Comparison of placental isoferritin levels in maternal serum and coelomic and amniotic fluids during first trimester human gestation

R. Maymon¹, E. Jauniaux², C. Rodeck³, L. Zaspin³ and C. Moroz³, ⁴

¹ Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin (affiliated with Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv) Israel, ² Department of Obstetrics and Gynecology, University College, London Medical School, London, UK and ³ Molecular Immunology Unit, Felsenstein Medical Research Center, Rabin Medical Center (Beilinson Campus), and Sackler Faculty of Medicine, Tel Aviv University, Petach Tikva, Israel
⁴ To whom correspondence should be addressed at: Molecular Immunology Unit; Felsenstein Medical Research Center, Rabin Medical Center, Beilinson Campus, Petach Tikva 49100, Israel

Placental isoferritin (PLF) is present in serum at high concentrations throughout normal gestation. The current study compared PLF concentrations in first trimester maternal serum with those of amniotic and coelomic fluids in 25 healthy, pregnant women. Use of a specific enzyme-linked immunosorbent assay indicated concentrations high (22 ± 3 U/ml) in maternal serum, whereas significantly lower values were detected in both coelomic and amniotic fluids (5 ± 2 and 3 ± 2 U/ml, respectively, P < 0.005). It was further demonstrated that when pure PLF was added to amniotic and coelomic fluids, the PLF levels remained low in each compartment, suggesting that bioproducts produced inside the gestational sac may interfere with the PLF immunoassay.

Key words: amniotic fluid/coelomic fluid/placental isoferritin/pregnancy

Introduction

Isoferritin from placental tissue, mainly syncytiotrophoblasts (Brown et al., 1979), exerts immunosuppressive activity (Matzner et al., 1985), and is involved in the down-regulation of the maternal immune system during pregnancy (Sirot et al., 1989). Placental ferritin (PLF) can be measured by a specific enzyme-linked immunosorbent assay (ELISA) using the monoclonal antibody (MAb), CM-H-9, which binds exclusively to a 43 kDa subunit (P43) associated with PLF (Moroz et al., 1987). PLF values do not correlate with serum ferritin and therefore are not related to maternal iron stores (Maymon and Moroz, 1996). High serum PLF values have been measured in healthy, pregnant women (Moroz et al., 1987), whereas low values (<10 U/ml), have been observed in various pathological conditions of early pregnancy, such as spontaneous miscarriage (Fisch et al., 1996), incomplete abortions (Maymon et al., 1996), complete molar gestations (Maymon et al., 1995a), and tubal pregnancies (Maymon et al., 1995b). The aim of the current study was to further analyse the patterns of PLF in the coelomic and amniotic cavities during first trimester, and compare them with maternal serum at the same gestational age.

Material and methods

Healthy, pregnant women (n = 25) with apparently normal gestations between 7 and 12 weeks of pregnancy were enrolled into this study. All underwent termination of pregnancy for psychosocial reasons. In all cases, the fetal heart beat was ultrasonographically confirmed and the crown–rump length was within the normal range for the gestational age. Informed written consent was obtained from each patient before the surgical procedure, which was performed under general anaesthesia.

After micturation, the patient was placed in a lithotomy position and the vagina was cleaned using iodine solution. A 5 MHz transvaginal transducer (Aloka SSD-680, Aloka Co., Tokyo, Japan) covered with a sterile glove was then inserted into the vagina. Exocoelomic and amniotic fluids were aspirated through a 20-gauge needle by transvaginal puncture under ultrasonographic guidance, as previously reported (Jauniaux et al., 1991). Simultaneously, maternal blood samples were collected from the forearm vein prior to pregnancy termination. All samples were stored at –20°C until assayed.

PLF was isolated from term placenta as previously described (Parhami-Seren and Moroz, 1986). Its concentrations were measured by a specific ELISA using alkaline phosphatase conjugated CM-H-9 MAb, specific for P43 associated with PLF (Moroz et al., 1987). The enzymatic reaction was carried out by adding p-nitrophenyl phosphate substrate and the results were read at 405 nm. The amount of P43 that bound 250 pg alkaline phosphatase conjugated CM-H-9 was arbitrarily defined as 10 U of PLF. PLF values <10 U/ml were considered negative. Results were expressed as units per millilitre of serum. This cut-off level was based on receiver-operating characteristics constructed by Rosen et al. (1995) by plotting sensitivity versus specificity.

PLF values were not distributed normally and a Mann–Whitney test with α = 0.05 (Bonferroni) was applied to compare results between the samples. The results are presented as mean ± SD.

Results

The mean level of PLF in the maternal sera was 22 ± 3 U/ml, whereas significantly lower PLF concentrations (P < 0.005) were measured in both coelomic and amniotic fluids (5 ± 2 and 3 ± 2 U/ml, respectively).

The question was raised whether, during the first trimester of gestation, these latter compartments were devoid of PLF or if the low levels of PLF in these fluids could be attributed to the presence of bioproducts which might interfere with this immunoassay. Therefore, varying amounts of purified PLF of up to 4000 ng/ml were added to each of three different
coelomic and amniotic fluid samples. The mixtures were titrated in ELISA, and then compared with equal amounts of PLF dissolved in phosphate-buffered saline (PBS).

As shown in Figure 1, the threshold of detection of P43 (PLF) dissolved in PBS was 12.5 ng/ml, which equals 10 units. However, 100 ng/ml of PLF dissolved in PBS equals 80 units compared with 45 units when dissolved in amniotic fluid (~50% activity). When the same amount was added to coelomic fluids, it could not be measured. A gradual increase in PLF concentration to 4000 ng/ml yielded only 30 units of PLF in the coelomic fluid, which equals 30 ng/ml of PLF in PBS (~1% activity). These data indicate a remarkable interference of the coelomic fluid with the immunoassay.

Discussion

The current study revealed that, whereas during the first trimester of gestation, high concentrations of PLF were detected in maternal sera, its level was very low or below detection in samples of amniotic and coelomic fluids.

The coelomic fluid contains high concentrations of specific trophoblastic and secondary yolk sac proteins and several products from placentl metabolism, suggesting that the coelomic cavity is a physiological liquid extension of the villous mesenchyme acting as a reservoir for nutrients needed by the developing embryo (Jauniaux et al., 1994). With rare exceptions, the concentration of most placentl or secondary yolk sac proteins is higher in coelomic fluid than in either amniotic fluid or in maternal serum, and the transfer through the amniotic membrane separating the fluid cavities is limited (Jauniaux et al., 1994).

The synthesis of some of these proteins, such as α-fetoprotein, is confined to embryonic compartments with little transfer to the maternal compartment during the first trimester (Jauniaux et al., 1993). Interleukin-6 is also normally present in coelomic and amniotic fluids of early pregnancy, but is rarely detectable in maternal serum (Jauniaux and Gulbis, 1997), suggesting that it may be involved with the fetal haemopoiesis function, and in the generation of new vessels in placental villous tissue (Jauniaux et al., 1996). In contrast, PLF, acting as an immunosuppressive cytokine, is primarily secreted into the maternal compartment (Maymon and Moroz, 1996).

Since a number of studies, including ours, have demonstrated interactions between human sera, plasma proteins with tissue ferritins (Celada et al., 1982; Covell et al., 1984; Cazzola et al., 1985; Bellotti et al., 1987), the increased concentrations of P43 (PLF) in maternal serum indicate that they are above the serum absorption limit. In comparison with the maternal circulation during the first trimester of gestation, the low concentrations of PLF in coelomic and amniotic fluids might be attributed to a lack of secretion into these compartments. Alternatively, the lack of P43 (PLF) detection could be due to assay interference, or it might be rapidly degraded or coupled, thus rendering this protein nearly undetectable in the ELISA assay.

The current study indicated that coelomic fluid exhibited a remarkably high interference and/or absorption capacity for PLF, and therefore it could not be measured in this compartment. However, in amniotic fluid, a low P43 (PLF) absorption capacity was exhibited. Therefore, in this compartment, the levels of P43 (PLF), measured at 7–12 weeks of gestation, were primarily low. This is in agreement with our previous observations that higher P43 (PLF) concentrations (19.4 ± 8.2 U/ml) were measured in amniotic fluid at more advanced gestational ages (17–22 weeks) (Moroz et al., 1987). The late appearance of P43 (PLF) in amniotic fluid may be attributed to the redistribution of this protein after the fusion of coelomic and amniotic cavities. The difference in assay detection (in coelomic versus amniotic fluids) indicates the importance of assay validation for different biological samples. Furthermore, such differences rule out the possibility of an assay artefact, but rather reflect physiological significance which should be further investigated.

References


R.Maymon et al.


Received on June 4, 1997; accepted on December 12, 1997