Small haemoglobin components accompanying Hb Bart's in newborns

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NORMAL Hb A consists of 2 alpha chains and 2 beta chains (Hb alpha 2 beta 2), Hb F of 2 alpha chains and 2 gamma chains (Hb alpha 2 gamma 2) and Hb A2 of 2 alpha chains and 2 delta chains (Hb alpha2 delta2). Hb Bart's is made up solely of gamma chains and Hb H solely of beta chains. They are thought to result from the suppression of alpha chain production, leading to surplus of gamma chains or beta chains which form Hb Bart's (Hb gamma 4) or Hb H (Hb beta 4). The presence of Hb Bart's in the newborn period is therefore thought to represent alpha thalassaemia since under alpha thalassaemia is understood impaired production of alpha chains without the synthesis of abnormal haemoglobin chains.

An excessive amount of Hb Bart's in the newborn was found to be the cause of non-immune erythroblastosis foetalis leading to death (Lie-Injo and Jo, 1960; Lie-Injo, 1962; Lie-Injo et al., 1962). Lie-Injo and colleagues thought that these cases represent homozygous alpha thalassaemia. This idea was supported by the findings of Pootrakul et al., (1967) in Thailand — Todd et al., (1970) in Hongkong, and Weatherall et al., (1970) in Singapore.

According to current concepts alpha thal$_1$ represents the severe type of alpha thalassaemia and is represented in the newborn period by the presence of Hb Bart's in appreciable amount, alpha thal$_2$ represents the milder type of alpha thalassaemia and is represented in the newborn period by the presence of Hb Bart's in trace amount. Accord-

ing to this concept, combination of alpha thal$_1$ with alpha thal$_2$ will result in Hb H disease (Wasi et al., 1964), and combination of 2 alpha thal$_1$ in hydrops foetalis.

In the present paper, the author wants to discuss the evidence that Hb Bart's in the newborn period is not specific and is often an accompaniment of abnormal haemoglobin synthesis of different types and that the above concept has to be revised.

Material and Methods

Cord blood obtained from the delivery room of the Maternity Hospital at Kuala Lumpur were examined for unusual haemoglobin components.

Haematological examinations followed standard methods. Methods of haemoglobin studies are the same as previously reported by the author (Lie-Injo et al., 1966; Lie-Injo et al., 1971). However, haemolysates prepared for analysis were much more concentrated than usual. Starch gel electrophoresis, using tris-EDTA boric acid buffer pH 8.0 and 8.6 and discontinuous tris-boric acid buffer pH 9.5, were routinely carried out for separation of haemoglobin components and benzidine was used for staining of haemoglobin patterns.

Results

Among 1,431 newborns studied (492 Malays, 501 Chinese and 438 Indians) 98 had Hb Bart's in the blood. Of these, 39 were found to have Hb Bart's level above 5% and 58 with Hb Bart's below 3.8% with only one with a level in between. The rest did not show Hb Bart's. Relatively more cases with trace amounts of Hb Bart's were detected than in our previous study (Lopez and Lie-Injo, 1971). This is probably due to the use of more concentrated haemolysates. In the group with trace amounts of Hb Bart's, a variety of abnormalities of haemoglobin production was found. Those findings will be published in detail elsewhere. In short, among the cord blood samples with Hb Bart's below 3.8%, many showed an additional abnormality.

This additional abnormality of haemoglobin synthesis is of different types. One type of abnormal haemoglobin synthesis is associated with the presence of a slow-moving abnormal haemoglobin slower than Hb A2 consisting of two components we tentatively called slow-moving Hb X components which are similar to those described earlier in association with Hb H disease and which have opened new aspects regarding the inheritance of Hb H disease (Lie-Injo et al., 1971). Newborns with such abnormal slow-moving components invariably had one parent with the same abnormal haemoglobin. These slow-moving components in newborns have apparently been overlooked in studies in Thailand and other areas, including our own earlier study (Lopez and Lie-Injo, 1971) using less sensitive methods. Structural studies of these X components showed them to have an abnormality in the alpha chains.

A second type of abnormal haemoglobin synthesis accompanying Hb Bart's in the newborn period, probably also overlooked in earlier studies, is an abnormal gamma chain variant which the author called Hb F Kuala Lumpur. A detailed report on the structural studies of this new haemoglobin will be discussed elsewhere. The abnormality was not found in either parents since adults do not produce gamma chains. This abnormal haemoglobin occurs in slightly less than one per cent of Indian newborns and is often found together with Hb Bart's. When the child grows older this Hb F Kuala Lumpur, as well as the small amount of Hb Bart's, disappeared and there are no signs of alpha thalassaemia in the blood of the child.

A third type of abnormal haemoglobin sometimes accompanying Hb Bart's in the newborn is Hb E, a beta chain variant. Four of 12 babies with Hb E in the cord blood had Hb Bart's as well. Hb E in the cord blood occurs in low concentrations and may be overlooked if studied only in the standard buffer pH 8.6 where it has the same mobility as Hb A_2 and not at pH 9.5 where it has a slightly faster mobility than Hb A_2. Follow-up study of one of the four babies with Hb E and Hb Bart's shows the child, when he was older, to be just a Hb E trait carrier without alpha thalassaemia.

Among the newborns with appreciable amounts of Hb Bart's, usually two haemoglobin components were detected in addition to Hb Bart's, Hb F, Hb A and sometimes Hb A_2. One moves slightly slower than Hb F and faster than Hb A_2 clearly seen at pH. 8.6, which the author tentatively designates the Y_1 component (Fig. 1). The other moves between Hb A and Hb Bart's which is clearly seen at pH 8.0 and is tentatively designated Y_2 component (Fig. 2). At pH 8.6, the Y_2 component cannot be seen properly because it overlaps with Hb A_2. Only after a prolonged run can an indication of the Y_2 component be demonstrated at this pH. At pH 8.0 however, the Y_2 component stands out clearly. Follow-up study of 8-day-old babies, who had the abnormality in their cord blood showed that fresh haemolysates, prepared immediately after bleeding from the washed red blood cells still showed the Y_1 and Y_2 components in

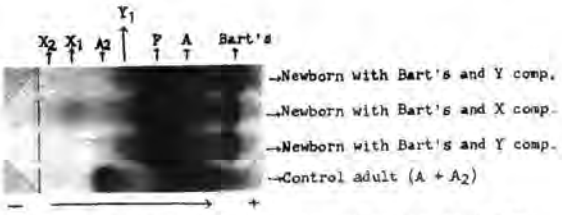

Fig.1. Starch gel electrophoresis in tris-EDTA-Boric acid buffer pH 8.6 showing the haemoglobin patterns of newborns with Hb Bart's and small moving haemoglobin components. Y_1 component is clearly seen at this pH , Y_2 not.

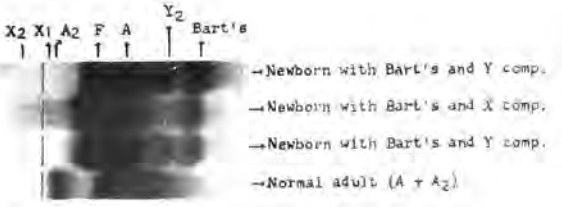

Fig. 2 Starch gel electrophoresis in Tris-EDTA-Boric acid buffer pH 8.0 showing the haemoglobin patterns of newborns with Hb Bart's and small haemoglobin components. Y_2 is clearly seen at this pH, Y_1 not.

addition to Hb Bart's, Hb F and Hb A. Our attempts to purify the Y_1 component was not very successful. Everytime the Y_1 component was eluted from the starch gel, concentrated and rerun, the electrophoretic pattern obtained from it curiously had the mobility of Hb A_2 with Hb F as contaminant.

The author was also able to show the Y_1 and Y_2 components stained with benzidin, in cases of hydrops foetalis thought to be due to homozygous alpha thalassaemia, or Bart's hydrops foetalis syndrome. In most of these cases, these small components could clearly be detected in addition to a large amount of Hb Bart's and small amount of Hb H (Fig. 3).

The component Y_1 and Y_2 could not be demonstrated in the parents.

Discussion

This present survey on newborns in Malaysia shows that Hb Bart's in the newborn period can accompany different types of abnormalities in haemoglobin synthesis involving an alpha chain variant (slow-moving Hb X components), a beta chain variant (Hb E), a gamma chain variant (Hb F

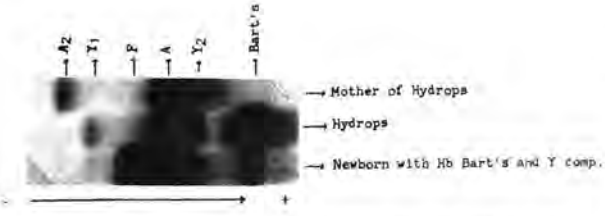

Fig. 3 Starch gel electrophoresis, Tris-EDTA-Boric acid buffer pH 8.6 showing the Hb pattern of a newborn with Hb Bart's and Y comp. compared with small Hb comp. in hydrops foetalis. The Y_1 comp. in the newborn has the same mobility as the slow comp in the hydrops foetalis.

Kuala Lumpur) as well as the synthesis of the Y_1 and Y_2 components, the significance of which is not yet clear. It can, therefore, be concluded that contrary to current belief, the presence of Hb Bart's is not specific for alpha thalassaemia since under alpha thalassaemia is understood the suppression of alpha chain production without abnormality of structure of any chain. The concept of Hb Bart's in the newborn being always the expression of alpha $thal_1$ or alpha $thal_2$ is therefore not anymore valid. Hb Bart's in the newborn seems to be the result of an aspecific imbalance of chain production, which may occur in various types of abnormality. A more detailed description and discussion of the different abnormalities which the author often found accompanying trace amounts of Hb Bart's will be published elsewhere.

Studies on Hb Bart's in the newborn period have so far failed to report the Y1 component although the Y_2 component may have been described before. Todd et al., (1970) and Weatherall et al., (1970) in studies of respectively 15 and 14 cases of Bart's hydrops foetalis, did not report any small haemoglobin component moving slower than Hb A. However, they did describe a component which migrates between Hb A and Hb Bart's and structural studies showed that this component is identical with Hb Portland described earlier by Capp et al., (1967). This component may be the same as the component we designate Y_2 in our healthy newborn babies with appreciable amounts of Hb Bart's and which we also found in our cases of Bart's hydrops foetalis syndrome.

However, the other component moving much slower, which the author designates Y_1 and which she found in healthy newborns with appreciable amount of Hb Bart's as well as in cases of Bart's hydrops foetalis syndrome has never been reported by others in newborns. This small component may be of theoretical importance. Todd et al., (1970) and Weatherall et al., (1970) were unable to find

any alpha chains in Bart's hydrop foetalis syndrome. Trace amounts of alpha chains detected by Weatherall et al., in 2 of their 14 cases were thought to be due to contamination with maternity blood. Todd et al., (1970) as well as Weatherall et al., (1970) showed that the component which moves in between Hb A and Hb Bart's (probably the same as our Y_2 component) had gamma chains combined with another type of chain which is not identical with normal alpha, beta, gamma or delta chains and which was also described earlier by Capp as Hb Portland (1967). Hybridisation studies in vitro showed that, when this Hb Portland was hybridisised with normal Hb A (Hb alpha 2 beta 2) it gives as one of the hybrids a haemoglobin which moves slightly faster than Hb A_2 and which had alpha chains combined with the unusual chains of Hb Portland. If the component we call Y_1, which also has a mobility slightly faster than Hb A_2, is identical with this last mentioned hybrid, it would mean that in the Bart's hydrops foetalis syndrome, contrary to general belief, alpha chains are present and that in this condition, the alpha chain synthesis is not completely suppressed.

If, on the other hand, the Y_1 contains abnormal chains not physiologically produced, than the term alpha thalassaemia for the condition would not be valid since under thalassaemia is currently understood the suppression of the synthesis of a particular chain without the production of abnormal non-physiological haemoglobin chains. In either case, it would not fit into current concepts. One has also to keep in mind the possibility that a trace amount of the mother's blood has entered the babies' blood through the placenta before delivery and that the alpha chains of the mother's Hb A (Hb alpha 2 beta 2) would have bound with the unusual chains of Hb Portland (Y_2 component) of the baby to form the hybrid haemoglobin. This is not very likely because, when a haemolysate was immediately prepared from blood, obtained in ACD as well as in EDTA solution, from an 8-day-old baby, who had the abnormality in his cord blood, the fresh haemolysate still showed the Y_1 and Y_2 components in addition to Hb Bart's, Hb F and Hb A, while no time for hybridisation was allowed in the preparation and study of the haemolysate prepared from the freshly-drawn washed red blood cells. If some mother's blood was still present in the baby at this age, her haemoglobin would have been found in the fresh haemolysate of the baby in the form of Hb A and not in the form of a hybrid.

Isolated Y_1 component resolved into a haemoglobin with the mobility of Hb A_2 with Hb F as contaminante when rerun on starch gel electrophoresis. This curious quantity switching over to Hb A_2 (or a haemoglobin with the mobility of Hb A_2) is also demonstrated by the Hb X component described earlier in Hb H disease (Lie-Injo et al., 1971) and is especially seen in the homozygous case for the X component discovered recently by Lie-Injo.

Summary

In an attempt to discover small haemoglobin components which might throw new light in the problem of alpha thalassaemia associated with Hb Bart's, a new survey of newborns was carried out. As in a previous study, two groups of newborns with Hb Bart's in the blood could be found, one with appreciable amounts of Hb Bart's and the other with trace amounts. It was found that Hb Bart's in the newborn may accompany different types of abnormal haemoglobin production involving alpha, beta, as well as gamma chains.

Further, new borns with appreciable amounts of Hb Bart's usually have two small haemoglobin components tentatively designated Y_1 and Y_2 components. Hb Y_1 component is clearly seen at pH 8.6 moving between Hb F and Hb A_2 and Hb Y_2 component is clearly seen at pH 8.0 moving between Hb Bart's and Hb A. Similar components with the same mobility as Y_1 and Y_2 components could also be demonstrated in cases of hydrops foetalis thought to be due to homozygous alpha thalassaemia.

It is concluded that contrary to current concepts, the presence of Hb Bart's in the newborn is not specific for alpha thalassaemia and that its presence in the newborn is the result of an aspecific imbalance of chain production which may occur in various types of abnormality involving haemoglobin synthesis.

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