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Haptoglobin development in newborn infants from diabetic mothers

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Abstract. Haptoglobin (Hp) development during the neonatal period has been studied in 325 newborn infants from normal pregnancies and in 242 infants from diabetic mothers. In infants from diabetic mothers Hp development is delayed as compared to infants from normal pregnancies. This delay is associated with a change in the pattern of relationship between Hp development and the polymorphism of acid phosphatase (ACP1) (an enzyme which shows phosphotyrosine phosphatase (PTPase) activity). In infants from normal pregnancies who show ACP1 phenotypes with the highest activity, the appearance of Hp is accelerated as compared to other infants. In contrast, infants from diabetic pregnancies who have ACP1 phenotypes with the highest activity, show delayed Hp development.
Key words. Hp development; diabetic pregnancy; newborn.

The high incidence of malformations in infants of diabetic mothers suggests that in these mothers the maternal environment interferes with developmental programs[1]. In infants of diabetic mothers a delayed γ-β globin gene switch has been reported[2–4].

Haptoglobin (Hp) is a polymorphic alpha-2-globulin, which develops gradually during the first weeks of life. In normal infants its plasma concentration, very low at birth, rises steadily during the first 2–3 days of life[5–8]. Hp development has been proposed as a postnatal maturation index. It has also been suggested that this protein confers on the newborn infant a non-specific protection against bacterial invasion[8].

In the present paper we have searched for possible effects of maternal diabetes on Hp development during the first days of life. In addition, we studied the polymorphism of acid phosphatase locus 1 (ACP1), and looked for interactions between this polymorphism and Hp development. ACP1 shows phosphotyrosine phosphatase activity[9], and may have important functions in the modulation of cellular growth and proliferation and in the control of developmental parameters.

Materials and methods

325 consecutive newborn infants from normal pregnancies and 242 infants from diabetic mothers were studied. All mothers were living in Rome. Of the diabetic women, 106 had IDDM, 39 NIDDM and 97 gestational diabetes. Classification of diabetes was carried out according to the criteria of the Diabetes National Data Group[10]. Diagnostic and therapeutic criteria have been discussed in a previous paper[11].

The serum haptoglobin pattern was studied by starch gel electrophoresis: under our experimental conditions the minimum detectable serum Hp concentration was around 5 mg/dl, measured as hemoglobin binding capacity. This limit was established by serial dilutions of several adult plasma samples in which Hp concentration had been previously determined. By this semiquantitative method it was possible to classify subjects into two categories: those with an Hp concentration lower than 5 mg/dl and those with an Hp concentration higher than 5 mg/dl. The Hp pattern was determined in the 3rd day of life.

Acid phosphatase (ACP1) phenotype was determined according to Harris and Hopkinson[12]. In some normal infants it was not possible to study ACP1, thus there are numerical differences between data in tables 1 and 4.

In infants from both normal and diabetic mothers the phenotypic distribution of Hp (3-day-old infants with detectable patterns) and ACP1 (all infants) did not appear significantly different from Hardy-Weinberg expectations.

Results

Table 1 shows that the presence of sufficient Hp for it to be electrophoresis after the second day of life is more frequent in infants from normal pregnancies than in infants of diabetic women. The delay in Hp development is highly significant in the subgroup of infants with a gestational age of 37 weeks or more. For this reason we can exclude the possibility that the delayed Hp development in infants from diabetic mothers is due to the well-known increased incidence of pre-term deliveries in these subjects.

The delay of Hp development is identical in the three types of diabetes (see table 2) and shows no association with maternal glycemic levels during the last three

Table 1. The effect of diabetes in pregnancy on haptoglobin development.

	Newborn infants from: diabetic mothers	non-diabetic mothers
	All infants	
Hp present in the third day of life	122	221
Hp absent	120	104
% of infants with Hp detectable pattern	50.4	68.0
Association between Hp appearance and diabetic pregnancy: $\chi^2 = 17.2236$ (1 df) $p \ll 0.001$		
	Infants with gestational age ≥ 37 weeks	
Hp present in the third day of life	90	215
Hp absent	91	94
% of infants with Hp detectable pattern	49.7	69.6
Association between Hp appearance and diabetic pregnancy: $\chi^2 = 18.3125$ (1 df) $p \ll 0.001$		

Table 2. Hp development in relation to type of diabetes.

	Type of diabetes		
	Gestational	IDDM	NIDDM
Proportion of infants with detectable Hp pattern in the 3rd day of life	48.5%	51.9%	51.3%
Total no. of infants	97	106	39
Association between Hp appearance and type of diabetes:			
χ^2	df	p	
0.2529	2	N.S.	

months of pregnancy (see table 3). When these individuals with a detectable Hp pattern were considered, no significant difference in the proportion of different Hp phenotypes in normal infants and infants of diabetic mother was observed (data not shown).

Table 4 shows that the relationship between haptoglobin development and ACP1 phenotype depends on the type of pregnancy. In infants from normal pregnancies carrying the ACP*C allele (which is associated with the highest enzymatic activity), the appearance of Hp is accelerated as compared to infants with other ACP1 phenotypes. On the contrary, in infants from diabetic pregnancies carrying the ACP1*C allele, Hp development is delayed as compared to infants with other ACP1 phenotypes. In both normal and diabetic pregnancies the distribution of Hp types among 3-day-old infants with a detectable pattern was statistically independent of ACP1 phenotype (data not shown).

Discussion

The infant's intrauterine to extrauterine transition requires the rapid maturation of biochemical and physiological systems in order to cope with the new requirements of extrauterine life. The delay of Hp development and that of the γ-β globin gene switch are both strong arguments in favour of the hypothesis that the diabetic environment exerts disturbing effects on neonatal maturation.

Table 3. Hp development in relation to metabolic control of diabetes during pregnancy.

	Mean glycemic levels during the last trimester of pregnancy		
	< 120 mg/dl	≥ 120 mg/dl	unknown
Proportion of infants with detectable Hp pattern in the 3rd day of life	50.8%	50.6%	48.5%
Total no. of infants	124	85	33
Association between Hp appearance and metabolic control of diabetes:			
χ^2	df	p	
0.0578	2	N.S.	

Table 4. The effect of diabetes in pregnancy on the relation between acid phosphatase phenotype and haptoglobin development.

	Newborn infants from diabetic mothers			Newborn infants from non-diabetic mothers		
ACP1 phenotype	AA + BA	BB	CC + AC + BC	AA + AB	BB	CC + AC + BC
Hp present in the third day of life	52	61	9	95	79	28
Hp absent	39	65	16	35	57	5
% of infants with Hp detectable pattern	57.1	48.4	36.0	73.1	58.1	84.8

Interaction among ACP1 phenotype, haptoglobin appearance and diabetic pregnancy: (three way contingency table analysis by log-linear model)

G = 8.62 (2 df) $p < 0.02$

Association between haptoglobin appearance and ACP1:

	χ^2	df	p
in newborn infants from diabetic mothers	3.928	2	0.140
in newborn infants from normal mothers	11.872	2	0.003

Association between haptoglobin appearance and diabetic pregnancy:

	χ^2	df	p
in ACP1-AA and ACP1-AB phenotypes	5.408	1	0.020
in ACP1-BB phenotype	2.087	1	N.S.
in newborn infants carrying ACP1*C allele	12.656	1	0.0003

Haptoglobin is a member of the serine protease superfamily. The aminoacid sequence of the Hp-α-chain also shows significant similarity to the sequences of plasminogen and prothrombin[13]. Serum haptogobin levels increase during inflammation, as do those of other acute-phase reactants. Interleukin-6 is one of the most important mediators of these events[14,5]. Hp limits the utilization of Hb by adventitious bacteria, preventing life-threatening haemoglobin-driven bacterial infections[9]. A delayed Hp appearance might therefore increase the risk of such infections in newborns from diabetic mothers.

The interaction with ACP1 seems to be of particular interest, in view of previous observations suggesting a role of this genetic polymorphism in intrauterine and early extrauterine development[16–20]. ACP1 (EC 3.1.3.2) is an enzyme found in the cytoplasm of many tissues, and shows polymorphism, with three codominant alleles (ACP1*A, ACP1*B, ACP1*C) at a locus on chromosome 2[21,22]. There are considerable quantitative differences of enzyme activity among phenotypes of ACP1. Spencer et al.[23] found the following activities: ACP1-AA = 122.4, ACP1-AB = 153.9 ACP1-BB = 188.3, ACP1-AC = 183.8 and ACP1-BC = 212.3.

ACP1 is a member of a family of low molecular weight acid phosphatases present in human erythrocytes, in rat liver and in other tissues of humans and other animal species. These animal enzymes have sequence similarities to human ACP1[9,24–27].

Based on experimental evidence, two important functions have been suggested for ACP1: flavin-phosphatase activity and tyrosine-phosphatase activity[9,28–30]. Catalysing the conversion of flavin-mononucleotide (FMN) to riboflavin, ACP1 may have a role in regulating the cellular concentration of flavin-adenine-dinucleotide (FAD), the activity of flavoenzymes, and energy metabolism. As phosphotyrosine phosphatase (PTPase), the enzyme may have an important role in cellular growth regulation and in modulation of the glycolytic rate through the control of receptor activities[9,28,31–33]. Given that variants of ACP1 with different activities are common, this enzyme could have a significant role in regulating a large spectrum of cellular functions and developmental programs, thus explaining its association with the appearance of Hp, and other neonatal parameters.

It has been shown that folates and some products of intermediate metabolism inhibit ACP1 activity. This effect is genotype-dependent and is much more marked in carriers of the ACP*C allele (AC and BC)[34].

Thus the abnormal biochemical environment of diabetes may influence developmental parameters through modulation of ACP1 activity. The percentage of Hp positivity in the third day of life is observed to be in the order [AC + BC] > [AA + BA] > [BB] in normal infants, but changes to [AA + AB] > [BB] > [AC + BC] in infants from diabetic mothers, which also supports this hypothesis.

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