Clinical significance of sialic acid in acute rheumatic fever with and without carditis

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Abstract

Objective: In Egypt, acute rheumatic fever (ARF) is the most common cause of acquired heart disease. ARF is a clinical syndrome without a specific diagnostic test or single pathognomonic feature, so the aim of this study is to investigate the utility of using serum total sialic acid (TSA) in differentiation between children suffering from ARF with and without carditis.

Methods: In the present study, 62 children were divided into three groups: first group, 16 healthy children; second group, 20 patients suffering from chronic rheumatic heart disease (RHD); and third group, 26 patients suffering from ARF, subdivided into three subgroups as (A) 10 patients suffering from arthritis without carditis, (B) 10 patients with carditis but without congestive heart failure (CHF), and (C) 6 patients with carditis and CHF. Anti-streptolysin O titre (ASOT) by latex agglutination test, erythrocyte sedimentation rate (ESR) by Westergren method, C-reactive protein (CRP) by turbidimetric immunoassay method, lactate dehydrogenase (LDH) activity by kinetic method, Immunoglobulin levels by radial immunodiffusion assay (RID), cardiac troponin I (cTni) by ELISA test kit and TSA by diphenylamine (DPA) method were performed.

Results: Significant elevated values of ESR, CRP, LDH, IgG and IgA were obtained in ARF as compared to both control and RHD groups; significant values of IgM were found between B and C as compared to control and RHD groups; cTni showed insignificant difference between all groups; values of TSA showed highly significant difference between ARF as compared to both control and RHD groups as well as between RHD and control groups; significant difference was found between B and C groups as compared to group A.

Conclusion: The results of the present study point out the possibility that the levels of TSA could be of help in differentiation between ARF patients with and without carditis.

Key words: Acute rheumatic fever; Heart disease; Immunoglobulin; Sialic acid; Troponin I

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Introduction

Acute rheumatic fever (ARF) is an inflammatory disease of childhood that occurs following untreated group A streptococcal pharyngeal infections. ARF is the most common cause of acquired heart disease worldwide [1, 2]. This multisystemic disorder can affect the heart, joints, skin and central nervous system. ARF remains highly prevalent in developing nations where crowding, poor hygiene and limited access to health care persist [3].

There are still many controversial points related to the clinical manifestation and diagnosis of ARF. Physicians who frequently deal with new cases of the disease often feel uncomfortable in establishing a definite diagnosis, especially if they strictly follow Jones’ criteria. This set of criteria was created in the mid 40’s, when incidence of ARF in the USA was high. The Jones’ criteria have been revised a few times since then; the last revision was performed by the WHO Expert Consultation panel on ARF and rheumatic heart disease (RHD) [4, 5].

There is no specific test available for establishing the early diagnosis of ARF [1, 6]. ESR and CRP are evidence of inflammation but they fail to determine slight changes in the activity of the rheumatic process and identify rheumatic carditis [7].

Detection of active rheumatic carditis is of great prognostic and therapeutic importance and is currently based on the Jones’ criteria. The diagnosis of rheumatic carditis by Jones’ criteria becomes difficult when carditis is the isolated manifestation
of ARF. This is true especially when carditis is subclinical, when it is apparent but supportive noncarditic criteria for diagnosis of ARF are not fulfilled, or when the previous cardiac findings are not known for documentation of interval change in cardiac findings during the recurrence of disease [8].

Clinically manifest mitral regurgitation (MR) and aortic regurgitation (AR) are diagnostic of acute rheumatic carditis. The rheumatic carditis is rarely diagnosed in the absence of valve regurgitation and precordial auscultation has been the usual modality for the diagnosis of MR and AR. However, studies done even in the golden era of cardiac auscultation have shown that valvular regurgitation may not always be detected by routine clinical auscultation. Recent studies have shown that clinical auscultation may be a dying art. Echocardiography, therefore, may allow earlier diagnosis but ultimately may not prove to be superior. In addition, the ability of echocardiography to detect subclinical recurrence of carditis in the presence of preexisting rheumatic heart disease remains obscure, unless there is an obvious interval change in echocardiography findings [8].

In the last few years, different workers all over the world have demonstrated that concentration of sialic acid in the human serum is abnormally high in a pathological state where the underlying pathology is either of tissue destruction, tissue proliferation, depolymerization or inflammation [9, 10]. Since necrotic, degenerative and inflammatory changes are all features of the pathologic picture of ARF and since in inflammatory diseases, the induction of glycoproteins and mucoproteins associated with sialic acid give clearly purple color with diphenylamine (DPA) [11].

In the present study it was decided to investigate the levels of total sialic acid (TSA) in children suffering from ARF and chronic RHD.

Materials and methods
The study was performed on 62 children (34 males and 28 females) with age range from 4-15 years, divided into three major groups. First group included 16 healthy children free from any symptoms or signs of ARF or any chronic illness (negative Antistreptolysin O titre, ASOT), second group included 20 patients suffering from chronic RHD (positive ASOT) and third group included 26 patients suffering from ARF (positive ASOT). This last group was subdivided into three subgroups: subgroup (A) included 10 patients suffering from arthritis without clinical signs or symptoms of carditis; subgroup (B) included 10 patients with clinical signs of carditis but without signs or symptoms of congestive heart failure (CHF); and subgroup (C) included 6 patients with clinical signs and symptoms of carditis and CHF. Diagnosis of cases was according to Jones’ criteria [4], this criteria have been validated in the ethnic population studied. Diagnosis of cases was confirmed with echocardiography. Differentiation of acute cases from chronic cases was depending on acute phase reactants.

Overnight fasting blood samples were obtained from each individual within the first two days of the admission at Pediatric Abo-Reich Hospital, Cairo, Egypt. Each blood sample was distributed into two tubes: first tube was used for determination of erythrocyte sedimentation rate (ESR) at (first and second hour) by Westergren method [12]; second tube was used for the following assassments: ASOT by latex agglutination test (Vitro Scient, Cairo, Egypt) [13], C-reactive protein (CRP) by turbidimetric immunoassay method (Giesse Diagnostic, Rome, Italy) [14, 15], lactate dehydrogenase (LDH) activity by kinetic method (Chronolab AG, Zug, Switzerland) [16], IgG, IgA and IgM by radial immunodiffusion assay (RID) (Biocintifica S.A., Buenos Aires, Argentina) [17], cardiac troponin I (cTnI) by ELISA test (United Biotech Inc., Mountain View, CA, USA) [18] and serum TSA by a colorimetric assay using standard chemical and reagents, in this method a protein precipitate of serum containing sialic acid will react with DPA producing a purple color which is quantitatively measured on a spectrophotometer at 540 nm [19, 20]. A written consent was given by all participants, and the study was approved by the ethical committee at Pediatric Abo-Reich Hospital and National Research Centre, Cairo, Egypt.

Statistical analysis
All results were presented as mean values ± standard error. Differences between groups were evaluated by the calculation of Student’s t-test and one-way ANOVA. Correlations between biochemical markers and other continuous variables were tested using the Spearman or the Pearson’s correlation coefficients. All reported p-values are based on two-sided tests and compared to a significance level of 5%. SPSS 17.0 software (Statistical Product and Services Solutions, version 17.0, SPSS Inc, Chicago, IL, USA) was used for all the statistical calculations.
Results
As shown in Table 1, significant elevated values of ASOT were obtained in ARF subgroups as compared to chronic RHD group; also significant values of 1st ESR, 2nd ESR, CRP, LDH, IgG, IgA were obtained in ARF subgroups as compared to both control and chronic RHD groups. The subgroups (A, B and C) of ARF showed insignificant differences between each other in all of the previous parameters except for LDH and IgA which showed significant difference in subgroups B and C as compared to subgroup A. Significant elevated values of 1st ESR and CRP were obtained in chronic RHD as compared to control.

Regarding the values of IgM, significant difference was found between B and C subgroups as compared to control and chronic groups; significant difference was found between B and C as compared to A. The data of cTnI showed insignificant difference between all groups.

As shown in Table 1 and Fig.1, the values of TSA showed highly significant difference between ARF subgroups as compared to both control and chronic RHD groups; also, TSA showed highly significant difference between RHD as compared to control group as well as significant difference was found between B and C groups as compared to group A.

As shown in Fig.2, negative significant correlation of cTnI against ESR and CRP (r = -0.458, p = 0.02), (r = -0.475, p = 0.014), also negative correlation between cTnI against ASOT and TSA (r = -0.339, p = 0.091), (r = -0.089, p = 0.664) were estimated.

As shown in Fig.3, positive significant correlation was obtained between TSA and ESR (r = 0.62, p = 0.001).

Table 1. Clinical data of the studied groups (one-way ANOVA)

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (n = 16)</th>
<th>Chronic RHD (n = 20)</th>
<th>Subgroup A (n = 10)</th>
<th>Subgroup B (n = 10)</th>
<th>Subgroup C (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASOT (IU/ml)</td>
<td>Negative</td>
<td></td>
<td>480 ± 61.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>540 ± 60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>666.7 ± 122.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>1st ESR (mm/h)</td>
<td>6 ± 0.26</td>
<td>8.6 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.7 ± 4.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>38.7 ± 4.5&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>41.6 ± 7.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2nd ESR (mm/h)</td>
<td>13.7 ± 0.56</td>
<td>16.1 ± 1</td>
<td>63.4 ± 7.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>67.7 ± 6.8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>72.5 ± 12.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.6 ± 0.1</td>
<td>2.45 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.12 ± 0.46&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.6 ± 1.08&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>8.53 ± 1.42&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>331.7 ± 9.8</td>
<td>338.9 ± 11</td>
<td>391.3 ± 15.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>550 ± 10&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>584 ± 14.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>870.4 ± 56.3</td>
<td>978.4 ± 47.5</td>
<td>1541 ± 185.5&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1861 ± 260.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2566.9 ± 402.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>121.20 ± 4.3</td>
<td>126.4 ± 5.5</td>
<td>176.9 ± 19&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>250.4 ± 25&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>306 ± 9.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>119.6 ± 10.4</td>
<td>137.6 ± 17</td>
<td>139 ± 13.9</td>
<td>193.7 ± 17.5&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>223.2 ± 16&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>cTnI (ng/ml)</td>
<td>0.058 ± 0.0036</td>
<td>0.059 ± 0.0028</td>
<td>0.06 ± 0.0045</td>
<td>0.066 ± 0.0039</td>
<td>0.099 ± 0.0035</td>
</tr>
<tr>
<td>TSA (mg/dl)</td>
<td>64.4 ± 1.2</td>
<td>77.5 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109.30 ± 0.66&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>127.6 ± 1.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>129 ± 3.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
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Negative; less than 200 Ig/ml; significantly different from *control group, *chronic RHD group, and *ARF subgroup A.
Discussion

There are still many controversial points related to the clinical manifestation and diagnosis of ARF. Physicians who frequently deal with new cases of the disease often feel uncomfortable in establishing a definite diagnosis, especially if they strictly follow Jones’ criteria [4, 5]. The aim of this study is to establish a simple technique that can be reliable in early diagnosis, detection of slight changes in the activity of the rheumatic process, prediction of RHD and differentiation between patients with and without carditis through measuring the values of serum TSA in two groups of children suffering from ARF and chronic RHD.

In the present study, regarding the values of ASOT significant differences were obtained in ARF subgroups as compared to chronic RHD group, also ESR and CRP showed significant differences in ARF subgroups as compared to both control and chronic RHD groups. These results are in harmony with Ayob et al [21] and Carvalho et al [22] who reported that the values of these parameters were markedly increased in patients of ARF.

Regarding the values of ASOT, ESR and CRP, insignificant differences between the three subgroups of ARF were in agreement with Alehan et al [23]; El Amrousy et al [24]; Roodpeyma et al [25] and Tavli et al [26]. Their prospective studies were performed on patients with acute rheumatic valvular disease using echocardiography and cTnI in order to detect myocardial involvement. Neither significant cTnI elevations nor echocardiographic systolic function abnormalities were found in their patients with rheumatic carditis. They concluded that, variable clinical presentation of disease and increasing intensity of cardiac involvement is not related to the ASOT, ESR or CRP levels in cases of ARF.

Regarding the investigation of serum LDH activity, significant difference was found between ARF subgroups as compared to both control and chronic RHD groups as well as in subgroups C and B as compared to subgroup A. Our results were in agreement with the report of Megahed and Yassin [27]. In this study, LDH can differentiate between ARF with and without carditis, but using of TSA for this purpose might be preferred since the TSA showed higher sensitivity (100%) than LDH (80%) in our results.

Regarding the values of IgG, IgA and IgM, our results were in agreement with Morad [28]. The percentage of cases in ARF subgroups showing increase in IgG, IgA and IgM were 69, 73 and 52%, respectively. Also, these rising of Igs levels in rheumatic patients were mostly associated with hyperimmune to streptococcal antigens are in agreement with many other authors [28, 29].

In ARF groups, IgG were increased more than IgM values and this can be explained that, the specific anti-streptococcal anti-bodies belong mainly to IgG class [30]. The excess IgM [31] is most probably due to antibody formation against streptococcal exogenous antigens cross reacting with endogenous antigens of different organs especially the heart and joints and hence leading to or perpetuating tissue destruction.

In the present study, the values of IgM were increased more in carditis with CHF than without CHF. Similar observation was observed by Kamel et al [32] in CHF where a sluggish blood flow and poor perfusion of different organs including where the site of IgM catabolised. This may delay the uptake and catabolism of IgM because it is mainly an intravascular macromolecule.

In this study, IgA and IgM can differentiate between ARF with and without carditis, but using of them for this purpose may not be preferred since the determination of Igs was found to be more expensive, cost and time consuming than TSA.

In agreement with Gupta et al [33], Oran et al [34], Tavli et al [26] and Cunningham [35], insignificant difference was found in cTnI levels between ARF children with arthritis and with carditis as compared to healthy controls. However, the absence of elevated cTnI throughout the course of ARF in particular during active carditis may be related to insignificant cardiomyocyte injury as reported by Oran et al [34], Gupta et al [33], Williams et al [36] and Kamblock et al [37].

In ARF subgroups, A, B and C, insignificant difference of cTnI was found between each other. In agreement with Kamblock et al [37], Alehan et al [23] and El Amrousy et al [24] who reported insignificant difference for cTnI levels also was found between their patients with and without carditis. Since serum cTnI concentration does not exceed above normal limits in rheumatic carditis due to the less destructive nature of rheumatic carditis on the myocyte, it has limited value in the diagnosis or the prognosis of rheumatic carditis. Whether slight increases of cTnI may be observed in rheumatic carditis would be a subject of further investigation [23].

In our result, the demonstration of slight increase in cTnI levels in our patients with carditis and CHF can be explained by five patients in subgroup C
which had pericardial effusion detected with echocardiography or may be related to only minimal myocardial injury. Missove et al [38] and Bonnefoy et al [39] also confirmed that, the pericarditis associated with ARF may cause limited sub-epicardial myocardial cell damage resulting in slight cTnI elevation. Moreover, it has been found that the elevation of cTnI is greater in ischemic injury (such as myocardial infarction), but it was less in non-ischemic injury (such as myocarditis) [23, 40]. The low cTnI values, especially in the presence of active carditis, disputes significant ischemic myocyte injury. In ARF, myocardial necrosis is not prominent despite intensive inflammation. This is supported by lack of myocardial necrosis observed in biopsy specimens of patients with ARF carditis [8].

In the present study, patients with ARF showed negative significant correlation of cTnI against ESR and CRP; also, negative correlation between cTnI against ASOT and TSA. Gupta et al [33], Kambluck et al [37] and El Amrousy et al [24] also found negative correlation between the levels of cTnI and ASOT as well as ESR and they suggested that cTnI levels are not influenced by the immune response during acute RF and a tendency to the decrease of cTnI with the importance of the inflammatory syndrome.

From our previous investigation, it has been found that there is no specific test for ARF diagnosis and whether the rheumatic process has become inactive during therapy. Although it is generally agreed that the ESR and CRP have a great value as a guide in the treatment of ARF, they fail to determine slight changes in the activity of the rheumatic process and identify rheumatic carditis [41].

The goal in treatment of ARF is the suppression of all rheumatic inflammation. But this goal is seldom attained because the amount of anti-inflammatory drug is unknown. The optimum dosage of these drugs probably varies with each patient and each attack. In general, treatment is administered to a degree sufficient only to induce relief of symptoms and a progressive fall in the ESR and CRP [7, 42, 43].

In the present study, it has been found that the reaction of DPA with sialic acid is a good index in the different degrees of ARF. Concerning the investigation of serum TSA level and its analysis, high significant difference was found between the values in ARF groups as compared to both control and chronic RHD groups, as well as between chronic RHD as compared to control group. In ARF groups, significant difference was found between the values in B and C subgroups as compared to group A. From our analysis it has been found that TSA in ARF subgroups was two folds higher than mean normal values so it can be a predictable and reliable test for diagnosis of ARF. This investigation is also supported by Lindberg et al [11], who reported that serum sialic acid was increased during inflammatory processes due to increased concentrations of richly sialylated acute phase glycoproteins [20, 44].

In our data, although all cases of ARF subgroups showed high level of TSA. The values of ESR in 15.5% and CRP in 42% in these cases were normal in level indicating the high sensitivity of TSA test to reflect mild rheumatic activity and subclinical inflammation above ESR and CRP tests. In chronic RHD group, TSA values were significantly higher than normal control group (the percent increase was 62.5% over the mean normal value), while ESR and CRP values showed insignificant difference. Hence, the TSA test was of value in reflecting the mild rheumatic inflammation as well as predicting a rheumatic exacerbation. Moreover, in ARF subgroups, the estimation of TSA in the two groups with carditis (B and C) showed highly significant values than group A without carditis. This data confirmed that the detection of TSA could be a reliable value and differentiating test between ARF patients with carditis from patients without carditis.

In our study, a positive significant correlation was obtained between TSA and ESR as acute phase reactant. This data was also in agreement with Crook et al [45] and Berkan et al [46] who reported that TSA levels correlates with the acute phase reactants that appear in acute inflammatory reaction. In addition, the level of serum TSA seems to correlate with inflammation, accelerated atherosclerosis and cardiovascular events in patients with heart diseases [47].

Although the investigation of TSA still unknown, this test affords perhaps the most useful laboratory guide and now it is available for the care of the rheumatic patients. It provides a wide range of values in healthy children and in patients during inflammation and can be used as an accurate and quantitative measurement. When the level of TSA has been returned to normal, one might be assume that rheumatic inflammation either has probably been completely suppressed by treatment with a drug or has spontaneously subsided. However if TSA values persist at a high level after a rheumatic attack, one may reasonably assume that fresh rheumatic lesions may occur and may lead to heart
damage. If these observations and their interpretation are correct, treatment should be directed as early as possible in the rheumatic attack to provide a drug dosage adequate at least to suppress all inflammation as reflected by a prompt fall of TSA to a normal level.

It is of interest that, the other diagnostic benefit of TSA is that its determination together with other parameters may indicate the development of carditis in patients with ARF. This may help and give the clinician an early chance to start treatment with corticosteroids therapy and would ascertain the great need of bed rest for patients. The simplicity and the sensitivity of the present method recommend the use of this procedure in our study.

In conclusion, although there is no specific test till now for ARF, there is no way to determine with certainty when activity has ended and the quiescent rheumatic state begun. No use of histological test is detected, and even if detected, one could not interpret with assurance its significance. The best that one can do is to trace the persistence of inflammation with the most sensitive laboratory method available. In present work the level of ESR and CRP beside the TSA test are all helpful, and the use of TSA test alone could be the most useful marker in the present study.

References


