Abstract: To investigate erythrocyte membrane protein abnormalities in Çukurova region we studied a family observed to be hereditary spherocytosis. Diagnosis was made basis of clinical features. By densitometric tracing of SDS-PAGE stained by Coomassie Brilliant Blue, we found two spectrin deficiency, one ankyrin deficiency, two spectrin-band 3 deficiency, one ankyrin-band 3 deficiency and one combined spectrin-ankyrin-band 3 deficiency.

Key Words: Hereditary Spherocytosis, Spectrin, Ankyrin, Band 3, Red Cell Membrane.

Introduction

Hereditary spherocytosis (HS) is a common hereditary hemolytic anemia with prevalence in the Northern European population of in 1:5000 (1). The severity of anemia is quite variable. About 80% of the affected families exhibit an autosomal dominant pattern, whereas the remaining 20% have autosomal recessive forms (2). This disorder is characterized by a variable degree of anemia, splenomegaly, spherical shape of erythrocytes and increased osmotic fragility of erythrocytes (1-3). HS is a disorder of the red cell membrane skeleton. Several lines of evidence suggest that the structural stability of the red cell membrane is almost entirely determined by the membrane skeleton. The skeleton is composed predominantly of spectrin, actin, protein 4.1 and ankyrin. When red cell ghost membranes are examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), spectrin appears as two discrete high molecular weight bands. Spectrin is directly involved in the three major protein-protein interaction in the membrane skeleton also it plays an important role in the determination of cell deformability and shape. Spectrin fastens to ankyrin, which tethers the skeleton to the membrane by means of its connection to protein 3. The lateral interaction is greatly enhanced by the association of protein 4.1 with spectrin (4-7).

In this report, we presented results of densitometric quantiations of red cell membrane proteins, which analyzed by SDS-PAGE, obtained from a family with HS from Çukurova region. As a result, we observed a varying amount of deficiencies of erythrocyte membrane protein of this family members.

Materials and Methods

Venous blood was collected in tubes containing EDTA as anticoagulant. The hematological data has been studied by Coulter analysis and then hemoglobin electrophoresis and osmotic fragility tests have been performed to all individuals (8). The erythrocyte ghosts of individuals were prepared with hypotonic lysis the method of Dodge et al (9). After the solubilization process, the membrane protein samples were analyzed depending on their molecular weights by SDS-PAGE in 8.3% gels according to Fairbanks et al. and stained with Coomassie Brilliant Blue; the gels were washed with a mixture of acetic acid-methanol-water (5:15). The amount of spectrin, ankyrin, band 3 and band 4.1 proteins were quantitated as a percent by densitometry of the stained gels at 545 nm (10).

Results

We studied one family from Çukurova region with HS (Figure 1). Diagnosis was made by clinical features, by the abnormal osmotic fragility test in the hematology clinic laboratory. Table 1 shows hematological and red cell membrane protein data from individuals studied.

By SDS-PAGE and densitometric tracing we detected in this family spectrin deficiency in two cases (II:2, III:1).
Red Cell Membrane Protein Abnormalities of A Family with Hereditary Spherocytosis in Adana

Table 1. Hematological and membrane protein data of family N.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Hb (g/dl)</th>
<th>Rbc (10⁶)</th>
<th>MCHC (g/dl)</th>
<th>HbE (%)</th>
<th>Sp (%)</th>
<th>Ank (%)</th>
<th>B3 (%)</th>
<th>B4.1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.N</td>
<td>I.1</td>
<td>60</td>
<td>13.9</td>
<td>4.1</td>
<td>34.2</td>
<td>AA</td>
<td>↑</td>
<td>16.7*</td>
<td>5.0</td>
</tr>
<tr>
<td>N.N(mother)</td>
<td>II.5</td>
<td>38</td>
<td>13.7</td>
<td>4.6</td>
<td>8.2</td>
<td>AA</td>
<td>N</td>
<td>24.8</td>
<td>6.2</td>
</tr>
<tr>
<td>A.N(father)</td>
<td>II.1</td>
<td>46</td>
<td>15.7</td>
<td>4.6</td>
<td>34.5</td>
<td>AA</td>
<td>↑</td>
<td>25.6</td>
<td>4.9</td>
</tr>
<tr>
<td>E.N</td>
<td>II.2</td>
<td>48</td>
<td>15.0</td>
<td>4.5</td>
<td>33.1</td>
<td>AA</td>
<td>↑</td>
<td>16.7*</td>
<td>8.0</td>
</tr>
<tr>
<td>Z.B.</td>
<td>II.3</td>
<td>35</td>
<td>12.1</td>
<td>3.4</td>
<td>33.7</td>
<td>AA</td>
<td>↑</td>
<td>18.4</td>
<td>3.5*</td>
</tr>
<tr>
<td>S.I.</td>
<td>II.4</td>
<td>40</td>
<td>10.8</td>
<td>3.3</td>
<td>33.5</td>
<td>AA</td>
<td>↑</td>
<td>17.6*</td>
<td>4.0</td>
</tr>
<tr>
<td>E.N(Proband)</td>
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<td>12</td>
<td>7.0</td>
<td>2.4</td>
<td>34.1</td>
<td>AA</td>
<td>↑</td>
<td>17.0*</td>
<td>6.4</td>
</tr>
<tr>
<td>E.N</td>
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<td>13.4</td>
<td>4.5</td>
<td>32.5</td>
<td>AA</td>
<td>N</td>
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<td>4.7</td>
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<tr>
<td>G.N.</td>
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<td>22</td>
<td>15.6</td>
<td>5.2</td>
<td>32.9</td>
<td>AA</td>
<td>N</td>
<td>18.1</td>
<td>3.2*</td>
</tr>
<tr>
<td>S.B.</td>
<td>III.4</td>
<td>8</td>
<td>11.4</td>
<td>3.9</td>
<td>32.9</td>
<td>AA</td>
<td>↑</td>
<td>14.6*</td>
<td>2.3*</td>
</tr>
</tbody>
</table>

*Sp<%18.1, Ank<%3.8, B3<%18.7

Figure 1. The pedigree of the family "N"

Discussion

In this study, we have investigated a family with HS. Hematological data of the members of this family were found to be in normal ranges except II:4, III:1, III:4 but...
the hematological data of E.N. was quite low (Table 1). Abnormality in membrane proteins were detected as spectrin deficiency. While in membrane proteins of the other members of family, mother, father, brothers, no any abnormalities were observed. In addition to this family, in the spectrin deficiency were detected E.N.'s relative, his uncle, (II:2) (Table 1). In the first generation, the individual numbered as I.1, the spectrin deficiency were found to be combined with band 3 deficiency. The same abnormality were observed in the second generation members of individual II.4. And in the third generation, for individual III.4 the spectrin-ankyrin-band3 deficiency were observed (Figure 1). But in the same generation for individual III.3 only ankyrin deficiency were observed. As a summary in this large family, spectrin deficiency in 2 individuals, combined spectrin-band3 deficiency in 2 individuals, ankyrin deficiency in 1 individuals, combined ankyrin-band3 deficiency in 1 individual, combined spectrin-ankyrin-band3 deficiency in 1 individual, and normal 3 individuals were observed.

Most patients with HS have a partial deficiency of spectrin, and the clinical severity of the disorder correlates with the degree of spectrin deficiency (12). However, spectrin deficiency may represent the primary defect in only a minority of HS patients. These include a rare HS kindred with an autosomal recessive form of the disease, a common deficiency of spectrin and ankyrin has been found (13). However, results of the present study did not confirm the expectations, that is, ankyrin or spectrin represent primary molecular defect in HS. Because in this study there are some combined spectrin-ankyrin-band3 deficiency cases; these are not explained only spectrin or ankyrin deficiency. The nature of defect in HS is still completely not explained. Therefore, we propose that studies of membrane protein synthesis would be done and also mRNA amounts of membrane proteins would be detected. Because the partial protein mRNA deficiency is a result of a point mutation leading to a reduced expression or a reduced stability of the protein mRNA. As a result, the deficiency at the amount of the protein mRNA affect the linkage among the proteins.

The main triggering effect for hemolysis in HS in known to be the decrease in area-volume ratio of erythrocytes as a result of abnormalities in its membrane structure. The main agents for this membrane structure abnormalities are membrane lipids and proteins. The individual spectrin, combined spectrin-ankyrin and band3 protein deficiencies can be attributed to the molecular abnormalities in HS.

Acknowledgements
This study was supported by Çukurova University Research Foundation (SBE 94.E.30 and TF 96-37).

References