GENETIC AND NONGENETIC VARIATION IN THE AB0 AGGLUTININ LEVELS OF PLASMA, SALIVA AND MILK

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The agglutinin levels of 250 parturient women and their newborn babies were studied and the modifying influence of 16 variables evaluated. The most important factor in this variability is the AB0 phenotype. The agglutinin titers are generally higher in milk than in plasma or saliva. Blacks always show salivary agglutinins in higher frequencies than Whites. Within each fluid the amount of anti-A and anti-B are always highly correlated (r *0.59-0.79*). *Associations were also observed between the titers in plasma and milk* (r *0.25-0.30*). *The plasma and milk anti-A levels of the 0 mothers are correlated with those of their 0 children* (r *0.28-0.37*), *but the anti-B are not. Socioeconomic conditions may affect the salivary anti-A and anti-B titers.*

INTRODUCTION

The possible causes of the variation found in the AB0 agglutinin levels in organic fluids are not well-known, the suggestions presented so far being the subject of discussion for some time (review in Race and Sanger 1975).

Recently, Grundbacher (1976) investigated the influence of some factors in the seric levels, concluding that 20-30% of the total phenotypic variability is genetic; but 50% of this variation remained unexplained. In saliva, the results are few in number and sometimes conflicting (Callegari et al. 1972), but the influence of the AB0 phenotype in the frequency of the agglutinins found is well established (Jakobowicz et al. 1966, Boettcher 1967, Denborough et al. 1967), as well as the occurrence of seasonal fluctuations (Otten 1966). In milk, AB0 agglutinins have been found in varying amounts (Marrack 1948, Tomasi et al. 1965, Denborough et al. 1967) and their frequencies differ depending on the time that elapsed between delivery and the study (Prokop and Uhlenbruck 1969). The AB0 blood group phenotype is also important (Denborough et al. 1967) but other possible sources of variation were not studied. On the other hand, the agglutinin levels of newborn children vary according to their mothers' blood groups (Denborough and Downing 1969, Fong et al. 1974), but more detailed analyses of the possible importance of other factors are scarce.

For these reasons, we decided to undertake a quantitative and comparative study of the levels of these agglutinins in plasma saliva and milk in a sample of parturient women and their children living in Porto Alegre. Results about the ABH and Le^a antigens secured in the same investigation were presented in another paper (Barrantes and Salzano 1978).

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MATERIALS AND METHODS

Blood, saliva and milk were obtained from 250 parturient women and cord blood from their newborn babies in the Hospital da Santa Casa de Porto Alegre. The sample of mothers was composed of 139 White and 111 Black women, with ages distributed in an interval between 14 and 42 years. Their socioeconomic level was classified as low or medium. The newborn children were considered on phenotypic grounds to be 135 White and 115 Black. The collections took place between September 1975 and August 1976.

The cord bloods were obtained in the delivery room immediately after the births of the children and directly from the umbilical cords. Five ml were collected in 75 × 15 mm tubes with ACD (formula A, 0.15 ml per ml of blood). Afterwards, at the laboratory, the plasmas were separated from the blood cells and the former stored at —20°C until testing. The AB0 and Rh blood groups were then determined. The antisera employed were anti-A, -B, -D, -A_1 (*Dolichus biflorus* lectin), all from Johnson and Johnson of Brazil; and anti-H from *Ulex europaeus* extracts prepared in our laboratory.

The samples from the blood, saliva and milk of the mothers were collected 48-60 hours after delivery, always between 10 and 11 a.m. The bloods were obtained by venipuncture and treated in exactly the same way as those of their children. The salivas were collected in paper cups without stimulation and subsequently divided in two portions. One was immediately placed in the deep freezer at —20°C for the agglutinin studies, while the other was boiled during 20 minutes before storage. The latter was used for the ABH and Le^a antigen determinations. The milk, collected directly from the mammary gland, was placed in 15 × 75 mm vials, centrifuged at 2,000 r.p.m. for 5 minutes and divided in two portions that were treated in the same way as the salivas. No differences were observed, however, between the tests performed with boiled or unboiled milk. Both saliva and milk were centrifuged at 2,000 r.p.m. for 3 minutes before testing.

For the titration studies, double serial dilutions were made in saline using twelve 75 × 10 mm tubes, to which a 2% red cell suspension was added. For the anti-A and anti-B studies bloods from B and A_1 donors, respectively, were used. The mixtures were kept at room temperature for 20 minutes and then centrifuged at 1,000 r.p.m. during 1 minute. The readings were macroscopic and the titer was considered as the last dilution showing agglutination. The strength of the latter was measured according to a scale ranging from zero to four. A control with a known titer and score was always tested at the beginning and at the end of each working session.

In the statistical treatment of the data the values of the titers and scores were transformed into logarithms of base two and ten respectively. The concomitant information obtained and analyzed using stepwise multiple regression analysis were, in the mothers: race, AB0 and Rh blood groups, ABH secretor status, date of collection, age, socioeconomic condition, parity, number of abortions and gestation length. In the newborn children: race, sex, birth weight, neonatal examination (Apgar and presence or absence of jaundice), mother's and their own AB0 and Rh blood groups, and date of collection. All the data were codified and processed in a Burroughs B-6700 electronic computer of the Centro de Processamento de Dados of our university.

RESULTS

The frequencies (in percentages) of the AB0 and Rh blood groups, as well as the ABH secretor trait in saliva and milk, in the samples studied, are as follows.

Mothers. Whites (N = 139): 0 44, A_1 32, A_2 9, B 10, A_1B 4, A_2B 1, Rh(+) 86, ABH Sec. 87. Blacks (N = 111): 0 50, A_1 21, A_2 8, B 18, A_1B 3, Rh(+) 91, ABH Sec. 82.

Newborn children. Whites (N = 135): 0 53, A 35, B 13, Rh(+) 87. Blacks (N = 115): 0 52, A 39, B 6, AB 3, Rh(+) 93.

There are no significant differences within each ethnic group between these frequencies in the mothers and their children; and the values agree with previous studies done in Porto Alegre (Salzano 1963, Salzano et al. 1967, Palatnik et al. 1969). Since the findings employ-

ing scores were almost always concordant with those obtained using titers, only the last set of data will be presented here. Information concerning the whole analysis is available on request.

Table 1 summarizes the results on the agglutinin levels in the plasma, saliva and milk of the individuals tested. In the mothers the plasmatic levels vary depending of the ABO phenotype. The higher values are present in O persons, their anti-A titer being significantly higher than that of B individuals (5.7 and 6.9 respectively; $p < 0.001$). Within O persons, anti-A occurs in larger amounts than anti-B (6.9 and 6.3; $p < 0.001$). These findings are similar to those obtained elsewhere (Grundbacher 1967, 1976) despite the fact that differences occur in the average titers of the series investigated. In the babies, the mean levels are always lower, but show the same relationships as those present among the adults. The only racial difference observed was in a higher titer of anti-B in A persons among the Black, compared to the White mothers of the same blood group (6.6 ± 1.5 and 5.8 ± 1.2 respectively; $p < 0.01$). Therefore, the results are presented in the table irrespective of ethnic group. As is shown in Table 1, generally the agglutinin levels are higher in milk than in plasma or saliva, independently of the person's blood group. These results are different from those

Table 1. *Agglutinin levels in the plasma, saliva and milk of the individuals tested*

Blood group	Agglutinins	Plasma-mothers			Plasma-children			Saliva-mothers			Milk-mothers		
		N	Titer (log 2) *m*	*s*	N	Titer (log 2) *m*	*s*	N	Titer (log 2) *m*	*s*	N	Titer (log 2) *m*	*s*
A	anti-B (A_1)	67	6.2	1.4	—	—		24	1.1	0.9	67	6.7	2.5
	anti-B (A_2)	22	5.9	1.3	—	—		10	1.2	0.5	22	6.5	2.1
	anti-B (Total)	89	6.1	1.4	45	1.3	1.4	34	1.0	1.2	89	6.6	2.4
B	anti-A	34	5.7	1.2	14	0.8	0.7	11	1.5	1.5	34	6.5	1.9
0	anti-A	117	6.9	1.2	92	2.1	1.5	83	1.3	1.1	117	8.1	1.9
	anti-B	117	6.3	1.2	93	1.5	1.4	83	1.5	1.0	117	7.4	2.2

Note: Since the cord bloods do not react well with *Dolichus* and *Ulex* extracts, the classification of the newborn children for the A subgroups was not performed.

obtained by Schicke et al. (1965) and Denborough et al. (1967), who observed lower titers in milk than in plasma. It is noteworthy that in the three organic fluids studied the anti-B levels of O individuals are similar to those of A persons, while the average titers of anti-A are, with one exception, always higher in O as compared to B subjects.

Information about the presence of agglutinins and their levels in the plasmas of the newborn children according to the blood groups of their mothers is shown in Table 2. Considering both the frequency of appearance and the titer, the combination O mother-O child is the one that shows the largest amount of agglutinins. This variation due to the mothers' blood groups was also found by Denborough and Downing (1969), Toivanen and Hirvonen (1969) and Fong et al. (1974); detailed comparisons with these studies are hindered by differences in the way the data were analyzed, but the frequencies of agglutinins present were always higher in Porto Alegre. In the combinations mother A-child O and mother B-child O, we observed anti-A and anti-B that could have only been produced by the baby. Similar observations were made by Jakobowicz et al. (1967) and Thomaidis et al. (1969).

Table 2. *Presence of agglutinins and their titers in the plasmas of the newborn children according to the blood groups of their mothers*

Blood group of the mother	Blood group of the child	Number studied	Anti-A			Anti-B		
			Frequency (%)	Titer (log 2)		Frequency (%)	Titer (log 2)	
				m	*s*		*m*	*s*
0	0	84	98	2.2	1.5	91	1.5	1.4
0	A	28	—			79	1.5	1.5
0	B	5	100	1.2	0.8	—		
A	A	51	—			45	1.1	1.3
A	0	33	9	1.3		49	1.3	1.4
A	B	3	0	0.0		—		
B	B	14	57	0.5	0.5	—		
B	0	14	50	1.0	0.8	7	1.0	
AB	A, B, AB*	10	0	0.0		0	0.0	

* Eight A, one B and one AB.

Table 3 presents the data concerning the occurrence of agglutinins in saliva, when we consider the race and blood groups of the individuals studied. Blacks always show higher frequencies than Whites (as ascertained by chi-square analysis, four contingency tables, $z = 2.30$; $p < 0.03$). On the other hand, 0 persons present higher frequencies than subjects with other blood groups, confirming previous investigations (Wilson and Green 1964, Boettcher 1967, Denborough et al. 1967, Phansomboon 1968, Callegari et al. 1972). There is evidence that A_2 individuals show a higher titer of agglutinins in saliva than A_1 persons (Boettcher 1967) and our results are in the same direction, but the differences found here are not statistically significant. Pooling all of our data, the frequency of 60% for the occurrence of salivary agglutinins is obtained. Other investigators observed higher or lower prevalences, but the reasons for this variability have not been completely defined. They probably are mainly physiological, environmentally induced, or purely technical (Otten 1966, Bell and Fortwengler 1971). As for the milk results, the frequency of agglutinins was always 100% in 0, A or B individuals, independently of race.

The correlation coefficients between the agglutinin titers in the plasma, saliva and milk of the group 0 mothers, and between them and those observed in the plasma of their 0 babies, are shown in Table 4. Starting with the within-fluid comparisons, it will be seen that the anti-A and anti-B levels are always highly correlated (0.59-0.79). As for the interfluid asso-

Table 3. *Presence of salivary agglutinins according to blood groups and race*

Blood group	Agglutinin	Whites		Blacks		Total	
		N	%	N	%	N	%
A	anti-B	57	32	32	52	89	40
A_1	anti-B	44	30	23	48	67	36
A_2	anti-B	13	38	9	56	22	46
B	anti-A	14	29	20	35	34	32
0	anti-A	61	64	56	79	117	70
0	anti-B	61	67	56	75	117	70

ciations, the most consistent relationships are observed between the titers in plasma and milk (0.25-0.30), a result that contradicts that reported by Schicke et al. (1965). The correlations between saliva and milk or plasma are lower and negative in sign. The plasma and milk anti-A levels of the mothers are correlated with those of their children (0.28-0.37), but the anti-B are not.

The results of an analysis performed on 51 A mother/child pairs yielded a similar result for the relationship between the mothers' anti-B of plasma and milk ($r = 0.31$; $p < 0.05$). The titers of this agglutinin in these persons are negatively correlated in saliva and milk (—0.28; $p < 0.05$), as well as in saliva and plasma (—0.31; $p < 0.05$). The results described in the last paragraph and this one agree with those reported by Denborough et al. (1967) with one exception: they observed positive correlations between the levels in saliva and milk of both group 0 and A women. It should be mentioned, however, that they measured these levels using scores, not titers. Our results with scores show positive, but not significant correlation coefficients between the values in these two fluids. The mother-child comparisons yielded nonsignificant numbers.

Table 4. *Correlation coefficients between the agglutinin titers in the plasma, saliva and milk of a sample of 84 group 0 women and their 84 group 0 newborn children*

Nature of the sample	Agglutinin	Mothers					Children	
		Plasma	Saliva		Milk		Plasma	
		anti-B	anti-A	anti-B	anti-A	anti-B	anti-A	anti-B
Mothers								
Plasma	anti-A	0.59***	—0.06	—0.05	0.30**	0.29**	0.28**	0.12
	anti-B		—0.19	—0.24*	0.26*	0.25*	0.12	—0.02
Saliva	anti-A			0.60***	—0.09	—0.25*	0.09	0.18
	anti-B				—0.13	—0.21*	—0.07	0.17
Milk	anti-A					0.79***	0.37**	0.26*
	anti-B						0.30**	0.19
Children								
Plasma	anti-A							0.36**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 5 gives information about the multiple regression analysis performed to verify the possible influence on the mothers' agglutinin titers of the four genetic and six nongenetic variables listed. The most consistent result is the influence of the ABO phenotype. Socio-economic conditions may affect the salivary anti-A and anti-B levels.

Similar calculations were done considering the babies' agglutinin titers and the ten variables mentioned in the material and methods section. The only consistent result, that explains most of the variation observed, was blood group of the mother (Anti-A: $b = -0.82$, $F = 133.98$, $p < 0.001$; anti-B: $b = -0.43$, $F = 33.53$, $p < 0.001$).

DISCUSSION

Independently of the fluid studied, the ABO phenotype is the most important factor influencing agglutinin levels. The titers we observed in milk are always higher than those obtained in plasma or saliva. But it should be pointed out that the amount of these substances in

Table 5. *Multiple regression analysis performed to identify the variables that would influence the agglutinin titers of a sample of adult women*

Independent variable	Plasma		Saliva		Milk	
	anti-A	anti-B	anti-A	anti-B	anti-A	anti-B
Age	ns	ns	ns	ns	ns	ns
Parity	—1.87*	ns	ns	—5.97***	ns	ns
Abortions	ns	ns	ns	ns	ns	ns
AB0 blood group	0.30***	ns	—0.70***	—0.28***	0.33***	0.10*
Date of collection	ns	ns	ns	ns	ns	—0.47*
ABH secretion	ns	ns	—2.75***	ns	ns	ns
Socioeconomic level	ns	ns	—1.74***	—0.94*	ns	ns
Gestation length	ns	ns	ns	ns	ns	ns
Color	ns	ns	ns	ns	ns	ns
Rh blood group	ns	ns	ns	ns	ns	ns
ANOVA N	88	121	88	121	88	121
F	2.59	1.16	3.51	2.29	1.04	1.19
P	<0.01	>0.10	<0.005	<0.02	>0.20	>0.20

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
ns = nonsignificant.

milk show a tendency to lower as time elapses from the child's birth (Schicke et al. 1965, Prokop and Uhlenbruck 1969). This seems to be the main cause for the discrepancies encountered among series in the frequency with which agglutinins appear in this fluid, as well as their average titers. We always collected the samples two days after delivery, and they should have included colostrum, which is rich in antibodies for the protection of the child against infections (Campogrande and Trompeo 1970). Schicke et al. (1965) and Denborough et al. (1967) observed lower titers than those reported here, but they collected their samples 3 to 14 days after delivery.

Grundbacher (1967, 1976) found that the following factors would affect plasmatic agglutinin titers: sex, age, race, AB0 blood groups and secretor status. We have been able to clearly confirm the influence of the AB0 phenotype only. In our sample, significant racial differences occurred exclusively in the anti-B of A subjects. But there are several differences between Grundbacher's series and ours with respect to size, age and sex composition. Specifically in relation to age, the variation in our sample (14-42 years) was much smaller than that of his investigation (5-95).

As for the salivary agglutinins, besides the AB0 phenotype effect we detected variable frequencies according to race, and an effect of the socioeconomic conditions in their levels. The latter can be explained if we consider that persons of low status would be more exposed to several kinds of infection and that the IgA immunoglobulins that form the salivary agglutinins have the function of reacting against this type of stimulation (Kraus and Konno 1963, 1965, Brandtzaeg et al. 1970, Fudenberg et al. 1972). It is possible that the racial differences observed could be explained in the same way, since in Porto Alegre Blacks are generally more exposed than Whites to these infectious agents.

The average agglutinin titers of saliva and milk are very different; this is not surprising if we consider that the IgA immunoglobulin, of the same type in both secretions, is produced in lower amounts in saliva (Brandtzaeg et al. 1970). As for the plasma, IgM and IgG predominate, and the agglutinins in this fluid are to a large extent IgM. This would explain

the poor correlation between the plasmatic and salivary levels. The association found between the titers in plasma and milk could be due to the fact that in the latter there are larger amounts of IgM and IgG (Brandtzaeg et al. 1970), since it is known that the milk IgA is produced by local, relatively autonomous synthesis (Tomasi et al. 1965, Tomasi 1969, Brandtzaeg et al. 1970, Fudenberg et al. 1972).

The positive correlations observed between the plasmatic agglutinins of mothers and their newborn children are of course expected, since the majority of those found in cord blood are of maternal origin. The association between the titers observed in the mothers' milk and those found in the babies may be just a consequence of the primary correlation between the maternal milk and plasmatic levels.

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