Studies on Abnormal Hemoglobins

XI. Sickle Cell–Thalassemia Disease in the Negro. The Significance of the S + A + F and S + A Patterns Obtained by Hemoglobin Analysis

By KARL SINGER, LILY SINGER AND SEYMOUR R. GOLDBERG

It is now well established that individuals who harbor one gene for sickle cell hemoglobin and one for thalassemia (double heterozygotes) may develop a severe hemolytic anemia with some of the characteristics of both sickle cell disease and thalassemia.1-4 This hereditary disorder has been called microdrepandocytic disease,1 or sickle cell-thalassemia disease.4 It has so far been recognized most frequently in Italians or Greeks,1-4 and either in Europe or in the United States, and only twice in American Negroes.5,6

This paper reports four Negro families, some members of which exhibit this condition. Our observations indicate that sickle cell-thalassemia disease may manifest itself in some individuals as a relatively mild disorder only, which, however, can be readily diagnosed by hemoglobin analysis.

Methods

Hemoglobin analyses were performed with the technique of alkali denaturation7 in combination with electrophoresis, either in the Tiselius apparatus at pH 6.5, or on paper with the set-up of Smith and Conley,8 as outlined in previous communications of this series.9,10 Paper electrophoresis was run either for two hours or for 15 hours. It was found that quantitative differences became more discernible with the longer run. For computing the hematologic indices, the conventional standardized procedures were applied.9

Report of Cases and Results of Hemoglobin Analyses

Case 1

A. H., a 61 year old colored female, was first seen at Michael Reese Hospital in December 1953. For several years she had been known to have hypertensive heart disease, necessitating constant digitalization. While on a visit to the outpatient department, she fell and fractured her right femur and wrist. Routine blood examination showed anemia and a positive sickling test. Past history disclosed a state of ill health for the last five years with frequent attacks of cardiac asthma and pains in both legs. She denied ever having had true joint pains, leg ulcers, jaundice, or episodes of abdominal cramps. She was not aware of having had any blood disease or of having received any blood transfusions. Physical examination showed a moderately enlarged heart. B.P.: 165/105. There were sclerotic changes in the retinal vessels. The liver edge was felt 3 cm. below the costal margin, but the spleen was not palpable.

The hip was pinned and she was further hospitalized for almost three months in an attempt to ambulate her to the limits of her cardiac disability. No blood transfusions were given during her hospital stay. Treatment with iron did not alter her anemia.

From the Department of Hematologic Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

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TABLE I.—Hematologic Data

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Hb Gm./%</th>
<th>RBC mill.</th>
<th>Retics.</th>
<th>Hematocrit</th>
<th>MCV (Cu. mm.) per cu. mm.</th>
<th>MCH (vg) normal</th>
<th>MCHC (g) normal</th>
<th>Osmotic fragility (normal 0.82-0.32 % NaCl)</th>
<th>Target cells %</th>
<th>Stippled cells or ovalocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A.H.</td>
<td>61</td>
<td>f</td>
<td>8.6</td>
<td>3.8</td>
<td>8.6</td>
<td>327</td>
<td>27</td>
<td>71</td>
<td>22.6</td>
<td>31.8</td>
<td>0.32-0.28</td>
<td>35.8</td>
</tr>
<tr>
<td>2. J.H.</td>
<td>41</td>
<td>f</td>
<td>11.3</td>
<td>4.9</td>
<td>3.7</td>
<td>181</td>
<td>35</td>
<td>72</td>
<td>23</td>
<td>32.2</td>
<td>0.32-0.22</td>
<td>51.3</td>
</tr>
<tr>
<td>3. G.W.</td>
<td>35</td>
<td>m</td>
<td>12.5</td>
<td>5.0</td>
<td>3.1</td>
<td>155</td>
<td>40</td>
<td>80</td>
<td>25</td>
<td>31.3</td>
<td>0.40-0.22</td>
<td>28.0</td>
</tr>
<tr>
<td>4. A.B.</td>
<td>40</td>
<td>f</td>
<td>12.3</td>
<td>4.1</td>
<td>2.8</td>
<td>115</td>
<td>38</td>
<td>92</td>
<td>30</td>
<td>32.0</td>
<td>0.40-0.22</td>
<td>12.0</td>
</tr>
</tbody>
</table>

The hematologic findings are summarized in table 1. Her hemoglobin level was 8.6 Gm. per cent. The values for the MCV and the MCH were definitely lower than normal, but the MCHC was only slightly decreased. The reticulocyte output was always elevated (8.6 per cent) in the absence of any gross bleeding. Target cells were conspicuous in the film (35.8 per cent), and the osmotic fragility of the erythrocytes was markedly decreased. These findings remained essentially unchanged during an observation period of almost seven months.

Hemoglobin analysis, repeated several times, showed electrophoretically a pattern indistinguishable from that found in classical sickle cell anemia (fig. 1). The amount of S (sickle cell) hemoglobin was 79.9 per cent, leaving a non-S hemoglobin fraction of 20.1 per cent. The alkalai denaturation technic demonstrated, however, only the presence of 8.5 per cent fetal pigment. Consequently, the patient’s hemolysate contained also 11.6 per cent type A (normal adult) hemoglobin which, at pH 6.5, has the same mobility as the fetal pigment.11 These studies revealed what we have become accustomed to call the S + A + F pattern (the various types of hemoglobin are always reported in the sequence of their quantitative representation in the hemolysate).12

The S + A + F pattern must be distinguished from the S + F pattern, encountered in homozygous sickle cell anemia, where the percentage of the non-S hemoglobin fraction, determined electrophoretically, is in reasonably close agreement with the values obtained by the alkalai denaturation technic.13 The non-S hemoglobin fraction in classical sickle cell anemia is entirely composed of F hemoglobin.19

Family studies were performed on the patient’s sister, her only living relative, who exhibited the pattern of the typical sickle cell trait (A + S hemoglobin, fig. 1).

On account of all these data, the diagnosis of sickle cell-thalassemia disease was made.
Case 2

J. H., a 41 year old Negro woman, was first seen at Michael Reese Hospital in May 1951, at which time she was scheduled to have a hemorrhoidectomy. Pre-operative routine blood examination revealed a positive sickling test and a mild anemia. In general, she has been well all her life, with no complaints possibly related to sickle cell disease. Since 1944, she had numerous attacks of "sciatic" pain and a diagnosis of a herniated nucleus pulposus at L 5 had been made by myelogram.

Physical examination revealed no jaundice, no hepato-splenomegaly, nor were there any skin ulcerations. Following hemorrhoidectomy, she continued to be active as a teacher and housewife, without any particular new symptoms or signs. There is no history of blood transfusions at any time during her life. Treatment with iron did not ameliorate the hemoglobin level.

The hematologic findings are compiled in Table 1. As can be seen, the hemoglobin was 11.3 gm. per cent with 4.9 M.RBC. The values for MCV and MCH were decreased, but the MCHC was within normal range. The reticulocyte count was always elevated, and more than 50 per cent target cells were seen in the smear. The osmotic fragility of the erythrocytes was decreased.

Hemoglobin analysis: The electrophoretic pattern in the Tiselius apparatus is depicted in figure 2. The value for S hemoglobin was 82.2 per cent, with a non-S hemoglobin fraction of 17.2 per cent. The alkali denaturation value was only 2.5 per cent; thus, the remainder (14.7 per cent) must be attributed to the presence of hemoglobin A. Therefore, the S + A + F pattern was again demonstrable.

This patient has one living son, Gerard H., age 17 years. His medical history is not suggestive of any hematologic abnormalities. Examination of Gerard's blood revealed a hemoglobin of 15.2 Gm. per cent, with 6.0 M.RBC. His hematocrit (Wintrobe) was 47, MCV: 78 eumicrons, MCH: 25.3 micromicrograms, but the MCHC was 32.3 per cent. The reticulocyte count was 1.2 per cent. Target cells were only moderately increased. Analysis of the hemolysate prepared from Gerard's red cells showed an alkali denaturation value within normal limits. The electrophoretic pattern in the Tiselius apparatus revealed 91.6 per cent of hemoglobin A, and 8.4 per cent of a faster component, the nature of which was not immediately apparent. Addition of pure S hemoglobin to this hemolysate demonstrated that the unidentified component was not sickle cell hemoglobin. It has been pointed out in a preceding communication of this series \cite{14} that patients with thalassemia frequently show small amounts

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{electrophoretic_patterns.png}
\caption{Electrophoretic Patterns}
\end{figure}
of such erythrocyte constituents which may or may not be a hemoglobin compound. The
finding that the son of Mrs. H. has a microcytic erythrocytosis provides additional support
to the diagnosis of sickle cell-thalassemia disease in the mother.¹

Case 4

G. W., a 35 year old Negro male, was first seen in 1951 when he was hospitalized with a
diagnosis of recurrent hepatitis. In view of the fact that he had a positive sickling test,
further hematologic investigation was instigated. The past history showed that he had
had five episodes of rather severe icterus, the first occurring at the age of 18. Prior to this
time, he had been completely well.

Physical examination revealed only a slight enlargement of the liver. Liver function
studies were negative. He has continued to be well after the short hospitalization in 1951,
and has been free of jaundice ever since. He does, however, complain of dyspepsia following
excessive fat intake. There is no history of blood transfusions at any time.

The hematologic findings are compiled in table 1. It may be noted that the MCV is 80
cubic with a MCH of 25 micromicrograms; the MCHC is only slightly decreased (31.3
per cent). The reticulocyte level is elevated (3.1 per cent) in the absence of any bleeding.
Target cells (28.0 per cent) were present, and the osmotic fragility of the red cells was
decreased.

_Hemoglobin analysis_ showed a normal value with the alkali denaturation method (1.6
per cent). The electrophoretic pattern with the moving boundary technic revealed 77.2
per cent S and 22.8 non-S hemoglobin, composed of hemoglobin A (fig. 3). This patient,
then, revealed the S + A pattern which has to be clearly distinguished from the A + S
pattern encountered in the sickle cell trait. In the latter, the pathologic S pigment is always
present in amounts not exceeding 50 per cent.

_Family studies_. Mrs. W., the propositus' wife, is hematologically normal and so is her
hemoglobin analysis. Of their three children, two, Geraldine and Patricia, aged 11 and 9,
are typical carriers of the sickle cell trait; Valerie, the third child, aged 5, is entirely nor-
mal. All these four individuals have normal alkali denaturation values, and their hemato-
logic indices are within normal range. Figure 3 shows the results of paper electrophoresis
with the five hemolysates of this family. As can be seen, the difference of the S + A pattern in
the father and the A + S pattern in his two children with the sickle cell trait is quite striking.
Case 4

Mrs. A. B., a 40 year old Negro woman, has always been in perfect health. When applying for a job in the hospital, routine blood examination showed a positive sickling test with a hemoglobin of 12.3 Gm. per cent and 4.1 M.RBC. Her reticulocyte count was 2.8 per cent in the absence of any gross bleeding. The saline fragility of the red cells was decreased, and the number of target cells in the smear was moderately increased (12.0 per cent). The hematologic indices were within normal limits (table 1).

Hemoglobin analysis revealed an electrophoretic pattern of 74.5 per cent S hemoglobin and 25.5 per cent non-S pigment, which was composed of 11.3 per cent F and 14.2 per cent A hemoglobin (fig. 4). This patient, thus, represents another example of the $S + A + F$ pattern, but with normal hematologic indices. No family studies were feasible.

Discussion

It is now recognized that patients with sickle cell-thalassemia disease are double heterozygotes, harboring one gene for sickle cell hemoglobin and one for thalassemia.\textsuperscript{1-4} Hematologically, the thalassemia gene is held responsible for the low MCV and MCH, and, at least in part, for the leptocytosis\textsuperscript{12} found in this disorder.\textsuperscript{1-4} Further studies signify that the thalassemia factor in such families expresses itself, in the absence of the gene for S hemoglobin, either as a mild microcytic anemia, or a microcytic erythrocytosis, or it may only produce a slight degree of leptocytosis, objectively demonstrable by a decreased osmotic fragility of the erythrocytes.\textsuperscript{1-4} The gene for S hemoglobin, if not accompanied by the thalassemia factor, manifests itself as a typical sickle cell trait in such sibships. Association of both genes in the same individual has been shown to result in a severe hemolytic process with hepato-splenomegaly, abdominal crises, and leg ulcers, besides the above mentioned red cell characteristics.\textsuperscript{1-4} This severe clinical picture has been explained as being due to the interaction of the non-allelic abnormal genes.\textsuperscript{1-4}

Our observations suggest, however, that sickle cell-thalassemia disease may not always exhibit such a severe clinical syndrome, but may appear as a mild disorder, hardly causing any discomfort to the afflicted individual.
Clinical and Hematologic Findings in our Patients

Only patient 1 showed a moderately severe microcytic anemia, but she never had any abdominal cramps or leg ulcers. Patient 2 was only slightly anemic. Studies of her red cells revealed a lowered MCV and MCH. Her son had a microcytic erythrocytosis. Propositus 3 had a hemoglobin of 12.5 Gm. per cent with 5.0 M. RBC. The MCH of his red cells was decreased. He was married to a normal individual. The offspring of patients with homozygous sickle cell anemia, married to a normal person, are all carriers of the sickle cell trait. Two of this patient's children exhibit the sickle cell trait, but his third child is hematologically normal. This may be taken as additional evidence that this propositus is not homozygous for the pathologic pigment. Patient 4 is not anemic but reveals some leucopenia. The hematologic indices computed for her red cells are within normal range.

All four patients have definitely elevated reticulocyte levels in the absence of any gross bleeding. This suggests an existing hemolytic process which, in cases 3 and 4, appears to be almost completely compensated. Unfortunately, erythrocyte survival time studies were not feasible.

All four patients had conspicuous numbers of target cells, and the osmotic fragility was definitely decreased. In only two cases (1 and 3) were oval and stippled red cells seen. No sickled erythrocytes were found in any smear.

Results of Hemoglobin Analyses

Hemoglobin analysis, consisting of the combination of quantitative electrophoresis and the alkali denaturation technic, has become indispensable in the clarification of these hereditary syndromes. It has been shown in this laboratory that, in classical sickle cell anemia, there exists a reasonably good agreement between the non-S hemoglobin fraction, as determined electrophoretically, and the values found by the alkali denaturation technic. In this malady, type A hemoglobin is, therefore, not demonstrable in the hemolysate. In the patients presented in this paper, the discrepancy between the results obtained for the non-S hemoglobin fraction with the electrophoretic and the alkali denaturation technics indicates the presence of the S + A + F pattern first noted by Neel et al. in sickle cell-thalassemia disease. Only in patient 3 was fetal hemoglobin absent and the non-S fraction entirely composed of type A pigment. It is of great interest that the amount of sickle cell hemoglobin in microdrenocytic disease may vary from 60 to over 80 per cent, although the patient is heterozygous for the abnormal pigment. In some instances, the percentages for S hemoglobin in the double heterozygous condition may thus be within the range of those encountered in homozygous sickle cell anemia which, in our 120 patients so far studied, vary from 76 to 100 per cent. Although the patient is heterozygous for the abnormal pigment, the hypothesis was advanced at that time that in these individuals the thalassemia gene may enhance the expressivity of the gene for the pathologic pigment. This hypothesis seems to be sup-
ported further by the findings in the cases reported here. We have pointed out that the S + A + F, or the S + A pattern may be pathognomonic for sickle cell-thalassemia disease. This statement is, however, only valid provided that the patient has not received any blood transfusion prior to the hemoglobin analysis. The S + A + F, or the S + A patterns are regularly found in classical sickle cell anemia following transfusion with erythrocytes containing A hemoglobin. These patterns are easily distinguishable, even by paper electrophoresis, from the A + S pattern encountered in the typical sickle cell trait (fig. 3). By adopting the system of reporting the various types of hemoglobin in the sequence of their quantitative representation in a hemolysate, confusion in terminology is easily avoided. Since individuals afflicted with sickle cell-thalassemia disease may not always show a severe hemolytic syndrome, hemoglobin analysis provides an objective test to demonstrate the presence of this disorder.

The Genetic Determination of F Hemoglobin Production

It is now generally recognized that in the majority of patients with homozygous sickle cell anemia, fetal hemoglobin may be present, varying in amounts from 2 to 24 per cent. F hemoglobin has also been encountered inhomogeneously in hemoglobin C-sickle cell disease and in Itano's two patients with hemoglobin D-sickle cell disease (up to 12.0 per cent) and in its D phenotype. The embryonic pigment does not seem to be present in pure homozygous hemoglobin C disease, nor in the A + S, A + C, or A + D traits. Fetal hemoglobin has been found in large amounts (up to 90 per cent) in thalassemia major and in smaller quantities in the milder manifestations of this disorder. Some adult patients with hereditary spherocytosis also show fetal hemoglobin in their red cells. After splenectomy, the production of the embryonic pigment continues, although the anemia disappears completely.

So far, geneticists have rather ignored this problem of the appearance of the F pigment in such a heterogenous group of hereditary disorders. However, it has been postulated that the production of type A hemoglobin is genetically conditioned, and that the pathologic hemoglobins probably arise due to activities of alleles of this normal gene. One may also postulate that the production of fetal hemoglobin is under genetic control. It should be recalled that, physiologically, fetal hemoglobin is present at birth in amounts up to 90 per cent of the total pigment and is replaced by normal adult hemoglobin to the greatest extent within the first year of life.

Chernoff has shown with an immunologic technic, that a large number of normal adults continue to produce minute amounts of fetal hemoglobin throughout their life. If this is true, one may assume that hemoglobin A suppresses the production of fetal hemoglobin almost completely, but that in sickle cell anemia (homozygous for hemoglobin S), and in hemoglobin C + S disease, this mechanism may be less effective. Furthermore, the manufacture of the fetal pigment may be influenced by the thalassemia gene as well as the gene for hereditary spherocytosis. This discussion does not consider directly the re-activation of the production of fetal hemoglobin at a larger scale, as observed irregularly in acquired hematologic conditions, such as pernicious anemia, leukemia, cancer metastases to the marrow, aplastic anemia, etc.
obscure. According to our point of view, the hypothetical gene for fetal hemoglobin is not an allele of the gene for normal adult hemoglobin like the gene held responsible for the appearance of the abnormal human hemoglobins. It is considered to represent a determinant whose suppression is physiologically almost complete, but which may be rendered incomplete by the presence of other pathologic genes. Figure 5 summarizes this concept of the genetic aspects of fetal hemoglobin. That other genetic modifiers are, most likely, also involved may be deduced from the inconstant appearance of F hemoglobin in the hereditary syndromes mentioned. The genetic situation outlined is thus similar to that encountered in the various thalassemia syndromes. There, individuals, heterozygous for the abnormal gene, show all kinds of graded transitions from thalassemia intermedia with marked anemia, hepato-splenomegaly, and leg ulcers to thalassemia minima with only a slight degree of leucytosis. Such modifying factors may also be postulated for the milder types of sickle cell-thalassemia disease, resulting from the interaction of the non-allelic genes for the abnormal hemoglobin and for thalassemia.

Correlation between the Percentages of S Hemoglobin in the Hemolysate and the Severity of the Clinical Condition

Repeatedly, attempts have been made to correlate the severity of the anemia in a patient with sickle cell disease with the percentage of S hemoglobin found in the hemolysate. We have voiced our disagreement with this simple correlation and have pointed out—as have others—that the severity of an anemia does not depend only on the rate of disintegration of the red cells but also on the ability of the bone marrow to compensate for this mechanism. Some of the individuals reported here clearly demonstrate that one may observe a high percentage of S hemoglobin without any anemia.
Incidence

Curiously enough, sickle cell-thalassemia disease has been described most frequently in patients of Italian or Greek descent,1-4 and we have found only two instances in the literature of microdysplastic disease in the Negro.5, 6 This communication draws attention to the existence of milder types of this specific syndrome which is probably not too rare in the Negro race. We have knowledge of four other families with sickle cell-thalassemia disease (one of Italian origin, and three Negro) which are not included in this paper since we performed only the hemoglobin analyses (revealing the S + A + F pattern), but did not have full access to all the pertinent clinical and hematologic data. Some of these patients exhibited the severe form of the disease, whereas others showed only such mild manifestations that they were considered to have an atypical sickle cell trait because fetal hemoglobin was found without any anemia. In such instances the value of hemoglobin analysis by means of electrophoresis and the alkali denaturation techniques becomes clearly established. It is easily predictable that with more frequent application of these procedures more such cases of mild sickle cell-thalassemia disease will be detected in the future.

Summary

(1) Four Negro patients with mild sickle cell-thalassemia disease (heterozygous for the genes for S hemoglobin and for thalassemia) are described. In contrast to reports in the literature, some of these patients are only mildly anemic, or not anemic at all. In three, the values for MCV and MCH are decreased, but in one, all hematologic indices are normal. All four individuals show leucocytosis and elevated reticulocyte levels.

(2) Hemoglobin analyses, consisting of a combination of electrophoresis and the alkali denaturation technique, demonstrate the S + A + F pattern in three, and the S + A pattern in the fourth. These patterns are considered pathognomonic for sickle cell-thalassemia disease. They may be sharply differentiated from the S + F pattern, encountered in classical (homozygous) sickle cell anemia, and from the A + S pattern found in the heterozygous sickle cell trait. The various types of hemoglobin are reported in the sequence of their quantitative representation in the hemolysate. Hemoglobin analysis is indispensable for the recognition of the different types of sickle cell disease.

(3) Evidence is cited that clinically almost asymptomatic sickle cell-thalassemia disease is probably not too rare in the American Negro population.

(4) The genetic aspects of the production of fetal hemoglobin are discussed. It is postulated that the production of fetal hemoglobin is also under genetic control. The genes for fetal hemoglobin are not alleles of the genes for normal adult hemoglobin and are physiologically almost completely suppressed by the latter. Pathologic genes may render this suppression incomplete.

Summario in Interlingua

1. Es describite le casos de quatro negre pacientes con leve morbo de cellulas falciforme plus thalassemia (heterozyg in re le gen del cellulas falciforme e le gen de thalassemia). In contrasto con le reportos trovate in le litteratura, alicunes
de iste patientes es solo levemente anemic o non anemic del toto. In tres casos le valores median del volumine corpuscular e del hemoglobina corpuscular es descrescite. In un caso omne le indices hematologic es normal. Omne le quatro individuos monstra leptocytosis e elevate nivellos reticuloctytic.

2. Analyses de hemoglobina—consistente de un combination del technicas de electrophorese e del denaturation a alcali—demonstra le configuration S + A + F in tres casos e le configuration S + A in un caso. Iste configurationes es considerate como pathognomonie e indicative de morbo a cellulas falciforme plus thalassemia. Illos pote esser claramente distinguite del configuration S + F, que es incontre in le forma classic (homozyge) de anemia a cellulas falciforme, a del configuration A + S, que es trovate in le tracto heterozyge de cellulas falciforme. Le varie typos de hemoglobina es reportate in le ordine de lor representation quantitative in le hemolysato. Le analyse hemoglobinie es indispensabile pro le recognition del diferente typos de morbo a cellulas falciforme.

3. Nos presenta datos que indica que un forma clinicamente quasi asymptotic de morbo a cellulas falciforme plus thalassemia as probabilemente non nimis rar in le negre population american.

4. Es discutite le aspectos genetic del production de hemoglobina fetal. Nos postula que etiam le production de hemoglobina fetal depende de factores genetic. Le genes pro hemoglobina fetal non es alleles del genes pro normal hemoglobina adulte. Physiologicamente iste ultimes supprime le genes pro hemoglobina fetal quasi completely. Le presentia de genes pathologic pote resultar in un forma incomplete de iste suppression.

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SINGER, SINGER AND GOLDBERG

Studies on Abnormal Hemoglobins: XI. Sickle Cell—Thalassemia Disease in the Negro. The Significance of the S + A + F and S + A Patterns Obtained by Hemoglobin Analysis

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