

4. Title: Pathology as Seen in Fatal Cases of Abnormal Hemoglobin Diseases

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OBJECTIVE:

Abnormal genes for Thalassemia as well as for hemoglobin E and other abnormal hemoglobins are prevalent among the Thai population. Intensive studies of the clinical, hematologic and genetic aspects of this condition have been done. This study attempts to obtain information on the morphological changes in the various organs of the patients who died of these conditions or their consequences.

DESCRIPTION:

Autopsy cases with the diagnosis of abnormal hemoglobin diseases in the file of the Department of Pathology, Siriraj Hospital Medical College, performed between 1961-1965 are collected and reviewed. The tissue had been fixed in Zenker's formalin, embedded in paraffin, sectioned and stained with hematoxylin eosin, Perl's stain for iron, PAS after diastase digestion. Oil red O and Acid fast stain were performed on some of the sections when indicated. Also some unstained sections were mounted and studied under fluorescent microscope.

RESULT:

Twenty-five autopsy cases of abnormal hemoglobin diseases have been collected. Twenty had Thalassemia hemoglobin E and twelve of this were female, eight were male. The age varied between one and thirty eight years. Two cases had Thalassemia major, both were male aged five and eleven years old respectively. Three cases had Thalassemia hemoglobin H disease, one male and two females whose ages were three, twenty-one and thirty-three respectively. Table I summarises the findings at the time of death. The pathological changes encountered in this series of cases are as follows:

Growth and development.

Eight out of twenty-five cases displayed definite evidence of retardation of growth and development. This is more prominent in some cases which reached the age of puberty but had poor development of secondary sexual characteristics. Cases with Thalassemia hemoglobin H did not show any striking retardation of growth while cases with Thalassemia major displayed very striking abnormality. Six cases showed a particular facie which, for lack of a definite anthropological definition, may be called Thalassemia mongoloid facie. They had sunken nose, broad and flat face and prominent cheek bones. The rest may show some of these characteristics but to a lesser degree.

Heart

The heart weights were increased in all of these cases. The weight ranged from 120 gms. in a one year old child to 340 gms. in a thirty three year old male. Gross description revealed marked anemia in most of the cases; fatty metamorphosis was noted in half of all the cases. In one case, mitral stenosis probably of rheumatic nature was discovered. Microscopic examination reveals non specific changes in twelve cases. While in eight cases, an increase in brown granular pigment in the myocardial fibers is noted in the perinuclear location. This pigment is iron negative and PAS positive and is probably lipochrome in nature. The amount of pigment is definitely more than one would expect from the heart of Thai patients within similar age groups. In three cases hemosiderin granules are seen in the heart. In one case the granules are in the myocardial fibers, in one there is little in the myocardial fibers but the subendocardial connective tissue especially elastic fibers gives strong reaction for iron. In another case there is iron reaction in the endocardium and pericardium. In one case there are senile Aschoff granulomata in the atrial appendages.

Pancreas

Gross examination of pancreas revealed the color to be dark brown in six cases. Microscopic examination reveals heavy deposit of hemosiderin in the tissue of pancreas in seven out of the fifteen examined cases. Hemosiderin granules are observed more intensely in the acinar cells, and less in the islet cells. All types of islet cells appear to be involved. Some of the ductal epithelial cells and interstitial connective tissue show deposit of hemosiderin but the degree of involvement is relatively mild. In some instances the acinar secretion has a bluish haze in sections stained for iron. There is no evidence to indicate that the acini and islets are destroyed. In two cases, some increase in interstitial connective tissue is present.

Adrenal glands.

The adrenal glands look brownish in seven cases. Nodular hyperplasia of the cortex is seen in three cases. Microscopic examination shows heavy deposit of iron in the adrenal glands in eight out of sixteen examined cases. In one case there is a deposit in the capsule while in others the intense iron reaction is present in cells of zona glomerulosa. In two instances cells in the upper most part of zona fasciculata reticularis may give some iron reaction but no iron is found in the deeper part of these two zones. In the adrenal medulla, a mild iron reaction is observed in the paraganglia in cases where iron is present in the adrenal cortex. The hyperplastic nodules in the adrenal cortex show positive iron reaction in the cells around the periphery.

Brain.

Gross examination of the brain reveals no significant changes. Microscopic examination reveals in general, non specific changes. Focal proliferation of glial cells is noted in the cerebral cortex in six cases and in one of these spongiosis is noted. An increase in the amount of brown granular pigment is noted in the cytoplasm of neurones of the cerebral cortex in four cases. This pigment is iron negative, partially acid fast, strong by PAS positive and gives reddish fluorescence under U.V. light. It is probably a lipochrome pigment.

Gastrointestinal tract.

In two cases, acute amoebic ulcerative colitis and diffuse pseudomembranous colitis were found respectively. Focal hemorrhage in the stomach, small intestines and large intestines were found in four cases. Others show no remarkable findings grossly. Microscopic examination reveals an increase in amount

of lipochrome pigment in the smooth muscle of the small intestine in one case. In one case a pigment whose fluorescence is blue white, is seen in the esophagus and probably is ceroid in nature. Small amount of hemosiderin pigment is present in the mucosa of the small intestine in eight cases. In the duodenum, Brunner's gland gives a faint bluish haze when stained with Perl's iron reaction.

Lungs

Lungs showed non specific findings namely congestion and edema in sixteen cases. Bronchopneumonia is noted in three cases, and interstitial pneumonia with hyaline membrane formation in one. A small amount of hemosiderin is present in eight cases while in one case a moderate amount is seen. In fourteen cases there are hemopoietic cells, nucleated red cells and megakaryocytes in the alveolar capillary capillaries.

Kidneys.

In one case there was a ureteral stone on the left side. The other cases show no remarkable findings grossly. Microscopic examination reveals that in most cases some degree of enlargement of glomeruli is present when compared to kidneys of children who died of other non renal diseases and do not have abnormal hemoglobin. In four cases marked glomerular enlargement with severe proliferation of endothelial cells of the glomerular capillary tufts, with occasional neutrophilic infiltration. Morphologically all four would be diagnosed as glomerulonephritis and one of these four cases had the clinical diagnosis of acute glomerulonephritis prior to death. Other three did not show urinary findings which would suggest the diagnosis and thus may be called "Subclinical glomerulonephritis" or "glomerulitis" (Bell). In addition another eight cases also show endothelial cell proliferation but the degree of involvement is lesser than the first four cases. Lobulation of glomerular capillary tufts are also prominent in three cases. The mesangial areas of the glomeruli are also widening and in sections stained with PAS, there is definite thickening of the mesangium containing PAS positive fibrils. Varying amount of hemosiderin granules is present in the tubules in eight cases. It is located mostly in the straight tubules more prominently in the thick limbs of Henle's loop and collecting tubules. Brown granular pigment which does not contain iron present in twelve cases mostly in the proximal convoluted tubules and may represent reabsorbed hemoglobin. Tubular degeneration and focal necrosis is noted in four cases. Bile cast is present in three cases and a mild degree of nephrocalcinosis in three cases. The calcific spherules also contain some iron.

Lymph nodes.

Lymph nodes which were collected were hilar and mesenteric lymph nodes. They were not enlarged, the largest one was 1 x 1 x 0.8 cm. in measurement. Two of the lymph nodes contain granulomatous foci probably tuberculous in nature. One display a foreign body granulomatous reaction around calcophorite structures resembling psammoma bodies. Some of these enclose crystalloid structures which stain strongly for iron. In all lymph nodes, there is marked hyperplasia of the cells lining the medullary sinuses. There is also marked phagocytosis of erythroid cells, notably in cases where splenectomy was done. The lymphoid tissue of these lymph nodes are depleted and germinal centers are somewhat shrunken and show no activities. Hemosiderosis in the form of granules in sinus macrophages is seen in twelve cases. Extramedullary hemopoiesis is observed in eight cases. This consists of erythroid, granulocytic and occasional megakaryocytic elements.

Bone marrow.

Bone marrow was obtained from sternum in all cases. Microscopic examination reveals marked hyperplasia of marrow elements in all of Thalassemia hemoglobin E and Thalassemia major while in Thalassemia hemoglobin H, one case shows depletion of all marrow elements. Another case shows irregular hyperplasia of marrow elements in certain places while in other places the marrow cells are diminished

in number. There is proliferation of macrophages in all cases. They show active phagocytosis of erythrocytes and some are seen ingesting nucleated red cells. Phagocytic activities are particularly strong in cases in which splenectomy was done. In six cases, a collection of polygonal cells with abundant homogenous, shiny, slightly acidophilic cytoplasm and small round nuclei is noted especially near the cortex of the bone. These cells show strong cytoplasmic PAS reaction after diastase and contain no iron. They are also present in the spleens and livers. Their significance is unknown. Small amount of iron is present in the marrow, notably in some of the young erythroid cells and in the macrophages.

Spleen.

The weights of the spleen were markedly increased, ranging from 80 gms in a one year old boy to 1420 gms in a 34 year old male. Splenectomy was performed in twelve cases and in six of these patients died either during operation or within 72 hours after operations.

Microscopic examination of the spleen reveals the presence of siderotic nodules in the connective tissue of the trabeculae in fifteen cases. Small amount of iron is noted in the sinusoidal lining cells or encrusting the ring fibers of the Bilot cords in most cases. Increased trapping of erythroid cells in the Bilot cords is seen in eleven cases. Lipochrome pigment deposit in the connective tissue cells of trabeculae is seen in seven cases. All cases show some depletion of lymphoid tissue in the white pulps. The germinal centers of the Malpighian corpuscles are inconspicuous and reactive centers are noted in only two cases (8% versus 74% in spleens of children and young adults died of other diseases). The sinusoidal lining macrophages show active erythrocytic phagocytosis. Extramedullary hemopoiesis is observed in sixteen cases. The cells consist of both erythroid, myeloid and megakaryocytes. In six of these very young erythroblasts are present in clusters in the sinusoids, indicating, probably, delay in maturation of these cells. Macrophages containing PAS material is seen in significant number in seven cases. On the whole, severe changes are noted in the spleens of Thalassaemia major and Thalassaemia hemoglobin E. Lesser amount of iron, lesser amount of extramedullary hemopoiesis with almost normal amount of lymphoid tissue but more active trapping and destruction of erythrocytes are observed in spleens of Thalassaemia hemoglobin H patients.

Liver.

The liver weights were markedly increased in all cases. The weight varies from 340 gms in a one year old boy to 3070 gms in a 34 year old male. The liver showed dark brown color in all cases and the cut surface was described as being granular in eight cases. In one case large nodule, 4 cm. in diameter, was found in the right lobe close to the falciform ligament. The nodule is partly encapsulated.

Microscopic examination reveals that most cases show a mild degree of unrest of the liver cells with an increase in number of binucleated liver cells. The Kupffer cells are hypertrophic and hyperplastic. Active phagocytosis of red blood cells is noted in most cases but more severe in cases where splenectomy was done. The sinusoids contain islands of extramedullary hemopoiesis in eighteen cases and in six of these, islands of young, erythroblasts are noted. The portal areas are enlarged in twenty-one cases, and fibrous septa containing small ductules are seen extending out into the lobules. All cases show deposit of hemosiderin granules in the liver Kupffer cells, as well as in macrophages which infiltrate portal areas and septa. Hemosiderin in homogenous and amorphous form is also present in some young erythroid cells in the hemopoietic islands in the sinusoids. In cases where heavy deposit of hemosiderin is encountered, all parts of the lobules are involved. In cases where a mild to moderate amount of hemosiderin deposit is present, the deposit in liver cells is more heavily in the periphery of the lobules and less intense toward the center. A few rows of liver cells next to the central veins may contain no hemosiderin granules at all. Also in these instances, hemosiderin granules tend to be deposited in peribiliary locations. In addition to hemosiderin pigment, an increase in the amount of lipochrome pigment is observed. It is

deposited in the liver cells, and in the connective tissue cells of the portal tracts. It may be difficult to evaluate this pigment if there is heavy hemosiderin deposit which would somewhat mask its presence. Still it can be seen in at least eighteen cases of this series.

Fibrosis and ductular proliferation appear to be frequently encountered and a semiquantitative grading was attempted. Cases with no enlargement of Portal tracts are graded as O (4 cases); cases with definite enlargement of portal tracts and proliferation of ductules are graded as Class I acquired hemochromatosis (11 cases); cases with enlargement of portal tracts and fibrous septa extending out the portal areas are graded as Class II acquired hemochromatosis (4 cases); cases with linking between septa are graded as Class III acquired hemochromatosis (4 cases); and cases with complete breaking up of the lobules with some regeneration of the liver cells are graded as Class IV acquired hemochromatosis (2 cases).

CONCLUSION:

The major pathological changes in Thalassemia patients as observed in this series show that in addition to the findings which may be encountered in other types of chronic hemolytic anemia there are other findings which need further clarification. These observations include the chronic progressive damage of the liver leading to fibrosis, ductular proliferation and cirrhosis, the increase in amount of lipochrome pigment in the heart, liver, smooth muscle, and connective cells, which is somewhat excessive for the age group of these patients, the glomerular changes of the kidneys which display enlargement of the mesangium and varying degree of endothelial cellular proliferation; and the relative atrophy of lymphoid tissue in the lymph nodes and spleen in the presence of active reticuloendothelial function.

Three other studies which form parts of a study on the pathology of abnormal hemoglobin diseases but detailed analysis has not been completed are:

1. Pathology of spleens and livers in cases which are treated by splenectomy. Tissues from 50 cases of Thalassemia hemoglobin E, Thalassemia hemoglobin H, Thalassemia major and other assorted hemoglobinopathies, who had splenectomy in 1964 were collected. Morphologic analysis has been made but the follow up data on the effect of splenectomy is being studied.

2. Pathology of homozygous and heterozygous E gene carrier. Since May 1965, heart blood from unselected autopsy cases done at Siriraj Hospital has been studied for the presence of abnormal hemoglobins. Up to March 31, 1966, two hundred and twenty-five cases were studied, thirteen cases were found to have hemoglobin AE, and one to have high level (almost 100%) of hemoglobin F. The rest were found to have hemoglobin A. These patients died because of other diseases. Detailed pathologic analysis has not been completed.

3. pathology of the livers of patients with abnormal hemoglobin diseases during hemolytic crisis.

During the past year only three liver biopsies were obtained from cases of Thalassemia hemoglobin E disease during crisis. In one case findings were suggestive of hepatitis which was rather unusual for this condition, in two others the findings were suggestive of increasing RE erythrophagocytosis activity in the livers.

TABLE I

Summary of Clinical Data at Time of Death.

Case No.	Age	Sex	Hb type & amount	Duration of Clinical Disease	Clinical Picture
1	13	M	EF 3.5	? One year	Normal development, hemorrhagic shock post splenectomy.
2	7	F	EF 3.5	5 years	Underdeveloped, Mongoloid facies, anemia, jaundice, pneumonia.
3	7	M	EF 2	6 years	Well developed, undernourished. Heart failure.
4	8	M	EF 5.2	2 years	Well developed, undernourished. Hemolytic crisis splenic infarction.
5	3½	M	EF 3.5	2 years	Underdeveloped, undernourished. Mongoloid facie. Pneumonia one year post splenectomy.
6	9	F	EF 8	8 years	Well developed, undernourished. Congestive heart failure 2 years post splenectomy.
7	1	M	EF 4.6	2 years	Underdeveloped, undernourished. Amebic ulcerative colitis
8	2 4/12	F	EF	1 year	Normal development, slight jaundice. Bronchopneumonia.
9	2	F	EF 7.3	3 years	Normal growth and development. One year post splenectomy ? hemorrhagic fever.
10	2	F	EF 4	6 months	Retarded growth and development. Mongoloid facie. Post splenectomy 6 months. Bronchopneumonia.
11	10	F	EF 4.8	7 years	Well developed, undernourished. Acute glomerulonephritis.
12	1	F	EF 2	6 months	Hemolytic crisis.
13	10	F	EF 2.3	7 years	Well developed, undernourished. Uncontrollable hemorrhage 8 hours after splenectomy.
14	14	M	EF	Not known	Well developed, undernourished 5 years post splenectomy, acute retention of urine.
15	28	F	EF 4.2	Not known	Well developed, undernourished. Died 24 hours after splenectomy, cholecystectomy.

Case No.	Age	Sex	Hb amount	Type & Duration of Clinical Disease	Clinical Picture
16	37	F	EF 3.3	27 years	Well developed, undernourished Sixth month of pregnancy-Dead fetus in utero. Died 24 hours after evacuation and curettage.
17	38	M	EF 8.1	38 years	Well developed, undernourished Jaundice. Died 72 hours after splenectomy.
18	21	F	EF 4	21 years	Underdeveloped, no secondary sexual characteristics, Mongoloid facie. Died of renal failure. Left ureteral stone, hydronephrosis and hydroureter.
19	34	M	EF 7.2	25 years	Underdeveloped, Mongoloid facie, Hemolytic crisis, gall stone, mared jaundice.
20	23	F	EF 4.9	10 years	Normal development, anemia. Rheumatic heart disease.
21	11	M	AF 4.3	5 years	Underdeveloped, undernourished Mongoloid facie. Cardiac arrest during splenectomy.
22	5	M	AF 2.2	4½ years	Underdeveloped, undernourished Hemorrhagic fever. Four years post splenectomy.
23	3	M	AH	1 month	Well developed, undernourished. Subdural hemorrhage. Ulcerative colitis.
24	33	F	AH 3.9	19 years	Well developed, undernourished jaundice. Developed pneumonia 3 days after splenectomy.
25	21	F	AH	10 years	Well developed undernourished Hemolytic crisis.