Relationship between Histochemical Alkaline Phosphatase Activity in the Placenta and Patterns of Serum Heat-stable Alkaline Phosphatase in Late Pregnancy

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YAMAGUCHI, R., NODA, K. and FUJIYAMA, T. *Relationship between Histochemical Alkaline Phosphatase Activity in the Placenta and Patterns of Serum Heat-stable Alkaline Phosphatase in Late Pregnancy.* Tohoku J. exp. Med., 1971, 105 (2), 129-135 — Alkaline phosphatase (AP) activity in the placenta was investigated histochemically in relation to the characteristic patterns of the heat-stable alkaline phosphatase (HSAP) in the serum of near-term women, which were classified in the previous report into four, *i.e.*, elevating, descending, flattening (plateau), and fluctuating (unstable). Most of the women who appeared entirely normal and showed either elevating or descending pattern in their HSAP activity, maintained a steady activity of AP in the chorionic epithelium of the placenta. On the contrary, most of the cases which are conceived as abnormal and showed fluctuating pattern in the HSAP activity revealed a decreased activity of AP in chorionic epithelium. Such decrease in the placental AP activity was observed predominantly in postdated women with the fluctuating pattern, but such relationship between AP activity in the placenta and the pattern of HSAP was less evident in cases with pregnancy toxemia. ——— alkaline phosphatase; placenta; placental function test

It has been known that heat-stable alkaline phosphatase (HSAP) contained abundantly in the serum of pregnant women originates from the chorionic epithelium and is heat-stable histochemically (Jensen *et al.* 1968). The present authors previously reported that the HSAP activity pattern, which was obtained through repeated examinations in the late pregnancy, might serve as an index of the placental function (Yamaguchi *et al.* 1968). This paper deals with the relationship between the patterns of HSAP in near-term women and the histochemical activity of alkaline phosphatase (AP) in the placenta.

MATERIALS AND METHODS

Materials were the serum in the late pregnancy and placenta from 26 women consisting of 12 normal, 7 postdated (43 weeks), and 7 toxemia cases.

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1. *Serum HSAP determination*: In women mainly over 36 weeks of gestation, 3 to 5 ml of blood was obtained from the cubital vein. Serum obtained after the natural separation was treated with heating in the water bath at 56°C for 30 min to inactivate other heat-labile isoenzymes of alkaline phosphatase. The activity of HSAP was then determined with a CicAPHOS kit which was a modification of the King and Kind's method. The examination was made 2 to 3 times a week in usual cases but more in specific cases. Patterns of HSAP activity were classified according to our classification reported previously: elevating, descending, flattening (plateau), and fluctuating (unstable) (Yamaguchi *et al.* 1968).

TABLE 1. *HSAP pattern and histochemical and histological*

Group	HSPA pattern	Histochem. AP activity			Histological findings					
		Peripheral villi	Stem villi	Inhomogeneity of AP activity among villi	Fibrinoid deposition	Microinfaret	Syncytial knot	Narrowing of intervillous space	Dilatation of intervillous space	Congestion of villous capillary
Normal pregnancy (12 cases)	Elevating	+	+	+	±	—	+	—	+	±
	Elevating	+	+	+	±	—	—	—	—	—
	Elevating	+	+	+	±	—	±	—	—	—
	Descending	+	—	+	±	—	±	+	—	—
	Descending	+	+	+	±	—	±	+	+	—
	Descending	+	+	+	±	—	±	+	+	—
	Descending	+	+	+	±	—	±	+	+	—
	Elevating	+	+	±	±	—	+	—	—	±
	Elevating	+	+	+	±	—	±	+	—	—
	Elevating	±	+	+	±	—	+	—	—	—
	Elevating	—	—	—	±	—	±	—	—	—
	Elevating	—	—	—	±	—	±	—	—	—

TABLE 2. *HSAP pattern and histochemical and histological*

Group	HSAP pattern	Histochem. AP activity			Histological findings					
		Peripheral villi	Stem villi	Inhomogeneity of AP activity among villi	Fibrinoid deposition	Microinfaret	Syncytial knot	Narrowing of intervillous spaces	Dilatation of intervillous space	Congestion of villous capillary
Postdate pregnancy (7 cases)	Fluctuating	—	+	—	±	—	±	—	+	±
	Fluctuating	+	+	—	+	—	±	+	—	—
	Flattening	+	+	—	+	—	±	—	—	—
	Fluctuating	+	±	—	+	—	±	—	—	—
	Descending	+	+	+	±	—	±	±	—	—
	Fluctuating	—	—	—	±	—	±	+	—	—
	Fluctuating	±	—	—	±	—	±	—	±	—

2. *Placenta*: Four chorio-decidual pieces, about 1 cm in size, were obtained from grossly normal portion of each placenta soon after delivery, referable to 12, 3, 6 and 9 on clock. Sections were made from each piece, and examined with the Gomori's histochemical stain for AP activity. The histological examination with hematoxylin-eosin stain was also performed with respect to the items shown in Tables 1-3. In applying the Gomori's staining procedure, the same substrate for incubation was used to obviate a variation in the staining effect. The histochemical and histological examinations were carried out without knowing of the HSAP results, and the results obtained were compared later with the HSAP data.

findings of the placenta in normal pregnancy group

by H-E staining				Pregnancy weeks	Birth weight of neonate (g)	Weight of placenta (g)	Neonatal asphyxia	Delivery and others
Mixing of ischemia and congestion in intervillous space	Hypovascularization of villous capillary	Increase of epithelium-capillary distance of villi	Thickening of basement membrane					
-	-	-	-	40	3,260	750	0	Spontaneous
-	-	-	-	41	2,660	420	0	Spontaneous
-	-	-	-	40	3,170	580	0	Spontaneous
-	-	+	-	40	3,320	600	0	Spontaneous
-	-	-	-	41	3,860	860	0	Spontaneous
-	-	-	-	40	3,320	530	0	Spontaneous
-	-	-	-	42	3,370	600	0	Vacuum extraction
-	-	-	-	41	3,380	700	0	Spontaneous
-	-	-	-	41	3,330	520	0	Spontaneous
-	-	-	-	41	2,740	430	0	Spontaneous
-	-	-	-	41	3,220	500	0	Spontaneous
-	+	+	-	41	3,220	600	0	Spontaneous

findings of the placenta in postdate pregnancy group

by H-E staining				Pregnancy weeks	Birth weight of neonate (g)	Weight of placenta (g)	Neonatal asphyxia	Delivery and others
Mixing of ischemia and congestion in intervillous space	Hypovascularization of villous capillary	Increase of epithelium-capillary distance of villi	Thickening of basement membrane					
-	-	-	-	43	2,790	700	1	Precipitated labor
-	-	-	-	43	3,880	870	1	Vacuum extraction
-	-	-	-	43	3,330	750	0	Spontaneous
-	-	-	-	43	3,470	680	0	Forceps (Fetal distress)
-	-	-	-	43	3,550	670	0	Spontaneous
-	-	-	-	43	3,140		0	Cesarean section
-	-	-	-	43	2,740	500	0	(CPD, fetal distress)
-	-	-	-	43	2,740	500	0	Spontaneous

TABLE 3. *HSAP pattern and histochemical and histological*

Group	Type of toxemia	HSAP patter	Histochem. AP activity			Histological findings					
			Peripheral villi	Stem villi	Inhomogeneity of AP activity among villi	Fibrinoid deposition	Microinfarct	Syncytial knot	Narrowing of intervillous space	Dilatation of intervillous space	Congestion of villous capillary
Toxemia of pregnancy (7 cases)	(AH)	Fluctuating	+	+	-	+	-	#	+	-	+
	(AH)	Fluctuating	+	±	-	#	+	#	+	-	-
	(Aoh)	Descending	+	+	-	+	-	+	+	-	-
	(AOH)	Flattening	+	+	-	#	-	-	-	-	-
	(AOh)	Elevating	#	+	-	+	-	+	+	-	#
	(oh)	Fluctuating	+	+	+	+	-	+	+	-	+
	(AoH)	Fluctuating	-	+	-	+	-	#	+	-	-

* Letters "A, O, H", and "a, o, h" represent the grades of symptoms of toxemia Obstetrics and Gynecology. A: albuminuria severe. a: same but mild. O: oedema

RESULTS

The results were summarized in Tables 1-3. All but 1 of 12 cases of the normal group had a steady activity of AP in the syncytium cells. Their HSAP patterns included none in fluctuating, all of 12 being shared by 8 in elevating and 4 in descending. In contrast to these, 4 of 7 cases of the post-dated group had the placental AP activity either almost negative or extremely weak. In their relation to the HSAP classification, 4 of 5 with the fluctuating pattern showed a decreased activity of placental AP. On the other hand, the placental AP activity was predominantly normal in the toxemia group; only 2 of the 4 with the fluctuating pattern in the total of 7 cases respectively showed a decrease in the AP activity distinctly and mildly.

The relationships between the patterns of HSAP and the histochemical AP activity in the placenta were summarized in Table 4. The AP activity higher than (+) in placental villi, at both stem and branch, was observed in 8 of 9 cases with the elevating, 5 of the 6 with the descending, all of the 2 with the flattening, but in only 2 of the 9 cases with the fluctuating pattern. The AP activity in these cases was kept fairly well as a whole, but the staining reaction of enzyme activity was not homogeneous in individual villi. This was evident in 7 of 9 cases with the elevating and 5 of the 6 with the descending pattern. In contrast to this, none of the 2 cases with the flattening and only 1 of the 9 with the fluctuating pattern showed the above evidence, and this suggested a possible relationship to a decreased metabolic activity of the placenta.

No relationship was observed in the placenta between histological findings and histochemical AP activity. Furthermore, the placental morphology had no

findings of the placenta in toxemia of pregnancy

by H-E staining				Pregnancy weeks	Birth weight of neonate (g)	Weight of placenta (g)	Neonatal asphyxia	Delivery and others
Mixing of ischemia and congestion in intervillous space	Hypovascularization of villous capillary	Increase of epithelium-capillary distance of villi	Thickening of basement membrane					
-	+	+	-	40	2,150	400	0	Spontaneous
-	+	+	-	38	1,020	180		Stillbirth (Autopsy: VSD)
-	+	-	-	28	610	140		Stillbirth (Mother: erythematodes)
-	+	+	-	33	880	280		Stillbirth
-	-	-	-	40	2,570	520	1	Spontaneous
-	-	-	-	40	3,560	680	0	Spontaneous
-	+	-	-	35	1,500	430	0	Spontaneous

according to the classification of the Committee of Toxemia of Japan Society of severe. o: same but mild. H: hypertension severe. h: same but mild.

TABLE 4. *HSAP pattern and histochemical AP activity*

HSAP pattern	Number of cases of AP activity higher than (+) both in peripheral and stem villi	Number of cases of inhomogeneous distribution of AP activity among villi
Elevating (9 cases)	8	7
Descending (6 cases)	5	5
Flattening (2 cases)	2	0
Fluctuating (9 cases)	2	1

meaningful relation to the HSAP patterns. Such incoincidence in histochemical and histological findings between two abnormal pregnancies suggested that the so-called 'placental dysfunction' was intrinsically different between the post-dated group and the toxemia group, eventually orienting separate course of fetal life.

DISCUSSION

There are several reports dealing with the activity of serum heat-stable alkaline phosphatase (Aoba *et al.* 1967; Curzen and Morris 1967, 1968; Levine *et al.* 1966; Messer 1967; Yamaguchi *et al.* 1968). We have previously emphasized the clinical importance of repeated examinations of HSAP activity in preference to a single test in each case (Yamaguchi *et al.* 1968). Thus, with serial values in our experiments, we classified the HSAP patterns into the following four groups; elevating, descending, flattening (plateau), and fluctuating (unstable). Among the 4 pattern groups the fluctuating pattern had a significantly high incidence in association with both acute and chronic (or latent) distress of the fetus.

The activity of AP in the placenta has been chemically and histochemically demonstrated to decrease after the calculated date of delivery (Thomsen 1955; Shiratori 1957; Humke 1962). The association of an increase in histochemical placental AP activity with pregnancy toxemia was observed by Thomsen (1955), but Noda (1960) noted that the AP activity in the placenta was almost unaffected by severe acute toxemia, only encountering some decrease in chronic and grafting type of toxemia. On the property of placental AP, Jensen *et al.* (1968) examined AP activity after treated with heat at 70°C, and claimed that AP in the syncytium cells was heat-stable, thus suggested that AP in chorionic epithelium would be a direct source of HSAP in the maternal blood.

In our present experiments, the histochemical AP activity in the placenta was found to have decreased markedly in the post-dated group, which agreed closely with other reports. In the toxemia group, however, individual differences were so great for indicating a general tendency. It was notable that most of the cases in the postdated group with the fluctuating pattern of HSAP revealed a decrease in histochemical AP activity of the placenta. In comparison with the normal and the postdated groups, the toxemia group was less clear in showing the relationships of the placental AP activity to the patterns of HSAP. To account for the difference noticed between the postdated and the toxemia groups in this investigation, the following factors can be considered as significant: First, the placenta in the postdated pregnancy is categorically considered as the 'aged' in contrast with the placenta in the toxemia as the 'damaged'. In the 'aged' placenta, decrease of AP activity appears to occur on an overall level, whereas in the 'damaged' placenta, the decreased AP activity in the damaged area conceivably bring about a compensatory increase of AP activity in the undamaged area. Second, there is a possibility that the Gomori's method, heat-treatment of serum, or CicaPHOS procedure, being used in this experiment, may have failed to distinguish correctly the 'placenta-specific' isoenzyme of alkaline phosphatase. Immuno-histochemical procedure may provide a more convincing explanation for this relation between the postdated and the toxemic placenta.

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