Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticulo peritonitis

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SUMMARY

The present study was designed to determine the diagnostic value of serum sialic acid (SA) concentration in cattle with traumatic reticulo-peritonitis (TRP). The study involved 12 cattle with TRP and 12 healthy cattle. Blood samples were collected from cattle for the determination of serum sialic acid, total protein, albumin, globulin concentrations, glutaraldehyde test and total leukocyte count. Mean concentration of total sialic acid (TSA), lipid bound sialic acid (LBSA) and protein bound sialic acid (PBSA) were 1.163 ± 0.123 g/l, 0.615 ± 0.112 g/l and 0.548 ± 0.120 g/l for the cattle with TRP and 0.761 ± 0.152 g/l, 0.333 ± 0.102 g/l and 0.428 ± 0.144 g/l for clinically healthy cattle, respectively. Serum TSA and LBSA concentrations in cattle with TRP were statistically higher (p<0.001) than that of healthy cattle. Increases in serum SA values were in parallel with the other inflammation markers (total protein, globulin, glutaraldehyde test, WBC) examined in this study. With a threshold of decision calculated by mean value + 1 standard deviation of control group, sensitivity of TSA and LBSA test were 100% and specificity were 91.7% and 75% respectively.

The results may suggest that serum SA concentrations might be a valuable marker for the determination of inflammatory process in TRP.

KEY-WORDS : Cattle, Traumatic reticulo peritonitis, serum sialic acid, inflammation.

RÉSUMÉ

Utilisation de l’acide sialique comme marqueur de l’inflammation lors de réticulo-péritonite traumatique chez les bovins. Par M. CITIL, V. GUNES, M. KARAPEHLIVAN, G. ATALAN et S. MARASLI.

Le but de cette étude est de déterminer la valeur diagnostique de la concentration sérique d’acide sialique chez les bovins atteints de réticulo-péritonite traumatique (RPT). Les mesures des concentrations en acide sia-lique, de la protéinémie, de l’albuminémie, de la globulinémie ainsi que le test au glutaraldéhyde et la numération leucocytaire ont été réalisées à par-tir des échantillons sanguins prélevés sur 12 bovins adultes atteint de RPT et de 12 animaux sains. Les moyennes des concentrations totales en acide sia-lique (TSA), et les concentrations d’acide sialique lié aux lipides (LBSA) et lié aux protéines (PBSA) obtenues chez les bovins malades étaient respectivement de 1.163 ± 0.123 g/l (TSA), 0.615 ± 0.112 g/l (LBSA) et 0.548 ± 0.120 g/l (PBSA) alors que les animaux sains présentaient des valeurs de 0.761 ± 0.152 g/l (TSA), 0.333 ± 0.102 g/l (LBSA) et 0.428 ± 0.144 g/l (PBSA). Les concentrations sériques de TSA et de LBSA étaient significa-tivement plus élevées chez les animaux atteints de RPT que chez les ani-maux sains (p<0.001). Les augmentations des concentrations sériques en acide sialique on été observées de façon parallèle à celles des autres mar-queurs de l’inflammation (protéinémie, globulinémie, numération leucocya-toire et test au glutaraldéhyde). Avec un seuil de décision correspondant à la moyenne de TSA ou de LBSA + une déviation standard obtenue dans le groupe témoins, les sensibilités des concentrations en TSA et en LBSA étaient de 100% et les spécificités de 91.7% et de 75% respectivement.

Ces résultats suggèrent que l’acide sialique sérique pourrait constituer un marqueur fiable du processus inflammatoire survenant lors de RPT.

MOTS-CLÉS : Bovin, réticulo-péritonite traumatique, acide sialique sérique, inflammation.

Introduction

Sialic acid (SA), an acetylated derivative of neuroaminic acid, is widely distributed in mammals’ tissue. N-acetyl neuroaminic acid (NANA) is the most common form of sialic acid. Since SA is usually bound to glycoproteins, glycolipids, oligosaccharides and polysaccharides, small amount of it is free in the body. Moreover, SA is an important structural component of biological membrane. It was also widely found in bacteria and animal tissues [17].

Sialic acid concentration increases rapidly following the inflammatory and injury process [9]. The mechanism inducing SA increase is not clearly understood. However, investigators have reported that SA localised at the end chain of many acute phase proteins can be used as marker for the determination acute phase protein concentrations [5, 9, 21, 24, 25] because serum acute phase proteins, especially $\alpha_1$-acid glycoprotein, are sialyted glycoproteins. Therefore the detection of SA particularly LBSA levels may be a valuable indicator for diagnosis and prognosis of inflammatory diseases [15].

The measurement of serum SA concentration is of importance in the diagnosis and prognosis of inflammation and cancer [13, 14]. Serum SA concentrations have been determined and they considerably varied during the course of many diseases such as nephritic syndrome, rheumatoid arthritis [17, 21], chronic tuberculosis [4], meningitis [18], trypanosoma vivax infection [8], bovine enzootic haematuria [19], distemper [2, 6], bovine leucosis (23), theileriosis, ana- plasmosis [7], and bovine leptospirosis [12].

Traumatic reticulo peritonitis (TRP) in cattle is an important inflammatory disorder induced by perforation of reticulum with sharp foreign objects. Moreover, TRP is usually followed by pericarditis, pleuritis, mediastinal lymphangitis, abscess formation in lung, spleen and liver. TRP causes eco-

nomical lose trough decreased milk production and cost of treatment [16]. Many researches have been carried out on different aspect of TRP. But, to our best knowledge, there have been no reports on the determination of serum SA levels in cattle with TRP.

The present study was therefore designed to determine SA concentrations (total, lipid and protein bound) in cattle with TRP.

**Materials and Methods**

**ANIMAL MATERIAL**

Twelve Brown Swiss cattle with TRP admitted to Clinics of Department of Internal Medicine Faculty of Veterinary Medicine, University of Kafkas, were included in the study as experimental group. Control group also consisted of 12 healthy Brown Swiss cattle, which were in various stages of lactation. Age of cattle in the study varied between 3 and 8 years old.

**CLINICAL EXAMINATION AND BLOOD SAMPLING**

Diagnosis of TRP was based on clinical and radiological examinations. Blood samples were taken from the jugular veins into a plain tubes for serum analyses. Serum samples were separated by centrifugation at 3000 g for 10 minutes at room temperature and stored at -20 °C until analyses. Blood samples were also collected in to EDTA treated tubes for the determination of total leukocytes count.

**SERUM SIALIC ACID ANALYSES**

Serum TSA and LBSA analyses were carried out colorimetrically on a spectrophotometer (UV-1201, Shimadzu, Japan) using the methods reported by SYDOW [22] and KATOPDIS et al., [11] respectively. The concentration of protein bound SA (PBSA) was calculated by subtracting LBSA from TSA.

**SERUM BIOCHEMICAL ANALYSES**

Serum glucose, total protein and albumin values were analysed on an autoanlyser (Ektachem DT 60 II, DTSC II, Kodak, USA) using commercial kits. The globulin concentration was calculated by subtracting albumin from total protein concentration. For the glutaraldehyde test (GA), 5 ml glutaraldehyde solution (1.4 %) was mixed with equal volume of blood and left for 15 minutes for coagulation [3]. Average coagulation time of each sample was recorded. Total leukocyte was counted by haemocytometric methods using Thoma slide.

**STATISTICAL ANALYSES**

Normal distribution of the data was tested using Anderson-Darling Normality test. Values were expressed as mean ± standard deviation. Data were subjected to statistical analysis using «t test» in SPSS [20]. Significant level was set at P < 0.05. Correlation coefficients of TSA and LBSA with other markers were determined. To determine test specificity and sensitivity cut off values were set as mean TSA and LBSA values plus their standard deviation (0.761+0.152=0.913 for TSA and 0.333+0.102=0.405 for LBSA). Yates corrected chi square test was used to compare the proportion of TSA and LBSA positive cattle in both groups [26].

**Results**

Normality test revealed that values of TSA, LBSA and PBSA in healthy group and TSA, LBSA, PBSA in TRP cattle were normally distributed.

The concentrations of serum SA (total, lipid bound, protein bound), total protein, albumin and globulin concentrations, albumin/globulin ratio, Glutaraldehyde total leukocytes counts are summarised in Table 1. Mean concentrations of TSA and LBSA (P<0.001) and PBSA (P<0.05) in cattle with TRP were significantly higher than those of control animals. Serum total protein and globulin concentrations, and albumin/globulin ratio were significantly higher in TRP cattle than in healthy cattle, whereas albumin concentration did not show any significant changes. In the same way, marked increases in Glutaraldehyde test and in total leukocyte counts were noticed in ill cattle.

Only statistically significant correlations were obtained between TSA and PBSA (r = 0.765, p = 0.004) and between TSA and total leukocytes count (r = -0.736; p = 0.024) in control group whereas no significant correlation between TSA or LBSA and other biochemical and haematological parameters was obtained in TRP group (Table 2).

When cut off point for TSA was set at 0.913 (mean value ± standard deviation of the control group), the sensitivity was 100% and specificity was 91.7%. The proportion of healthy and ill cattle positive for TSA significantly differed (X2=16.8, p<0.001). As for LBSA, when the cut off value was set at 0.405 (mean value + 1 standard deviation of the control group), the sensitivity and specificity were 100% and 75%, respectively. The proportion of LBSA positive healthy and ill cattle also significantly differed (X2=11.4, p<0.001).

**Discussion**

In this study, changes in SA concentrations were evaluated as an indicator for the determination of inflammatory process associated with TRP, a major health problem in cattle in Kars [10].

In the present study, TSA, LBSA and PBSA concentrations in cattle with TRP were higher than those of healthy cattle. These results are in agreement with the previous studies [1, 7, 12, 23, 27]. It is well documented that serum SA concentrations rapidly increase following the onset of inflammatory disease or injury [9, 21]. Serum SA concentrations in the present study revealed apparent tissue damage and inflammatory disorders. This is supported by increases of other parameters examined (TP, globulin concentrations, GA, and total WBC) in this study. High mean concentration
EVALUATION OF SERUM SIALIC ACID AS AN INFLAMMATION MARKER

Increase in serum SA concentrations may be a good indicator of inflammatory process associated with TRP in cattle as evident with high test sensitivity and specificity.

**TABLE I.** The concentrations of serum sialic acid, biochemical and haematological parameters in healthy cattle and cattle with traumatic reticulo-peritonitis (TRP) (n=12). Results are expressed as means ± standard deviations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Cattle</th>
<th>TRP Cattle</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sialic acid (g/l)</td>
<td>0.761 ± 0.152</td>
<td>0.1163 ± 0.123</td>
<td>***</td>
</tr>
<tr>
<td>Lipid bound sialic acid (g/l)</td>
<td>0.333 ± 0.102</td>
<td>0.615 ± 0.112</td>
<td>***</td>
</tr>
<tr>
<td>Protein bound sialic acid (g/l)</td>
<td>0.428 ± 0.144</td>
<td>0.548 ± 0.120</td>
<td>*</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>69.0 ± 6.0</td>
<td>86.0 ± 5.0</td>
<td>**</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>32.0 ± 2.0</td>
<td>33.0 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Globulin (g/l)</td>
<td>37.0 ± 4.0</td>
<td>53.0 ± 2.0</td>
<td>**</td>
</tr>
<tr>
<td>Glutaraldehyde test (min)</td>
<td>15.8 ± 0.7</td>
<td>1.3 ± 0.5</td>
<td>***</td>
</tr>
<tr>
<td>Albumin/Globulin ratio</td>
<td>0.62</td>
<td>0.86</td>
<td>**</td>
</tr>
<tr>
<td>Total leucocytes (10^9/l)</td>
<td>7.9 ± 3.1</td>
<td>11.8 ± 2.9</td>
<td>**</td>
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</table>

**TABLE II.** The correlation coefficients between TSA and LBSA concentrations and biochemical or haematological parameters in healthy and TRP cattle.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control cattle</th>
<th>TRP cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSA</td>
<td>LBSA</td>
</tr>
<tr>
<td>Lipid bound sialic acid</td>
<td>0.412</td>
<td></td>
</tr>
<tr>
<td>Protein bound sialic acid</td>
<td>0.765**</td>
<td>-0.271</td>
</tr>
<tr>
<td>Serum Total Protein</td>
<td>0.999</td>
<td>-0.003</td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.089</td>
<td>0.496</td>
</tr>
<tr>
<td>Globulin</td>
<td>0.265</td>
<td>-0.145</td>
</tr>
<tr>
<td>Alb/Glob</td>
<td>-0.260</td>
<td>0.228</td>
</tr>
<tr>
<td>GA test</td>
<td>0.140</td>
<td>-0.320</td>
</tr>
<tr>
<td>WBC</td>
<td>-0.736*</td>
<td>-0.347</td>
</tr>
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*; P<0.05, **; P<0.01

References


20. — SPSS FOR WINDOWS: 5.0.1 SPSS inc., 1993.


