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Incidence of erythroblastosis in the babies of one hundred RH negative women

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THE INCIDENCE OF ERYTHROBLASTOSIS IN THE BABIES OF ONE HUNDRED RH NEGATIVE WOMEN

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INTRODUCTION

DeLee and Greenhill (1) define Erythroblastosis Fetalis as a disease of the fetus and of the newborn. Included in the definition are three clinical types; 1. Icterus Gravis which is the most common form of the disease; 2. Fetal Hydrops which is the rarest form, but at the same time, one-hundred per cent fatal; and 3. Hemolytic Anemia of the Newborn which is rare by itself, but commonly found in combination with the other two forms of the disease.

During the past several years since the discovery of the Rh factor, much has been learned concerning its role in the etiology of Erythroblastosis Fetalis. Many papers have been written concerning this disease; the case histories, pathology, diagnostic standards, and therapy. Physicians and the layity alike have been warned repeatedly of the potential danger to the children of marriages between Rh negative women and Rh positive men.

This question then arises; what is the incidence of Erythroblastosis Fetalis in the children of such marriages. This paper demonstrates the incidence of Erythroblastosis Fetalis in one-hundred marriages between Rh negative women and Rh positive men.

DISCOVERY AND HISTORY

The discovery of the Rh factor is generally attributed to the work of Landsteiner and Weiner (2). From laboratory animals, immunized with the red blood cells of the Rhesus monkey, these workers obtained sera which they found would agglutinate the red blood cells of approximately eighty-five per cent of white Americans. They designated this agglutinable property the Rh factor.

Although this was the actual discovery of the Rh factor, previous observations of clinical conditions had been made which undoubtedly were due to the Rh factor. In 1905 Dienst (3) and in 1923 Ottenberg (4) both suggested that immunization of the mother by fetal blood might be the cause of eclampsia. Ottenberg also hinted that jaundice of the newborn might be due to, "accidental placental transfusion of incompatable blood." In a report on observed abnormal agglutination of human blood, Landsteiner and Levine (5) stated that there apparently was a range of differences in the blood of different people. In 1939, Levine and Stetson (6) reported the now well known case of a twenty-five year old woman whose blood showed iso-agglutination with about eighty per cent of the donors within her

own blood group. Following the delivery of a premature, still-born macerated fetus, the woman received three transfusions of whole blood. A Hysterectomy was performed between the second and third transfusions. Nineteen hours after the first transfusion, the patient voided 240 cc. of bloody urine. Sensitive cross agglutination tests showed that her blood was compatable with only eight of fifty donors in her own blood group. Levine and Stetson postulated that the patient had become immunized by some antigen in the fetus. This antibody, now known as the anti-D antibody, was almost identical with the previously mentioned antibody produced by Landsteiner and Wiener (2).

One of the first observations of the clinical importance of the discovery by Landsteiner and Wiener came when Wiener and Peters (7) obtained blood samples from patients who had shown hemolytic reactions after several blood transfusions. The serum of these patients contained anti-Rh iso-agglutinins and the Rh factor was not present in their red blood cells.

In 1941, a study made by Levine and associates (8) reported that Erythroblastosis Fetalis was due to an Rh blood group incompatability between mother and

child. They stated that, "Erythroblastosis Fetalis resulst from: 1. the iso-immunization of the mother by dominant hereditary blood factors in the fetus, as evidenced by the production of immune intra-group agglutinins, and 2. the subsequent passage of these maternal agglutinins through the placenta, and their continuous action on the susceptible fetal blood." In this paper was first described the antibody known at the present time as the anti-c antibody.

Also first demonstrated in 1941 was the first apparently pure anti-C antibody, by Landsteiner and Wiener (9). However, in 1944, Race (10) showed that this must have contained some incomplete anti-D antibody in it.

Landsteiner and Wiener, 1941, (9) using three sera carried out an heredity study on sixty families with a total of 237 children. From this study they concluded that the Rh factor is inherited in the blood as a Mendelian dominant characteristic. Later, these same two men (11) proved that the differences shown by the anti-D and anti-C sera were inherited, and they theorized that there were three major genes. They also believed that there might be a fourth gene.

In 1943, Race, Taylor, Boorman and Dodd (12),

described the first two examples of the antibody now called the anti-E antibody. The next year, 1944, Race, Taylor, Cappell, and McFarlane (13) defined seven alleomorphs and eleven phenotypes in man.

Working independently, and using all of the knowledge on the Rh factor available up to that time, Fisher (14), 1943, showed that the work previously done demonstrated that the reactions of anti-C and anti-c were antithetical. He believed that the genes of these two antibodies were allelomorphic, and called them C and c. Fisher also saw that the reactions of the remaining two sera, anti-D and anti-E, were not antithetical, and he therefore theorized that allelomorphs of these were also present. According to his theory, there were forty-eight possible antigen-antibody reactions. At the time of his paper, twenty-six of these reactions had been demonstrated.

With the discovery of the anti-e antibody in 1945 by Mourant (15) and the first report of the anti-d antibody in 1946 by Diamond (16), Fisher's theory has proven to be completely true.

According to this theory, there were eight possible phenotypes, thus giving a possibility of sixty-four genotypes. Fisher and Race (17) suggested that

the genes were located close together on the chromosome and therefore made unlikely the possibility of very much crossing over. For this reason some of the genotypes are quite rare.

DIAGNOSIS AND LABORATORY FINDINGS IN ERYTHROBLASTOSIS FETALIS

On this series of one-hundred Rh negative women, routine antibody titres were performed. Three tests were employed: 1. Agglutinins, to detect the presence of anti-Rh antibodies acting in a saline suspension; 2. Conglutinins, to detect the presence of anti-Rh antibodies acting in an albumin suspension; 3. Coombs' test, for the specific detection of anti-Rh antibodies acting in an anti-human globulin serum.

Coombs, Mourant and Race (18) considered this a more sensitive method for the detection of weak and incomplete antibodies, because it demonstrated the ability of an anti-C serum to further absorb, even after a heating process had caused it to become non-reactive to the agglutinating and blocking tests.

These worker (18) observed cases in which the antibody titres were as high as 1:2048, while the agglutinating and blocking titres remained low. The Coombs' test is also used on the blood of the infant,

a positive test aiding in the diagnosis of Erythroblastosis Fetalis.

Nelson (19) states that the diagnosis of Erythroblastosis Fetalis depends on the demonstration of a maternal agglutinin which is incompatable with the infants red blood cells. He also states that during the first few days after birth there is a great increase in the number of nucleated red blood cells. The excess number of nucleated red blood cells tends to diminish, and at the same time a very profound anemia develops.

In all of the cases of Erythroblastosis Fetalis which are presented in this report the diagnosis of the disease was based on laboratory evidence obtained shortly after delivery.

CLASSIFICATION OF THE GROUP

The typing of the blood of the mothers and fathers in this series of patients was performed by routine laboratory methods. In the case of the mothers, both the A B O group and the Rh genotype was determined. The fathers are all known to be Rh positive, but in thirty-six cases the Rh genotypes are not known.

The mothers were divided according to their A B O group and each group was considered separately. Table

I shows the number of mothers, pregnancies, and erythroblastotic babies in each blood group.

GROUP	A	B	O	AB	Total
Mothers	35	8	49	8	100
Preg.	95	19	129	19	262
EBF Babies	6	3	10	0	19

TABLE I. Grouping according to blood group of mothers, pregnancies, and Erythroblastotic babies.

The nineteen babies with Erythroblastosis Fetalis represent 7.25 per cent of the 262 total pregnancies in the group. In all cases except two; the three erythroblastotic babies of group B and the lack of any erythroblastotic babies in group AB; the figures compare closely with what would be expected. Using the Chi square formula on this table, with the Yates correction, Chi square is 1.25 and P equals seven tenths. This shows that the variations are not significant.

GROUP A

In table II the data compiled on the thirty-five mothers with group A blood is shown. The information included here is; the number of pregnancies of the different mothers, the pregnancies in which the erythroblastotic babies were born.

GRAVIDA NUMBER	1.	2.	3.	4.	5.	Total
Erythroblastotic babies	0	3	1	1	1	6
Rising Titre in Mother EBF in baby	0	1	1	0	1	3
Rising Titre in Mother No EBF in baby	0	0	1	0	0	1
Total Gravida of the Mothers	3	15	11	2	2	34

TABLE II. Erythroblastotic babies, rising titres, and total gravida of mothers in blood group A.

In the ros, total gravida of the mothers, in Table II, there was one mother not shown. She was a gravida six. All of the children were normal. The husband in this case was heterozygous Rh positive.

In this group there were thirty-five mothers. There were six babies with Erythroblastosis Fetalis, an incidence of 6.3 per cent of the ninety-five pregnancies in the group. These six babies were all of different mothers, these representing 17.8 per cent of the thirty-five mothers. The fathers in the erythroblastotic group were all Rh positive; half were homozygous and half heterozygous. In three of the eases of erythroblastotic infants a rising titre was demonstrated in the mother prior to delivery. It is also interesting to note that in one case a rising

antibody titre was seen in the mother, but the child did not demonstrate Erythroblastosis Fetalis.

GROUPS B AND AB

Similar data as in Table II is shown in Table III for the mothers in glood groups B and AB which were combined due to the small numbers involved. There were only eight mothers in each group, each group having nineteen pregnancies. The only large diference in the two groups was in the number of babies demonstrating Erythroblastosis Fetalis. By working the Chi square formula on Table I, it has already been shown that this is not significant.

GRAVIDA NUMBER	1.	2.	3.	4.	5.	Total
Erythroblastotic babies	0	2	1	0	0	3
Rising Titre in Mother EBF in baby	0	0	0	0	0	0
Rising Titre in Mother No EBF in baby	0	0	1	0	0	1
Total gravida of the Mothers	1	9	5	1	0	16

TABLE III. Erythroblastotic babies, rising titres, and total gravida of mothers in blood groups B and AB.

In this group of thirty-eight term pregnancies, three babies demonstrated Erythroblastosis Fetalis. This represented 7.8 per cent of the babies. Three of the mothers in this group of sixteen had erythroblasto-

tic babies, or a per centage of 18.75. Of the three erythoblastotic babies two had fathers who were heterozygous Rh positive. In only one case was a rising antibody titre demonstrated in the mother before delivery. In this one case the baby was not erythroblastotic.

GROUP O

This is the largest of the four groups in this series. The forty-nine mothers in this group represent nearly half of the mothers in the entire series of one-hundred mothers. Table IV contains data on the mothers with group O blood.

GRAVIDA NUMBER	1.	2.	3.	4.	5.	Total
Erythroblastotic babies	1	3	5	1	0	10
Rising Titre in Mother EBF in baby	1	1	1	0	0	3
Rising Titre in Mother No EBF in baby	0	0	1	0	0	1
Total gravida of the m mothers	5	21	13	7	3	49

TABLE IV. Erythroblastotic babies, rising titres, and total gravida of mothers in blood group O.

The O group included forty-nine mothers, of which, seven, or 14.28 per cent, had erythroblastotic babies. The ten babies with Erythroblastosis Fetalis represented 7.75 per cent of the total of 129 pregnancies. Five

of the ten babies had fathers who were heterozygous Rh positive. In three of the pregnancies resulting in an erythroblastotic baby a rising antibody titre was demonstrated in the mother. A normal infant was born in one instance where a rising antibody titre was seen in the mother during her pregnancy.

Probably the most important single fact in this group is the erythroblastotic baby in the first pregnancy. The father of this baby was a group O Rh positive heterozygous. A previous history of blood transfusions was present in this patient. In the case under consideration the agglutinin test was negative in a dilution of 1:5, the conglutinin test was positive in a dilution of 1:160, and the Coombs' test was positive in a dilution of 1:1280. The pregnancy ended spontaneously at five and a half months. This is a good illustration of the type of phenomonen Coombs and his co-workers (18) noted. Here the titre with the Coombs' test was high, and at the same time the other titres were low.

TOTALS OF ALL GROUPS

Table V shows the totals for all four A B O groups for the number of mothers having erythroblastotic babies, and the number of cases in which a rising

titre was demonstrated.

GROUPS	A	B	AB	O	Total
Mothers with Erythroblastotic babies	6	3	0	7	16
Rising titre in mother, EBF in baby	3	0	0	3	6
Rising titre in mother, No EBF in baby	1	0	1	1	3

TABLE V. Totals of mothers having erythroblastotic babies, and rising titres.

Sixteen per cent of the group of one-hundred Rh negative mothers had babies that were erythroblastotic. Those women having a normal child after one which was erythroblastotic all had husbands who were heterozygous Rh positive. In nine of the 262 pregnancies a rising antibody titre was demonstrated in the mother.

Table VI reports the relationship between the number of pregnancies, and the pregnancy in which the erythroblastotic infants occured.

The information given in Table VI shows that the per centage of erythroblastotic infants in each pregnancy group correlated well with the per centage of the mothers in that group. The only unexpected fact was the 5.21 per cent in the first pregnancy, where there

would ordinarily have been none.

GRAVIDA NUMBER	1.	2.	3.	4.	5.	Total
Erythroblastotic babies	1	8	7	2	1	19
Per cent of all EBF babies	5.21	42.17	36.78	10.63	5.21	100
Total gravida of mothers	9	45	29	10	6	99

TABLE VI. Relationship between pregnancies, erythroblastotic babies and the total gravida of the mothers in all four blood groups.

Ten of the nineteen babies demonstrating clinical erythroblastosis fetalis had fathers who were heterozygous Rh positive.

Although it is well known that a true abortion rate is almost impossible to determine, there were twenty-seven known abortions in this group of women. The term abortion here refers to the expulsion of a non-viable fetus. The known rate in this series is then 10.3 per cent, which could not be considered significant.

SUMMARY

A series of one-hundred Rh negative women was studied. All of these women had Rh positive husbands, and all of them had at least one pregnancy. An analysis of the pregnancies of these women was

undertaken and various facts were determined. It was found that: 1. The average number of pregnancies per woman in the series was 2.62; 2. In the 262 pregnancies occuring in the series, nineteen infants showed clinical evidence of erythroblastosis fetalis, an incidence of 7.25 per cent; 3. there was no significant difference in the number of erythroblastotic babies born to mothers of the different A B O blood groups; 4. sixteen women in the series gave birth to infants which were erythroblastotic, an incidence of sixteen per cent; 5. one woman in the series gave birth to an infant with erythroblastosis fetalis in her first pregnancy; 6. in this series there was a known abortion rate of 10.3 per cent.

CONCLUSION

Nelson (19) states that about eleven marriages out of one-hundred occur between Rh negative women and Rh positive men. If one should theorize on the basis that this group of 262 pregnancies represented eleven per cent of all pregnancies in a hypothetical series of marriages picked at random, one-hundred per cent would equal 2,382 pregnancies. In such a hypothetical group, the incidence of erythroblastosis

fetalis would be 0.798 per cent of all of the pregnancies. If this should be recalculated excluding the abortions in this group the incidence would still be only 0.889 per cent.

The incidence of sixteen per cent of Rh negative women in this series who gave birth to one or more erythroblastotic infants is relatively high, and re-emphasizes the point that all obstetrical patients should be typed for the Rh factor. Those who are Rh negative and have Rh positive husbands should be tested for anti-Rh antibodies. This is necessary so that proper precautionary measures may be taken for the infant at birth.

Another point to be re-emphasized here is that no Rh negative person should ever be given an Rh positive blood transfusion. This is demonstrated by the woman in this series who had previously been transfused with Rh positive blood, and subsequently, her first pregnancy resulted in an infant showing clinical erythroblastosis fetalis.

The question posed in the introduction can be answered in this manner: the incidence of the disease in children of marriages between Rh positive men and Rh negative women is relatively low, less

than eight per cent; however, in this series the incidence of women who gave birth to erythroblastotic children was sixteen per cent.

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