A STUDY OF THE HAEMATOLOGIC ASPECTS OF PREGNANCY AT THE BIR HOSPITAL

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INTRODUCTION

The haematologic problems associated with pregnancy are numerous, although in many areas of the world the primary source of concern has been anaemia. Specimens for evaluating the problem of nutritional anaemia of pregnancy in Kathmandu were obtained during this study. However, the data from these are not yet available. On the other hand, the types of other haematologic disorders encountered were unexpectedly varied. Therefore we are submitting this preliminary report in order to bring these disorders to the attention of local medical paractitioners.

Subjects and Methods

One hundred and fifty-one unselected, but for the most part consecutive admissions to the Maternity Service, Bir Hospital, from 25 August to 1 October, 1972, were studied. The only requirement for admission to the study was that the subjects could potentially deliver a viable newborn; i.e., were at least 28 weeks pregnant. Venipuncture bloods collected in EDTA were obtained from the study subjects on admission, at the time of delivery, or, rarely, shortly thereafter. In addition, cord blood specimens from the majority of newborns were also investigated. Umbilical cord blood specimens were obtained by expressing blood from the placental end of the cord into EDTA anticoagulated tubes.

Hematocrits, blood smears, white cell counts and differentials were performed by standard techniques and haemoglobin levels were determined by a standard cyanmethaemoglobin technique (Cartwright, 1968). Platelets were enumerated using phase contrast microscopy, with normal values ranging from 140,000 to 440,000/mm³ (Brecher and Cronkite, 1950). Reticulocyte counts and haemoglobin H were studied after incubation in Brilliant Cresyl Blue at room temperature for 3—4 hours. Haemoglobin electrophoresis of maternal blood was performed on acrylamide gel, pH 8.9 (Smith and Evatt, 1967), or on cellulose acetate strips using a tris-borate buffer, pH 8.6. Foetal haemoglobin was determined by an alkali denaturation technique (Singer et al, 1951) and haemoglobin A2 was quantitated by elution from cellulose acetate. Normal values are less than 1% for foetal haemoglobin and less than 4.0% for haemoglobin A2. Umbilical cord bloods were subjected to starch gel electrophoresis using a 0.4M phosphate buffer, pH 7.0, and quantitation of obviously excessive amounts of haemoglobin Barts was performed by elution from cellulose acetate strips. The percent of nucleated erythrocytes per 200 white cells was calculated on cord blood smears. The upper limit of normal for nucleated erythrocytes is 10% (Mollison, 1951). Hereditary abnormalities of certain erythrocyte enzymes were detected using the ascorbate-cyanide technique (Jacob and Jandl, 1966) on selected cases. ABO and rhesus blood grouping and Coombs tests were kindly performed by the Nepal Red Cross Blood Bank.

Results

A. **Subjects:** Of the 151 study subjects, seven did not deliver after admission. The reasons for these admissions were premature contractions (4), sepsis (1), haemoptysis (1) and anasarca and severe anaemia (1). The remaining 144 subjects delivered newborns. Of 144 newborns, 4 were stillbirths. Three were due to mechanical difficulties, such as compound presentations, and one was a macerated foetus.

B. **Anaemia:** Thirty-eight (25%) of the admissions were anaemic as defined by the World Health Organization for pregnant women (i.e. haemoglobins less than 11.0 gm %) (W.H.O., 1968). If allowance is made for the higher altitude of Kathmandu, this value should probably be increased to 11.5 gm % (Hurtado et al, 1945). In the latter instance, 50 (33.3%) were therefore anaemic. The anaemic subjects were arbitrarily divided into two groups; those moderately anaemic (haemoglobins of 7.5 to 11.5 gm %) and those with severe anaemia (haemoglobins less than 7.5 gm %). The number of subjects in the former group was 45 (30%) and, in the latter, 5 (3.3%). An illustration of the distribution of haemoglobin levels is presented in Fig. 1.

In the "moderate anaemia" group, iron and/or folate deficiency seemed to be a major cause of anaemia. Reticulocyte counts tended to be low, although a few may have had a haemolytic process or recent blood loss based on a mild elevation of reticulocyte counts and/or the presence of nucleated red cells on peripheral smear. Table 1 gives the probable cause of nutritional anaemia in subjects with haemoglobin less than 10 gm % based on peripheral smear evaluation and other listed haematologic data. Combined iron and folate deficiency appeared to be the most common etiology.

Regarding those with definite or probable haemolytic anaemia (Table 11), it should be noted that in severe iron or folate deficiency, the reticulocyte count is typically very low, although it can increase in the presence of acute blood loss or acute hypozia. Neither were present in any severely anaemic subject. A specific diagnosis of a haemolytic anaemia was not possible in certain of these cases. However, one should be aware that iron deficiency can lower haemoglobin A2 values, thus making the diagnosis of Beta thalassemia, and can also prevent the formation of haemoglobin H inclusion bodies in red cells, the hallmark of Alpha thalassemia in the adult. Most cases of severe anaemia were associated with reticulocytosis and nucleated red cells in the peripheral blood, and as no tachycardia or evidence of recent acute blood loss was present, this indicates a chronic anaemic process, probably basically haemolytic. Superimposed iron or folate deficiency most likely worsened these anaemias to some degree. But only one of the subjects with severe anaemia appeared to have uncomplicated iron deficiency as the primary cause of anaemia.

One severely anaemic woman was strongly suspected of having haemoglobin H disease, a severe form of Alpha thalassemia. However, this subject left the hospital before delivery and before iron repletion could be completed. A second severely anaemic woman had rare haemoglobin H cells in her blood smear and delivered a macerated infant. Unfortunately a cord blood specimen was not obtained, so it is unknown whether or not this represented a case of Alpha thalassemia *hydrops foetalis*. It is possible that this woman had haemoglobin H disease, but that iron deficiency greatly reduced the number of circulating haemoglobin H containing cells. Her husband also had a moderate hypochromic anaemia which could not be specifically diagnosed..

C. Thrombocytopenia: Three cases of thrombocytopenia were diagnosed. One occurred in a woman with a term pregnancy who entered with sepsis and her platelet count reverted to normal with therapy of the infection. Another was in a young woman with splenomegaly, of unknown etiology, but who otherwise had a being hospital course. Her platelet count remained low, and, while not anaemic, her reticulocyte count was slightly elevated. The third case of thrombocytopenia occurred in a woman who delivered within 20 minutes after admission and who had retained placenta. The initial blood specimen was drawn after delivery and the platelet count was repeated the next morning before operative removal of the placenta. Both platelet counts were low. The degree of thrombocytopenia was not severe ($100,000-120,000/\text{mm}^3$) in any of these cases, and no haemorrhagic diathesis was noted.

D. Thalassemia in the Newborn: Seventy-five cord blood specimens were studied by starch gel electrophoresis for evidence of increased levels of haemoglobin Barts, diagnostic of Alpha thalassemia in the newborn. One case was discovered, the child of a woman with haemoglobin H disease. The concentration of haemoglobin Barts was 6.4 %, indicating this child has Alpha thalassemia minor (Wasi et al, 1969). A similar concentration of haemoglobin Barts was found in another newborn just prior to the official start of this study. However, the mother and child were discharged before this was known, so no family study was obtained. The child of one study subject with Alpha thalassemia minor had a normal electrophoretic pattern, indicating the child had not inherited the gene (s) for Alpha thalassemia minor.

E. Haemolytic Disease of the Newborn: The percent of nucleated erythrocytes per 100 white cells in normal newborns should not exceed 10%. In 95 newborns so studied, 11 (12%) exceeded this number, although the increase was minimal (between 10-15%) in 6. Of these, one child had evidence of Alpha thalassemia minor. The range in the other five was 20-33%, and was associated with a leukocytosis. These were studied in some detail. In one instance, both mother and her male child had an abnormal ascorbate-cyanide test as compared to controls. The father was normal. There was no evidence of Rh or ABO incompatibility and a test for syphilis in the mother was negative. Thus, this case appears to be haemolytic anaemia of the newborn secondary to an erythrocyte enzymatic defect, most likely glucose-6-phosphate dehydrogenase deficiency or pyruvate kinase deficiency. Two other cases were due to ABO incompatibility, as the mothers belonged to blood "O", the newborns were group "A" and "B", and a positive direct Coombs test was demonstrated on the cord blood specimens. There was no Rh incompatibility nor evidence of an erythrocytic enzyme defect. The remaining two cases remain undiagnosed. Studies of the fathers might have provided indirect evidence for an enzyme defect (glucose-6-phosphate dehydrogenase deficiency is sex-linked), but no blood specimens could be obtained from them.

Serious jaundice did not develop in any of the newborns that were kept for observation, although some were discharged before the results of the tests were known.

F. Abnormal Haemoglobins: No abnormal haemoglobins were detected in the mothers with the electrophoretic techniques used.

Discussion

This study of the haematologic status of pregnant women was notable in two ways: one was the relatively low incidence of severe anaemia, and the second was the variety of abnormalities encountered. A discussion of each of these abnormalities seems indicated.

1) **Anaemia pregnancy:** Although further evaluation of the nutritional status of the mothers studied is planned (i.e. serum iron, serum B12 and red cell folate levels), it seems safe to assume that, for the most part, deficits of iron or folate produced primarily moderate anaemia. The presence of reticulocytosis, normoblastic nucleated red cells in the peripheral blood and the absence of associated thrombocytopenia or leukopenia are strong evidence against folate deficiency being a major cause of the severe anaemias. Likewise, except for one case, iron deficiency was apparently no more than a secondary problem in these patients. This certainly does not mean to minimize, for example, severe hookworm anaemia in pregnant women in or outside Kathmandu. And, whenever possible, iron and folic acid supplementation is indicated in pregnancy, not only to prevent anaemia, but to ensure a healthy newborn. Severe maternal folate deficiency has been reported as producing a variety of abnormalities in newborns (Cooper et al, 1970). However, from this study it is apparent that there may be an underlying chronic haemolytic process which requires the practitioner's attention. The propensity of haemolytic anaemic patients for folate deficiency is well known (Lindenbaum and Klipstein, 1973). It is likely that associated iron deficiency makes the

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Diagnosis of thalassemia in some patients, and correction of this deficit should enable the diagnosis of thalassemia to be made in those afflicted subjects at a later date.

2) **Haemolytic disease of the newborn:** Two causes of this state were discovered in the present day, one being an enzymatic defect, probably glucose-6-phosphate dehydrogenase or pyruvate kinase deficiency, and the other ABO incompatibility. The latter occurs almost exclusively in blood group "O" mothers who have children of blood groups "A", "B" or "AB" (Dacie, 1967). This occurs with the first pregnancy and may not occur in subsequent ones, in contrast to Rh incompatibility. A positive standard Coombs test is not always demonstrable in cord blood, but present, gives further weight to the diagnosis of ABO incompatibility. Although neonatal jaundice due to ABO incompatibility is usually not serious, exchange transfusion is occasionally required. The same can be said about erythrocyte enzyme defects such as glucose-6-phosphate dehydrogenase deficiency which produce haemolytic anaemia. A further reason for diagnosing such enzyme defects is the propensity for haemolytic anaemia after affected subjects ingest certain medicines, such as sulfa drugs, primaquine, etc. Vitamin K administration to an affected newborn has been reported to cause severe haemolysis.

3) **Thrombocytopenia:** No case of thrombocytopenia was found due to folate deficiency. In severe iron deficiency, thrombocytosis usually results. There appeared to be different causes of thrombocytopenia for each of the thrombocytopenic mothers diagnosed. However, as coagulation tests or bone marrow aspirations were not performed, these diagnoses are necessarily presumptive. One was thrombocytopenia associated with septicemia. In this instance platelet survival is typically shortened, and may or may not be associated with disseminated intravascular coagulation. Control of infection and any associated hypotension and hypoxia will remove the cause of thrombocytopenia. The thrombocytopenia of another case appeared to be due to splenomegaly. Although there may be some shortening of platelet life span, basically this situation is due to sequestration of platelets in the large spleen. Infusion of adrenalin in splenomegalic subjects with thrombocytopenia produces a marked percent increase in peripheral platelet count as compared to normal individuals. In other words, "hypersplenism", as applied to platelets, implies primarily splenic sequestration rather than platelet destruction (Aster, 1966), and, if liver function is normal, a haemorrhagic diathesis is not a problem. Indeed, the total body platelet count may be normal or even increased despite peripheral thrombocytopenia. In the case of retained placenta, release of placental thromboplastic factors may initiate disseminated intravascular coagulation with consequent utilization of platelets and other clotting factors. Hypofibrinogenemia and an increase in fibrinolytic activity have been reported (Phillips and Mendenhall, 1962). The thrombocytopenia and other complications such as abruptio placenta, amniotic fluid embolism, and retained placenta and also of septicemia can be severe. Furthermore, as coagulation is a major stimulus for activation of the fibrinolytic system, these states may be associated with marked clot lysis. Therapy, which is paradoxical, includes heparin to stop further intravascular coagulation, and a fibrinolytic inhibitor such as epsilonaminocaproic acid (Amicar) to control excessive clot lysis.

Thalassemia: As mentioned above, iron deficiency can make the diagnosis of both Alpha

and Beta thalassemia very difficult, if not impossible. Yet the most severe anaemias encountered in this study had evidence compatible with a chronic haemolytic process, and, as all subjects had normal adult haemoglobin (i.e., haemoglobin AA) thalassemia remains a strong possibility for the severe maternal anaemias and perhaps for the macerated infant born to one of the severely anaemic mothers. Alpha thalassemia in its most severe form is a common cause of foetal wastage in Thailand (Wasi et al, 1969). A discussion of the various forms of thalassemia was recently published in the Journal (Admas, 1972).

5) Abnormal Haemoglobin: It is notable that no abnormal haemoglobins were found in this series. 0.8% of routine bloods examined from in-patients and out-patients at the Bir Hospital were previously noted to have abnormal haemoglobins, primarily haemoglobins D and E (Adams, 1972). Therefore, while not diagnosed in the present study, homozygous haemoglobinopathies remain another cause of anaemia which may be associated with pregnancy and mistakenly diagnosed as mere iron deficiency anaemia.

Conclusion

In the study purely nutritional deficits were not demonstrated to be a main cause of severe anaemia of pregnancy encountered at the Bir Hospital. It seems more likely that in the severe anaemias, nutritional deficits were often contributory rather than primary problems. In addition, a variety of other haematologic problems were diagnosed, and, while not producing life threatening situations in the six weeks of this study, the potential lethality of thrombocytopenias, intravascular coagulation, and haemolytic disease of the newborn must be taken seriously.

It is of practical importance to realize that, with the exception of starch gel electrophoresis, every technique used in this study is quite simple to perform. Indeed, starch gel electrophoresis itself seems superfluous, as elevations of haemoglobin Barts in the range of 5% or greater in the newborn can be easily detected by cellulose acetate electrophoresis. The techniques used to diagnose all varieties of disorders discussed in this paper can be performed with minimal expense, with the exception of cellulose acetate and starch gel electrophoresis. All that is required is an expenditure of time.

To summarize, of 151 admissions to the Maternity Service, Bir Hospital, the following diagnoses, exclusive of nutritional deficiencies, were made:

1) Beta thalassemia minor	3
2) Haemoglobin H disease	1
3) Alpha thalassemia minor (maternal)	2
4) Alpha thalassemia minor (newborn)	1
5) Thrombocytopenia associated with sepsis	1
6) Thrombocytopenia associated with splenomegaly	1
7) Thrombocytopenia associated with retained placenta (?due to intravascular coagulation)	1

8) Haemolytic disease of the newborn secondary to an erythrocytic enzymatic defect (?Glucose-6-phosphate dehydrogenase deficiency)	1
9) Erythrocytic enzymatic defect (maternal)	1
10) Haemolytic disease of the newborn secondary to ABO incompatibility	2

In addition, there was one other possible case of Alpha thalassemia, one possible case of Beta thalassemia minor, and one possible case of **hydrops foetalis** due to Alpha thalassemia. Nutritional (folate and/or iron deficiency) anaemia was common but appeared to be associated primarily with moderate anaemias.

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TABLE I

Suspected Nutritional Deficiencies, Hb less than 10.0 gm %

Subject	Haemoglobin	MCHC [‡]	Reticulocyte Count	Fetal Hb*	Hb A ₂ †	Nutritional deficit suspected★
M3	7.9	30.4	0.9%	1.47	2.2	FA/Fe
M29	9.0	37.9	0.4%	0.86	2.5	FA
M37	9.75	32.5	0.9%	0.9	2.2	FA/Fe
M40	9.6	35	0.8%	2.7	2.2	FA
M65	9.5	32.7	0.5%	1.0	3.3	FA
M69	9.5	28.0	3.6%	0.5	3.0	Fe
M79	9.25	31.8	3.1%	1.4	3.3	Fe/FA
M82	8.75	30.2	3.0%	0.8	1.6	Fe
M89	9.75	33.6	3.1%	0.7	3.6	FA
M95	8.4	30.6	4.1%	1.1	2.6	Fe
M100	9.0	28.9	3.6%	1.0	3.8	Fe/FA
M105	8.9	30.7	4.6%	1.0	1.3	Fe/FA
M113	9.6	31.0	1.8%	0.6	3.6	Fe/FA
M117	8.6	30.7	1.8%	0.5	3.9	Fe/FA
M125	9.25	30.8	3.6%	1.2	3.2	Fe/FA
M126	9.25	30.8	2.7%	1.0	3.0	Fe/FA
M128	6.4	27.8	3.4%	0.9	3.0	Fe
M132	9.1	32.5	3.6%	0.5	3.6	Fe/FA

‡Normal mean corpuscular haemoglobin concentration (MCHC) values are between 32 & 36

*Elevation of foetal haemoglobin in some normal pregnant women has been previously reported, and its cause is unknown. Thus the necessity for simultaneous Hb A₂ estimations to rule out Beta Thalassemia.

†Normal Haemoglobin A₂ values are less than 4.0%.

★FA= Folic acid; Fe= Iron.

TABLE II

Diagnoses of Haemolytic Anaemia

A. Confirmed Diagnoses:

Subject	Diagnosis	Hb	MCHC	Foetal Hb	Hb A ₂	Reticulocyte count	Peripheral nucleated erythrocytes
M-7	B Thal. minor	9.0	30.5	1.08	5.7%	5.2%	+
M-13	B Thal. minor	9.5	32.7	3.04	5.3%	1.6%	+
M-15	B Thal. minor	11.5	—	2.05	5.9%	5.2%	+
M-31	Hb H disease	4.9	27.2	1.17	3.4%	6.9%	21/100 leucocytes
M-81	A Thal. minor*	6.75	29.3	0.90	1.5%	3.2%	—
M-94	A Thal. minor	9.6	29.1	1.30	2.9%	2.3%	+

B. Suspected Diagnoses:

M-36	B Thal. minor	8.0	29.1	0.8	4.3%	4.1%	+
M-68	Thal., type?	5.25	27.5	0.8	3.1%	7.0%	+
M-73‡	Hb H disease	2.25	21.5	1.0	1.8%	6.8%	9/100 leucocytes

*Delivered macerated infant. Mother may have had Hb H disease plus iron deficiency.

‡Splénomegaly present. One week after parenteral iron therapy, reticulocyte count was 17% and Hb H inclusions appeared to be developing in the peripheral blood smear. However, patient left hospital against medical advice before delivery.

Figure 1
Distribution of Maternal Haemoglobin Values