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Difference in the placental ferritin levels measured by a specific monoclonal antibody enzymoassay in preterm and term delivery

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SUMMARY

The use of a new monoclonal anitbody (MoAb) enzymoassay for the specific measurement of placental ferritin (PLF) enabled it to be quantitatively determined in the sera of pregnant women, women at full-term and preterm delivery, and in their newborns. High levels of PLF were measured in the sera of pregnant women at 17 weeks of gestation up to full-term delivery as compared with normal adult women who mostly lack PLF. The level of PLF in the serum was irrespective of the total ferritin level. In full-term newborns, PLF concentration was lower than that found in their mothers although it increased compared to healthy adults. It was further found that in the blood of women who delivered at 29–36 weeks, PLF was undetected or was very low. Accordingly their preterm infants also exhibited very low or undetectable PLF in their serum. These results suggest the possible use of PLF as a prognostic indicator in pregnancy.

Keywords placental ferritin monoclonal antibody ELISA pregnancy preterm delivery

INTRODUCTION

Ferritin is an iron-storage protein of wide distribution in eukaryotic cells. The relationship between ferritin levels as an indicator of iron stores in mothers and full-term newborns was previously investigated (Celada *et al.*, 1982). Some investigators found a correlation between the ferritin value in cord and mothers' blood (Fenton, Cavill & Fisher, 1977; Kelly, MacDonald & McDougal, 1978; MacPhail *et al.*, 1980) whereas others did not (Celada *et al.*, 1982; Bratlid & Moe, 1980; Hussain *et al.*, 1977; Jansson, Holmberg & Ekman, 1979; Rios *et al.*, 1975; VanEijk *et al.*, 1978). Celada *et al.* suggested that there is a preferential transport of iron from mother to the child irrespective of the maternal iron stores.

Multiple molecular forms of ferritin have been isolated from different tissues such as liver, spleen, heart and placenta. Ferritins derived from adult human heart and placenta as well as from several neoplasms show more acidic isoferritins on isoelectric focusing than liver and spleen ferritins (Drysdale, 1970; Drysdale *et al.*, 1971). Recently we developed two hybridomas (CM-G-8 and CM-H-9) which secrete monoclonal antibodies (MoAb) against human placental ferritin (Moroz *et al.*, 1985). It was found that CM-G-8 MoAb binds to placental, liver and spleen isoferritins whereas CM-H-9 MoAb binds to placental ferritin only. Using the two new MoAb we developed enzyme-

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Table 1. The levels of ferritin and PLF during pregnancy

Female blood donor	*n*	Ferritin (ng/ml)	PLF (u/ml)
Adult females	16	50·3 ± 59·8	4·5 ± 7·7
Pregnant 17–22 weeks	23	63·7 ± 48·9*	45·5 ± 52·9†
Pregnant 30–39 weeks	15	50·0 ± 39·4*	81·6 ± 89·3†
Term delivery	25	46·1 ± 37·3*	54·8 ± 53·0†
Preterm delivery	18	61·2 ± 29·4*	15·8 ± 15·7†‡
Amniotic fluid 17–22 weeks	15	86·4 ± 78·5	19·4 ± 8·2

* Not significantly different from adult female.

† Significantly higher than adult female ($P = 0{\cdot}0025$).

‡ Significantly lower than in pregnant women and women at term delivery ($P = 0{\cdot}02$).

The results are expressed as mean ± s.d.

linked immunosorbent assays (MoELISA) which enabled the quantitative determination of total ferritin and a specific placental ferritin (PLF) isoform (Moroz *et al.*, 1987). It was found that PLF was not detected in the majority of healthy adult blood donors whereas it was elevated in the sera of patients with lymphoproliferative diseases (Moroz *et al.*, 1987).

In the current study we determined the PLF levels in the serum of pregnant women, in women at term and pre-term delivery and in their newborns. In addition the results of PLF levels in the serum were compared to those of the total ferritin.

MATERIALS AND METHODS

Subjects. Twenty-five full-term newborns (14 males and 11 females) aged 38–41 weeks of gestation (mean weight 3350 ± 3000 g), 25 preterm newborns (12 males and 13 females) aged 26–36 weeks of gestation (mean weight 1820 ± 503 g) and their respective mothers were examined. The gestational age was calculated from the first day of the mother's menstrual bleeding preceding pregnancy and was confirmed by clinical examination (Dubowitz scoring). Twenty-three women at 17–22 weeks of gestation who underwent amniocentesis because of their age and fifteen women at 30–39 weeks of normal pregnancy were investigated. Forty healthy volunteers (16 females and 24 males) aged 20–40 years served as controls.

Methods. Blood was withdrawn from the umbilical cord of preterm and full-term newborns after ligation of the cord from the newborn side of the placenta. Concomitantly venous blood was obtained from the respective mothers. Amniotic fluid was withdrawn from pregnant women at 17–22 weeks of pregnancy and venous blood was collected from all other groups as shown in Table 1.

Monoclonal antibodies. MoAb CM-G-8 and CM-H-9 were produced against human placental ferritin as previously described (Moroz *et al.*, 1985). MoAb were obtained from ascites fluid following precipitation with 50% saturated ammonium sulphate solution. Placental ferritin used for standard was obtained following purification on DEAE-cellulose column as described previously (Moroz *et al.*, 1985). Liver ferritin standards were obtained from McELISA ferritin kits (Elias Medizin-technik GmbH, D-7800, Freiburg). The amount of placental ferritin which bound 250 pg of alkaline phosphatase conjugated CM-H-9 MoAb was considered as 10 units of PLF.

MoAb ELISA for PLF and common isoferritins. The enzyme-linked immunosorbent assays measuring the serum ferritin and PLF in the serum (MoELISA type A and MoELISA type B respectively) have been described (Moroz *et al.*, 1987). In both assays, the MoAb CM-G-8 which binds to all ferritins was coupled to the solid phase. For the second site CM-G-8 MoAb-enzyme conjugate was used in MoELISA type A and CM-H-9 MoAb-enzyme conjugate in MoELISA type B.

Table 2. The levels of ferritin and PLF in the sera of full-term and preterm newborns and their mothers

Group	*n*	Ferritin (ng/ml)*		PLF (u/ml)*	
Full term newborns	25	289·4 ± 167·5	$P = 0·0000$	20·3 ± 25·8	$P = 0·0075$
Mothers	25	46·1 ± 37·3		54·8 ± 53·0†	
Preterm newborns	25	208·2 ± 191·5	$P = 0·0005$	9·4 ± 15·1	$P = 0·018$
Mothers	18	61·2 ± 29·4		15·8 ± 15·7	
Blood bank donors	40	85·3 ± 65·9		8·1 ± 14·8	
Male	24	108·0 ± 58·0		10·0 ± 10·0	
Female	16	50·3 ± 59·8		4·5 ± 7·7	

* The results are expressed as mean ± s.d.

† Significantly higher than PLF in the sera of mothers of preterm newborns and adult female ($P = 0·02$, $P = 0·0025$ respectively)

The MoELISA type A and B were performed as follows: The wells of a microtitre plate (Dynatech m-129B) were coated with 150 μl CM-G-8 MoAb (100 μg/ml phosphate-buffered saline (PBS), pH 7·2) and incubated overnight at 4°C. The plate was washed three times with PBS-Tween (PBS, 0·05% Tween 20) and shaken dry.

Test sera (100 μl) diluted 1:2 in MoELISA type A and 1:4 in MoELISA type B in PBS-Tween 0·025%, were added in duplicates to the wells. Serum diluent and ferritin standards were also added in duplicates. A serum sample with an elevated ferritin concentration was diluted in the diluent to determine recovery at high dilution. The plates were incubated at 4°C for 1 h in MoELISA A and overnight in MoELISA B, washed three times with PBS-Tween and then 100 μl of alkaline phosphatase (AP) MoAb conjugate (0·4 μg) was added to each well. The plate was incubated further for 120 min at room temperature and washed again three times. The enzyme substrate (*p*-nitrophenylphosphate 1 mg/ml of diethanolamine buffer pH 8·0, 0·5 mM $MgCl_2$) was added and the reaction was stopped after 10–30 min by addition of 0·05 ml of 2 M NaOH. The amount of coloured product was measured by absorbance at 405 nm.

Statistical analysis. This was performed according to the Mann–Whitney *U*-test (non-parametric).

RESULTS

Serum levels of ferritin and PLF during pregnancy and at delivery determined by two different MoELISA assays. The mean concentration of total serum ferritin in pregnant women and in women at delivery was similar to that measured in the sera of adult blood bank female donors (Table 1). The mean concentration of ferritin ranged from 46 to 63 ng/ml as determined by MoELISA type A (Table 1). In comparison, pregnant women exhibited elevated serum concentration of PLF, as measured by MoELISA type B significantly higher than controls ($P = 0·025$, Table 1). It is noteworthy that 70% of the sera of the normal female tested contained no detectable PLF.

In this study it was found that high PLF levels were detected in the serum of women as early as 17 weeks of gestation (45·5 ± 52·9 u/ml). PLF levels did not change significantly during 30–39 weeks of gestation (81·6 ± 89·3) and were high at full-term delivery (54·8 ± 53). However, in women who had preterm delivery the concentration of serum PLF was significantly lower (15·8 ± 15·7 u/ml) than that measured in the sera of pregnant women at similar gestational period and in women at term delivery ($P = 0·02$) (Table 1). Measurement of ferritin concentrations in the amniotic fluid at 17–22 weeks of gestation revealed that the total ferritin concentration (86·4 ± 78·5 ng/ml) was not

significantly different than that of the serum (63·7 ± 48·9 ng/ml) whereas the PLF level (19·4 ± 8·2 u/ml) was significantly lower than that measured in the serum (45·5 ± 52·9 u/ml, $P = 0·02$).

Determinations of ferritin and PLF in the sera of term and preterm newborns. The mean concentration of total ferritin in the sera of full-term and preterm newborns (289·4 ± 167·5, 208·2 ± 191·5 respectively) was significantly higher than that measured in the sera of their mothers (46·1 ± 37·3, 61·2 ± 29·4 respectively, $P = 0·0005$, Table 2). Yet no difference in serum ferritin concentration was observed between term and preterm newborns (Table 2).

High PLF levels were detected in the sera of term newborns (20·3 ± 25·8 u/ml) as compared to normal healthy adults (8·1 ± 14·8 u/ml). However, the levels of PLF were significantly lower than that of their mothers (Table 2, $P = 0·0075$). In contrast in preterm newborns the serum PLF levels (9·4 ± 15·1) were low, similar to those of healthy adults but were significantly lower than those of their mothers ($P = 0·018$). The PLF levels in preterm newborns were not significantly lower than those found in term newborns ($P = 0·26$).

DISCUSSION

The development of a new ELISA (MoELISA B) for the specific determination of PLF enabled us for the first time to identify and measure placental ferritin. Using this assay it was found that PLF was undetectable in the serum of the majority of normal healthy women (Moroz *et al.*, 1987) yet it was exhibited in the serum of pregnant women (at 17 weeks of gestation up to full-term delivery). The level of PLF in the sera of pregnant women was irrespective of the total ferritin level measured by MoELISA A and was similar to that of healthy females. Since it is known that most of the pregnant women are iron-deficient, it is suggested that neither total ferritin nor the level of PLF in the serum can be used as an indicator of iron stores. This is in agreement with other studies which have shown no correlation between iron stores and serum ferritin levels (Celada *et al.*, 1982).

In the present study we found that total level of serum ferritin in the newborns was increased compared to that found in maternal blood. No significant difference in serum ferritin concentration was found between newborns of various gestational ages. For the entire group the mean ferritin level at birth was 250 ng/ml and this is compatible with other reports in which different assays were used for measurement of total serum ferritin (Halliday, Lappin & McClure, 1984; Puolakka, Janne & Vihko, 1980; Bruyere & Bienvenu, 1983). Contrary to total ferritin, the PLF levels in serum of term newborns were lower than the levels found in their mothers although increased compared to healthy adults. These results suggest that different regulatory mechanisms are involved in the production and secretion of the two ferritin isoforms identified in the current study.

The lack of the fetus rejection during pregnancy has been attributed to the presence of maternal serum factor(s) which may have an inhibitory effect on a variety of maternal immune processes.

Products such as human chorionic gonadotropin (HCG) or immunoregulatory substances such as oestrogen, progesterone, corticosteroids and pregnancy-associated growth factor (PAGF) are secreted by the placenta into the blood of pregnant women (Caldwell, Stites & Fudenberg, 1975; Fabris, Piantanelli & Muzzioli, 1977; Beer & Billingham, 1979; Siiteri, Febres & Clemens, 1977; Morse, Ehrlich & Canfield, 1982). Ferritin was demonstrated within placental syncytiotrophoblast particularly localized towards the microvillus plasma membrane (Brown *et al.*, 1979). It is conceivable that the placenta is a source of PLF found in the serum of both the pregnant women and the embryo. The significant difference in PLF levels between them may suggest a preferential secretion of PLF into the maternal circulation.

Rather surprisingly, it was found in the current study that in blood of women who delivered at early gestational age (29–36 weeks) PLF was undetected or was very low compared to the high levels of PLF found during pregnancy and full-term delivery. Although direct association between the preterm delivery and absence of PLF in the serum has not been proven, it is suggested that the finding of low PLF levels in the serum during pregnancy may serve as a prognostic indicator for early termination of the pregnancy which may result in preterm delivery or spontaneous abortions.

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