentage of rats infected with endoparasites according to the different group of infections and variations in different plasma amino acids in different group of infections were investigated (Table 1). It has been observed that the percentage of rats in liver infection and liver with blood (sporozoan) infection were high than other group of infections. Liver (cyst) and blood (sporozoan + trypanosome) with double infection only histidine was present while all other amino acids were totally absent. The Percentage of infected rats divided into different groups according to the parasitic infections in liver with and without involvement of intestine and / or blood in different region of the Sindh province (Fig. 1).
The percentage of rats having infection only in liver is given in Fig. 2. The cyst infection ranged from 1-4 cyst according to the intensity of infection. These single infections were found in 20% rats. 71.42% rats have arginine, histidine and proline in their plasma chromatograms. Isolucine, methionine and valine have not been found in any of the infected rats. 42.85% rats have lysine and thronine in their plasma. 57.14% plasma chromatograms of the rats having only infection in liver have tyrosine. Leucine is present in 14% of plasma chromatograms of the infected rats. It is also observed that those rats which have only single cyst infection have less missing of amino acids, in presence of 3 cyst only phenylalanine, proline and tyrosine are present while all others are absent in the plasma of the rats. In the presence of 4 cysts in liver infection only histidine is present, while all other amino acids are missing. These result show that when more infection in liver more amino acids are missing.
The percentage of infected rats was found to be only 14.28% (Fig. 3) of the total infected rats investigated, infection have been found in liver along with intestinal (cestode) infection. Only one liver cyst was present in the liver of infected rat while in some rats intestinal cestode ranged between 1 to 113 cestodes. 60% plasma chromatograms of the infected rats have arginine. Histidine, lysine, tryptophine and tyrosine were present in 80% rats. 20% infected rats have methionine, phenylalanine, proline and thronine. Isolucine, leucine and valine are missing from the plasma of the infected rats. Plasma amino acids variation in liver (1-3 cysts) with double intestinal parasitic infections namely cestode and nematode. Cestode, which ranged from 1 to 2 while nematode ranged from 1-100.

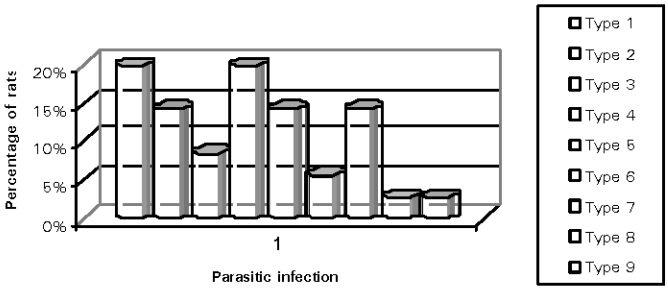


Fig. 1: % of liver along other tissues infection in rats.

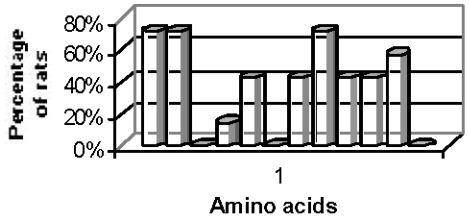


Fig. 2: Plasma amino acids in liver (cyst) infection.

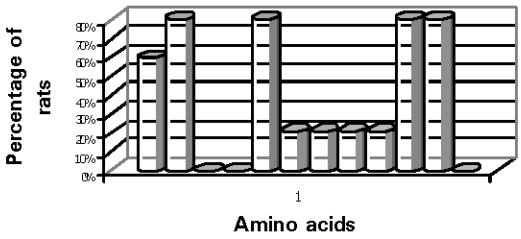


Fig. 3: Plasma amino acids in liver (Cyst) and intestine (cestode) infection.

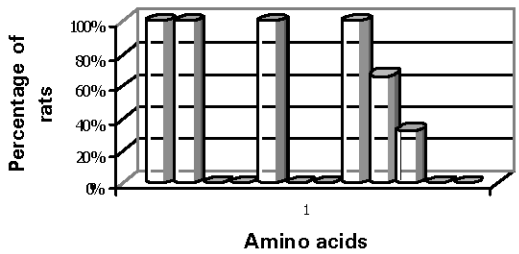


Fig. 4: Plasma amino acis in liver (Cyst), intestine (Cestode + nematode) infection.

This type of infection is found in 8.57% rats (Fig. 4). Arginine, histidine, lysine and proline are present in 100% of the infected rats. Tyrosine is present in 33.33% rats. Thronine

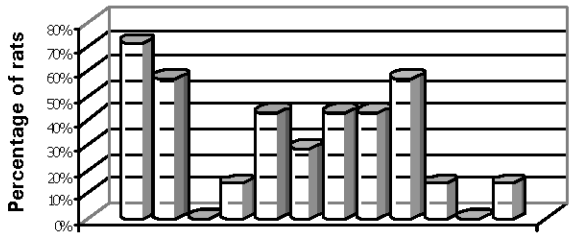


Fig. 5: Plasma amino acids in liver (Cyst) and blood (Sporozoan) infection.

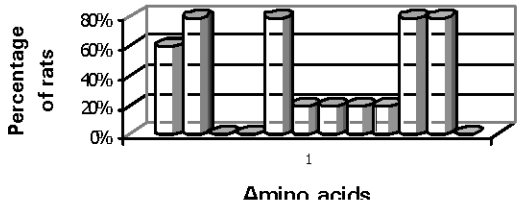


Fig. 6: Plasma amino acids in liver (Cyst) and blood (Trypanosome) infection.

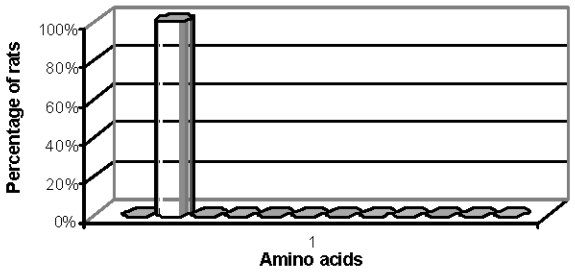


Fig. 7: Plasma amino acids in liver (Cyst) and blood (Trypanosome + Sporozoan) infection.

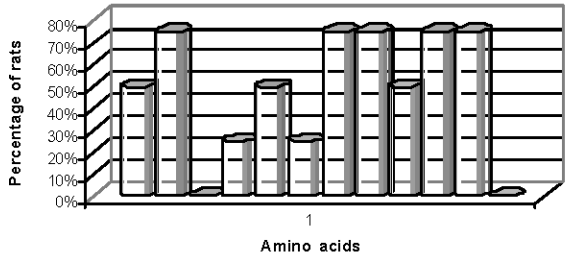
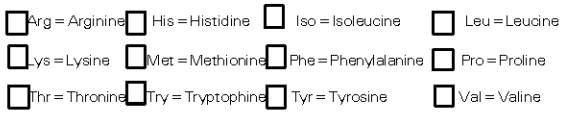


Fig. 8: Plasma amino acids in liver (Cyst), intestine (Cestode) and blood (Sporozoan) infection.



shows their presence in 66.66%rats. Isoleucine, leucine, methionine, phenylalanine, tyrosine and valine is totally absent in infected rats as compared to normal values.

It is observed that severity of the nematode infection does not give any effect on the plasma amino acids of the infected rats. From the plasma amino acids in infected liver (1-4 cyst) along with infection in blood (sporozoan). It was investigated that 20% rats are involved in this type of double infection (Fig.5). It has been found that when blood is infected then more amino acids are missing. Arginine is present in 71.42% infected rats. 57.14% rat 's plasma contains histidine and thronine.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% infected rats. Plasma chromatograms of the 14.28% infected rats have leucine, tryptophane and valine. Isoleucine was missing in plasma of the infected rats.

5.71% rats have infection in which they have plasma amino acids in liver (cyst) with blood (trypanosome) infection (Fig.6). Arginine, tryptophane and tyrosine are present in 20%-infected rats.100% rats have histidine. 40% rats have lysine and thronine. Proline is present in 60% rats. Phenylalanine and valine are absent in all. It is observed that trypanosome infection effect more on plasma amino acids.

5.71% rats have infection in which the plasma amino acids in liver (1-2 cyst) with double blood (trypanosome + sporozoan) were present (Fig.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 absent 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.

14.28% rats with plasma amino acids in liver (1-7 cysts) with intestinal (cestode 3-13) and blood (sporozoan) infections were found (Fig.8). The liver cyst ranged from 1-2 cyst, cestode (intestine) ranged were 3-13 cestode. 75% plasma chromatograms of the rats have histidine phenylalanine, proline, tryptophan and tyrosine. Arginine, lysine, thronine 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. Infection in liver (1 cyst), intestine (2 cestode) and blood (trypanosome) only found in one rat, histidine, lysine thronine and valine are present in the plasma of the infected rat while other A. acid are totally absent. Besides the group discussed above other group of infection investigated in which infection is in liver (1 cyst) and intestinal (1 nematode only) found in one rat, plasma chromatograms contain only arginine, phenylalanine, and proline while others plasma amino acids are totally absent. In this case as there is less infection but more amino acids are missing may be because of any other infection besides parasitic infection.

The protocol of the above infections showed that in all the cases amino acids are utilized for synthesis of different proteins. Hence the different infections showed absence of different amino acids in infected rats. Every organ has different requirement for amino acids, hence these amino acids are utilized during these infections differently. Our results consider with the results of Memon *et al.* (2001). But they found the Amino acid variation in Bacterial infection while our work is an parasitic infection. In any kind of parasitic infection variation has been observed.

If we look into the amino acids picture in case of trypanosome infection in blood still more amino acid, are missing as compared to sporozoan. If again both of these infections are found in blood of the rat still more missing of amino acids have been found. Absence of amino acids are correlated with

increasing manner of infection. It has also been observed that severity of the nematode in intestine does not show any effect on the plasma amino acids. Infection has been found in less no. of rats but more amino acids are missing in the infection with the (blood) sporozoan and trypanosome along with (liver) cyst infections. In infected rats with liver (cyst) and blood (trypanosome and sporozoan) / double infection only histidine has present in the plasma of the infected rats while all other amino acid which were present in normal rats, were totally absent in these cases. Leucine, methionine and valine were mostly absent except in a few rats, infected with all type of endoparasitic infection.

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