

Title: The Karyotypes of the Hemoglobinopathies and Glucose-6-Phosphate Dehydrogenase Deficiency in Thai.

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Period of Studied: July 1965 — December 1966

Background of Study:

It is known that the incidences of the hemoglobinopathies and the G-6-PD deficiency are quite high among the Thai population. Various investigations of these problems, including the genetic patterns, have been carried out.

Since the etiologies of these disorders are as yet undetermined, it is desirable to investigate as many aspects as possible.

Our purpose here is to evaluate the karyotypes of these two diseases.

Materials:

In our original plan, five groups of subjects will be submitted for chromosome studies:

1. One hundred control studies from normal, healthy individuals.
2. Twenty subjects who manifest definite G-6-PD deficiency, both clinically and by the assay method for the enzyme level.
3. Twenty subjects with Thalassemia major.
4. Twenty subjects with Hemoglobin E disease.
5. Twenty subjects with Hemoglobin H disease.

All of these subjects will be of various ages with no history of any significant irradiation, nor any detectable abnormalities or any other diseases at the time of the studies.

All the subjects will also have a complete blood count, hemoglobin electrophoresis and the G-6-PD determination performed at the time of the culture of the leukocytes.

Methods:

1. Chromosome study:

Peripheral white blood cells or bone marrow was used as indicated, in order to obtain the best results.

Techniques of R.G. Schertz et al and K.A. Kiossoglou were selected for peripheral blood and bone marrow respectively.

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a. Peripheral blood: Eight to ten ml. of heparinized peripheral blood was collected into a sterile culture tube. The tube allowed to stand in a refrigerator for 3-4 hrs. A few ml. of buffy coat were aspirated and poured another sterile tube containing: TC 199 media, phytohemagglutinin penicillin & streptomycin. This mixture was incubated at 37°C for 72 hrs. To stop the growing cells at metaphase, colchicine was added. The mixture was again incubated at 37°C for another 4 hrs. The tube was centrifuged at 800 r.p.m. for 10 min., and then the supernatant poured off. The hypotonic solution, 0.95% sodium citrate, was then added to the "button" of the cells. This mixture was incubated at 37°C for 10 min. Following incubation the tube was centrifuged and the precipitate saved. The sediment was washed with glacial acetic-methanol fixative for 3-4 times. Two-three drops of a suspension were then delivered on to the surface of a clean slide. The surface of the slide was ignited for a few seconds. The prepared slides were then dyed with Giemsa's stain, and Mounted in Permount. The preparations were now ready for the microscopic examination.

b. Bone marrow: The technique is more or less the same as used for the peripheral blood except the stage of culture in the TC 199 media was not needed.

2. Hemoglobin electrophoresis:

The starch gel method was selected.

3. The alkaline denaturation test:

Followed the method of Singer & Chernoff.

4. The G-6-PD determination:

a. Spot test using Biochemia kit or Matulsky's technique was used as available.

b. For the bioassay technique, C.F. Bochringer & Sochne's method was used.

Results:

As shown in tables 1,2, and 3 for the Hemoglobin E disorders, Hemoglobin H disease and Thalassemia major respectively.

The results, though, are not yet conclusive. For the majority of cells studied, 176 out of 234 cells (72.5%) in Hb E disorders and 114 out of 115 cells (73.5%) of the Hemoglobin H disease 46 chromosomes were observed.

Thirty nine of 46—chromosome containing cells in Hemoglobin E disorders were karyotyped, of these 4 cells show certain, but not constant abnormalities.

In hemoglobin H disease series, the number of karyotyped cells is still too small for any discussion. Though the incidences are less common in our hospital, quite a number of Thalassemia major and the G-6-PD deficient patients were submitted to the study. The results were, unfortunately, not satisfactory.

Since there may be chromosomal aberrations of varying types associated with the hemoglobinopathies studied, a continuation of this study is planned. Interpretation of the significance of these observations must be deferred until studies of control preparation are completed.