Diagnostic Value of 5'-Nucleotidase on Kala-Azar Patients

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Summary:

5'-Nucleotidase activity was measured in the sera of (67) Kala-azar patients before treatment and at different stages of treatment with pentostam as well as in (30) age matched normal children. The changes in 5'-NT isoenzyme profile were also followed among the above cases.

A change in the enzyme activity with the progress of the disease was observed. Our results suggest also that there are changes in the 5'-NT isoenzyme profile with the severity of the disease.

Introduction:

5'-Nucleotidase (5'-NT, Ec 3.1.3.5), specifically catalyzes the hydrolysis of the 5'-monophosphates of purine and pyrimidine ribosides. Commonly, most tissues contain two isozymes of 5'-NT, the intrinsic membrane one (5'-NT-I) and a soluble cytosolic 5'-NT-II.

The enzyme has important physiological functions, one of them is the production of adenosine from extracellular nucleotides (11-13). In routine clinical chemistry, practice 5'-NT has been measured in serum where its level is increased in hepatobiliary disease and malignancy (14). Kala-azar disease has a vast geographic distribution involving millions of people, is caused by species of leishmania (L-donovani complex, L-chagasi, L-infantum) tki disseminate hematogenously infected macrophage in the liver, spleen, bone narrow and lymphnodes. Demonstration of the parasite preferably by culture b essential before starting treatment (15, 16, 17). The parasite can be demonstrates if the spleen, liver, bone marrow, lymphatic gland & less common blood (3), evenhough this technique has a good results but complications as hemorrhage make it of less use in practice (15, 18, 19). Many immunologoes rest were performed which were based either on cellular method as in lishman—a skin test, or on humoral methods depending on the deinoistriarol. Circulating antibodies, Among these the methods mostly used in practice an immunofluorecent antibody test (IFAT) & micro-enzyme linked immuno absent assay (ELISA) & now days, the specific monoclonal antibodies, liis later method found to be positive even when the IFAT & ELISA are negative (20).

We have previously purified and characterized the enzyme (5'-NT) from sera of intiresed Kala-azar patients (21). Throughout this study we followed the changes of 5'-NT activities and isoenzymes pattern with the severity of the disease and UDH treatment of the disease.

Material and Methods:

Thirty two untreated Kala-azar patients were included in this study, they were admimcra different pediatric hospitals in Baghdad during the period (12/1994-6/1995) and thirty two patients received treatment: with pentostam (20 mg / Kg) of body weight for 21 days. These patients are from different areas of Iraq and their ages ranged between 3 months - 5 years. The diagnosis was based on the clinic grounds supplemented by IFAT test and / or the demonstration of the parasite of the amastigote form in direct smear of bone marrow, or the promastigote form in culture of bone marrow . Thirty samples were obtained from healthy children attending the maternal and child welfare units in Baghdad and were used as control for comparison. Blood samples were obtained by venipuncture from different sites depending on the age of the child involved it was collected carefully and slowly in order to avoid any hemolysis that could interfere with the results obtained.

Blood samples were left at room temperature to clot, then centrifuged at 3000 rpm for 15 minutes and the separated sera were used throughout this study.

Chemicals:

The chemicals used were from effferent companies and as follows: Adenosine 5'-monophosphate (5'-AMP), hydrochloric acid, stammous chloride, sodium hydroxide, sulfuric acid,
ammonium molybdate, potassium dihydrogen orthophosphate, tris-hydroxy methyl unimomethane, disodium hydrogen orthophosphate, sodium dihydrogen orthophosphate, sodium dodecyl sulfate and magnesium sulfate were from (BDH). Loxine serum albumin and hydrazine sulfate were from (SIGMA). Nickel cloride from Hopkin and Williams LTD 5'-AMP sephorose 4.2 from phannacia the chemicals. Sodium barbital from (Coming AC1 special).

Methods:
Separation of 5' NT isoenzyme:
Isoenzyme forms of an enzyme va- in their affinity to their substrate, so we chose affinity chromatography as a echnique to separate 5' NT isoenzyme present within the sera of Kala-azar patients. And in order to do so batch sample o of the sera of each group of patient used throughout this study (protein concentration of 10 mg) were applied on a 5' AMP sepharose 4 B columns (1.7x1.5 cm) preequilibrated with tris-HCl buffer (10 M, pH 8).
Fractions of one mliter volume were collected upon the enzyme elution from the column using 10 mM tris containing 10 mM 5' AMP (TRIS tris butler pH 8) at a flow rate of 0.25 ml/min.
The enzyme activity and the protein concentration were determined as described below.
Assay of 5' AT activity:
5' NT activity was measured by following Wood and Williams method (1981)22. Unit of enzyme activity was defined as the amount of the enzyme that produce 1 u mole of phosphate by hydrolysis of ester bond present in 5' AMP when potassium dihydrogen phosphate (KH2PO4) was used as a standard.
Protein determination:
Determination of serum protein was performed as described by Lowry et al (1951)10 using bovine serum albumin as a standard.
The specific activity of 5' NT; expressed as unit of enzyme activity / mg of proteins.

Results and Discussion:
Previous study carried out in our laborator21 showed that 5' NT activity in sera of normal children ranged between (2.5-11.901). An increase in the activity was observed in 10% of the Kala-azar patients studied where the activity reached up to 70 U/l. Throughout this study, we looked on the relationship between the enzyme activity and the severity of the disease in which the IFAT titre was used as an index of the disease severity. It is obvious from table (1) that 5' NT activity in the sera of Kala-azar patient changed with the disease severity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage of the Studied Cases</th>
<th>IFAT Titre</th>
<th>5'N'T Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Kala</td>
<td>17.4%</td>
<td>1</td>
<td>8.9-11.9</td>
</tr>
<tr>
<td>Azar patient</td>
<td>39.1%</td>
<td>1</td>
<td>11.9-30.0</td>
</tr>
<tr>
<td>Case</td>
<td>43.5%</td>
<td>1</td>
<td>30.0-70.0</td>
</tr>
</tbody>
</table>

Table (1) Relationship between 5' NT activity in the sera of patients and the severity of the Kala-azar disease.

5' NT activity was found to be equal to the upper limit of normal (ULN) activity when the IF AT titre was (1/16) while it increased three fold the upper limit of normal (ULN) when the titre was (1/32) and a maximum 5' NT activity was observed [seven ibid (ULN)] when the titre was (1/64). These values coincide with the value obtained in cases o1 intrahepatic biliary obstruction. The increase in 5' NT activity observed could reflect the pathological picture of non-specific granulomua formation in the liver of these patients.

It has been noticed by many investigators in Iraq, that the Kala-azar patients show differences in their disease severity as well as in their response to chemotherapy. AMP sepharose 4B has been widely used as a general ligand for affinity chromatography to separate many desire nucleotide metabolizing enzyme which differ in their kinetics properties related to the value of Km for AMP and its analogous compounds, and in an attempt to study the possibility of using the changes in 5' NT isoenzyme profile as a diagnostic tool to follow up the severity of the kala-azar and the progress of the treatment. Hatch sample of sera of the Kala-azar patient at different stages of the disease before and after their treatment, were applied on 5' AMP sepharose chromatography column and as described in the materials and methods section. The changes in isoenzyme profile were followed where it was assumed that there may be a specific elution pattern of these isoenzyme from the column depending on their Km values for 5' AMP. In our previous study17, the results showed that two isoenzyme of 5' NT were present. If the sera of the Kala-azar patient each present in more than one form. Throughout this work, Figures (1, 2, 3 and 4) show the changes in the 5' NT isoenzyme pattern upon elution from the column. Depends on the severity of the disease. A new 5' NT peaks with low Km for AMP appear in
the patient sera during the early stage of the disease. Upon treatment with pentostam the isoenzyme profile changes (Fig 2 A and B) toward the normal pattern (Fig. 1) and it is clear that with the treatment isoenzyme II disappears with an increase in the specific activity of isoenzyme I, when the child received the treatment at the beginning of the infection (i.e., when IF AT titre was 1/16): while the activity of is enzyme I increases Eighty with a decrease in one form of isoenzyme II to reach zero and a reduction of 62.5 % in the other form when the IFAT titre was (1/32) (Fig.3 A and B). When the IFAT value was (1/64), the picture seems to be different (Fig. 4 A) although four peaks of S' NT again were observed and the isoenzyme profile change again upon the treatment with slightly different profile (Fig. 4 B). Further work is carrying on in our laboratory to characterize these differences.

The change in the S' NT isoenzyme profile that has been reported here may reflect involvement of different organs in the infection which leads to the appearance of different isoenzyme (forms) of S' NT in the sera of Kala-azar patients. It is known that the parasite attack the reticuloendothelial system in the liver (Kupffer cells') thus the liver parenchyma is not attacked at the beginning of the infection and this explain why some patients shows normal acivity, but with the progress of the disease extensive, hypertroph and hepatomegaly, the liver parenchyma is slowly damaged so the elevation of S' NT activity observed in 28.6 % of the cases which has higher IAHT titre.

From this study we can conclude that following the changes in S' NT isoenzyme profile may be used as 2 diagnostic tool to follow up Kala-azar infection and the progress of their treatment. The possibility of the overall diagnostic value of S' NT would be changed by studying of the changes in S' NT isoenzyme pattern in patients suffering from different disease including hepatobiliary disease, a work being carried out in our laboratory.

References:
Legend to the figures:
Fin 1 The isoenzyme profile of 5' NT from sera of normal children. 5' NT isoenzymes were separated by applying the sera of normal children (5-10 ml) on 5'-AMFsephrose-4B column (1.5 x 1.7 cm) all details are explained in the material and method section.
Fin 2 The isoenzyme profile of 5' NT from sera of LDA-azar patient with IFAT value of (1/16): (A) Before treatment, (B) After the treatment with Fio3 The isoenzyme profile of 5' NT from sera of xula-azar patient with IFAT value of (1/32).
(A) Untreated patient, (B) J-jated patient.
Fig 4 the isoenzyme profile of 5' NT from sera of Lila-azarpatient (IFAT value 1/62) (A) Untreated patient, (B) Treated illienL.
Fig. 1: The isoenzyme profile of 5'NT from sera of Kala-azar patient (IFAT Value: A) untreated patient, (B) treated patient.