Cytokine 114 (2019) 143–148

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Preadipocyte factor-1 in maternal, umbilical cord serum and breast milk: The impact of fetal growth

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ARTICLE INFO

Keywords:
Extremes of fetal growth
Adipokines
Preeclampsia
Gestational diabetes
Metabolic disorders

ABSTRACT

Background/Objective: To study the concentrations of preadipocyte factor-1 (Pref-1) -an inhibitor of adipocyte differentiation, implicated in adipose tissue metabolism, late metabolic disorders and fetal growth- in maternal and umbilical cord serum, as well as maternal milk and correlate above concentrations with intrauterine growth and other perinatal parameters.

Material and methods: Pref-1 concentrations were determined by ELISA in antepartum maternal and umbilical cord serum, as well as day 3 to 4 postpartum breast milk, deriving from 80 women, who delivered 40 appropriate (AGA), 20 large for gestational age (LGA) and 20 intrauterine growth restricted (IUGR) neonates, classified by the use of customized birth-weight standards adjusted for significant determinants of fetal growth.

Results: Umbilical cord serum Pref-1 concentrations were significantly higher than antepartum maternal ones ($p < 0.001$), while breast milk concentrations were the lowest ($p < 0.001$ concerning umbilical serum, $p < 0.001$ concerning maternal serum). Umbilical cord serum Pref-1 concentrations were significantly lower in the LGA group than in the AGA one ($p = 0.044$). Breast milk and maternal serum Pref-1 concentrations did not differ between the three intrauterine growth groups. Maternal serum and breast milk Pref-1 concentrations did not correlate with maternal age, body mass index before and after gestation, birth weight, body length, and customized centile. A positive weak correlation was recorded between maternal serum and milk Pref-1 concentrations ($r = 0.238$, $p = 0.034$).

Conclusions: Pref-1 concentrations in umbilical cord serum are higher than in antepartum maternal serum, probably pointing to its fetal origin and role in intrauterine growth. Breast milk concentrations, being extremely low, and possibly implying infant protection from metabolic disorders, positively correlate with maternal serum ones, conceivably suggesting a transfer of the substance from the circulation to the breast. Umbilical cord serum Pref-1 concentrations were lower in LGA fetuses/neonates, as compared to respective AGA ones.

1. Introduction

Preadipocyte factor 1 (Pref-1) -also referred as Delta-like 1 homologue (Dlk 1), Fetal antigen 1 (FA1), or Zona glomerulosa-specific factor (ZOG)- is a 385 amino acids protein, encoded by an imprinted (paternally expressed) gene. Pref-1 is a preadipocyte secreted factor synthesized as a transmembrane protein that undergoes proteolytic cleavage to generate two distinct soluble forms [1,2]. In vitro assays have demonstrated that only the large 50 kDa soluble form of Pref-1 is biologically active and inhibits adipocyte differentiation [1,2].

The expression of Pref-1 is very high in preadipocytes; however, it decreases as their differentiation proceeds, resulting to its absence in mature adipocytes [3,4]. Embryonic tissues, as liver, lung, tongue, hypophysis, developing vertebra, adrenals, pancreas, skeletal muscles and placenta highly express Pref-1 [3] reflecting its role in intrauterine development and growth [5]. In this respect, its serum levels [6] and its

Abbreviations: Pref-1, Preadipocyte factor-1; AGA, appropriate for gestational age; LGA, large for gestational age; IUGR, intrauterine growth restricted; SGA, small for gestational age

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https://doi.org/10.1016/j.cyto.2018.11.010
Received 18 August 2018; Received in revised form 7 November 2018; Accepted 12 November 2018
Available online 18 November 2018
1043-4666/

placental expression [7] rise as pregnancy progresses. Following birth, Pref-1 expression is lost from the majority of tissues and is limited to specific cells, like preadipocytes [3], thymocytes [8], pancreatic islet cells [9] and adrenal cells [10]. According to literature reports, Pref-1 exerts a strong inhibitory effect on angiogenesis [11] and soluble Pref-1 prevents the differentiation of multipotent mesenchymal cells into adipocytes, chondrocytes, and osteoblasts [12] thus, it inhibits, besides bone formation, adipogenesis [2].

Published data [13] refer to the importance of normal differentiation of adipose tissue and appropriate lipid storage, which prevents ectopic lipid deposition to non-adipose tissues resulting to insulin resistance. Therefore, Pref-1 by negatively regulating preadipocyte differentiation, on the one hand reduces adipose tissue mass, and on the other, worsens glucose control [14,15].

This study was based on the hypothesis that circulating Pref-1 concentrations in maternal and umbilical cord blood, as well as in maternal milk, might differ in pregnancies complicated by intrauterine growth deviations, taking into consideration Pref-1 implication in adipose tissue metabolism and fetal growth. Therefore, we aimed to determine Pref-1 concentrations in maternal and fetal blood, as well as maternal milk in cases of abnormal fetal growth and correlate Pref-1 concentrations with several perinatal parameters, such as maternal age, parity, gestational age, mode of delivery, birth weight, customized centile and neonatal gender.

2. Material and methods

Our study, which conformed to the Declaration of Helsinki, was approved by the Ethics Committee of “Alexandra” University and State Maternity Hospital and informed consent was acquired by participating mothers.

2.1. Study population

Our cohort has been previously reported [16].

During a six months period (October 2017-April 2018) we consecutively recruited 80 parturients and subsequently puerperal women, as well as their infants at birth. We excluded pregnancies complicated by perinatal infection, congenital malformations and preterm deliveries.

Participating women comprised three groups: those having delivered appropriate for gestational age (AGA, centiles 10–90) (n = 40), intrauterine growth restricted (IUGR, centiles < 10) (n = 20) and large for gestational age (LGA, centiles > 90) (n = 20) infants. Related causes to abnormal fetal growth were late mild preeclampsia [n = 3], mild pregnancy-induced hypertension [n = 3], smoking more than 10 cigarettes per day [n = 7], thrombophilia [n = 5], obesity [n = 6], gestational diabetes mellitus (GDM) [n = 6], excessive weight gain during pregnancy [n = 5], or unidentifiable [n = 5]. However, mothers were appropriately treated. Customized centiles were calculated by applying the Gestation Related Optimal Weight computer-generated program (Software version 5.15 and Centile calculator software v5.12.1 March 2007, www.gestation.net), which adjusts for important determinants of birth weight (maternal height and booking weight, ethnic group, parity, gestational age and gender) [17,18].

Demographic data concerning mothers and neonates are presented in Table 1.

2.2. Data collection

Peripheral blood (3 ml) was drawn from parturients during the first stage of labor or before receiving anesthesia in cases of cesarean section. Blood (3 ml) was also drawn at birth from the doubly clamped umbilical cord, reflecting the fetal state. On the 3rd to 4th day postpartum mothers provided us with 3 ml of breast milk, expressed by an electric pump, following morning breastfeeding (between 8:00 to 10:00 h). Mothers completed a questionnaire giving familial, individual and obstetrical information of themselves and their infant(s).

2.3. Measurements

Milk samples were centrifuged at 4 °C (1500 g) for 20 min, and blood samples at room temperature for 10 min. Following centrifugation, blood supernatant and milk undernatant layers were divided into aliquots, which were stored at −80 °C until assay. Milk and serum concentrations of Pref-1 were determined by Enzyme-Linked Immunosorbent Assay (ELISA) [Human DLK-1 (Pref-1) ELISA (Delta Like 1 Homolog), Elabscience Biotechnology Co., Ltd, Guandong Science and Technology Industry Park, Wu Han, P.R.C]. The samples had undergone the following dilutions (kit sample diluent) according to their type, so that concentration values fall within the detection range of the kit: 1/1 for milk, 1/10 for maternal peripheral serum, 1/20 for umbilical cord serum. The sensitivity of the assay (minimum detectable dose of Human DLK-1, defined as the lowest protein concentration that could be differentiated from zero) is 0.094 ng/mL. The detection range of the assay is: 0.156–10 ng/mL. Intra- and inter-assay variation (CV%) are < 10% and < 10%, respectively.

Determined concentrations of Pref-1 were associated with various maternal, gestational and neonatal data.

2.4. Statistical analysis

Normality was examined using the Kolmogorov-Smirnov test. As Pref-1 variables in maternal serum and milk do not follow a normal distribution, Mann-Whitney tests were employed for comparisons between genders, parities (one or more than one) and modes of delivery (vaginal or caesarean section) and Kruskall-Wallis tests to compare the three intrauterine growth groups (IUGR, AGA, LGA) for each variable separately. Umbilical cord Pref-1 concentration follows a normal distribution. Median and interquartile range (IQR) were used for comparability reasons among the three Pref-1 concentrations (maternal serum, umbilical cord serum and breast milk). For the three intrauterine growth groups (IUGR, AGA, LGA) umbilical cord serum Pref-1 concentrations were compared, by using parametric tests (mean and standard deviation). Levene’s test was used to check the equality of variances. Correlations were estimated using the Pearson’s coefficient in case of normally distributed data and Spearman’s otherwise. A $p < 0.05$ was considered statistically significant. Data were analyzed using the statistical package IBM SPSS v.20.

3. Results

Umbilical cord serum Pref-1 concentrations (median: 77.58, IQR: 15.32 ng/ml) are significantly higher ($p < 0.001$) than antepartum maternal ones (median: 18.70, IQR: 9.16 ng/ml), while breast milk concentrations are the lowest (median: 0.029, IQR: 0.20 ng/ml), ($p < 0.001$ concerning umbilical serum, $p < 0.001$ concerning maternal serum) (Fig. 1).

Umbilical cord serum Pref-1 concentrations were significantly lower in the LGA group (mean ± SD: 73.81 ± 7.76 ng/ml), than in the AGA one (mean ± SD: 80.55 ± 9.41 ng/ml,) ($p = 0.044$). Pref-1 concentrations in the IUGR group (mean ± SD: 81.33 ± 12.32 ng/ml) do not differ significantly from the AGA group ($p = 0.055$) (Fig. 2).

Furthermore, umbilical cord serum Pref-1 concentrations did not differ between genders, modes of delivery, or parities for each growth group (IUGR, AGA, LGA) separately or for the entire sample. In addition, umbilical cord serum Pref-1 concentrations did not correlate with maternal age, body mass index (BMI) before and after gestation, gestational age, birth weight, body length, and centile for each intrauterine growth group (IUGR, AGA, LGA) separately, or for the entire sample.

Breast milk Pref-1 concentrations did not differ between the three

Table 1
Demographic data of participating mother/infant pairs [16]

	TOTAL	AGA	p-values AGA-IUGR	IUGR	p-values IUGR-LGA	LGA	p-values AGA-LGA
	80	40		20		20	
Birthweight (grams)	3317 ± 607	3381 ± 288	< 0.001	2509 ± 292	< 0.001	3999 ± 301	< 0.001
Gestational Age (weeks)	38.5 ± 1.2	38.8 ± 1.0	0.010	38.0 ± 1.1	0.314	38.4 ± 1.3	0.211
Centile (%)	57.8 (99.8–0)	57.8 (82.8–36.2)	< 0.001	3.5 (8.4–0)	< 0.001	94.5 (99.8–90)	< 0.001
Maternal age (years)	30.1 ± 6.4	30.0 ± 6.4	0.596	32.2 ± 6.4	0.155	28.2 ± 6.3	0.984
Gender							
Male	47 (58%)	20 (50%)		14 (70%)		13 (65%)	
Female	33 (42%)	20 (50%)		6 (30%)		7 (35%)	
Mode of delivery							
Vaginal	57 (71.2%)	29 (72.5%)		13(65.0%)		15 (75.0%)	
Caesarean Section	23 (28.8%)	11 (27.5%)		7 (35.0%)		5 (25.0%)	
Parity							
First	40 (50%)	17 (42%)		15 (75%)		8 (40%)	
Other	40 (50%)	23 (58%)		5 (25%)		12 (60%)	

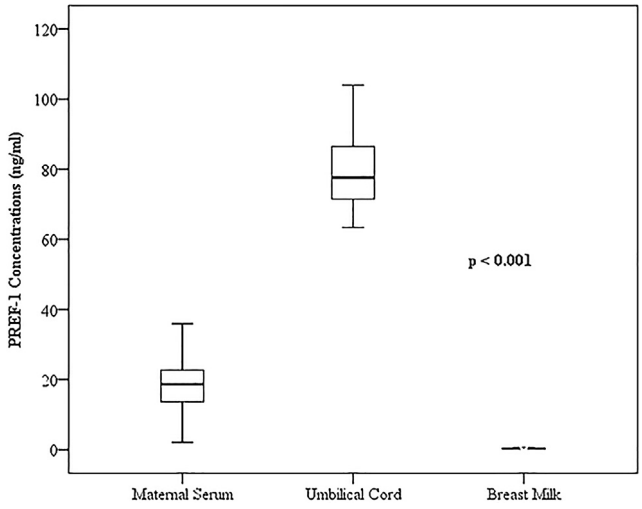

Fig. 1. Pref-1 concentrations in maternal, umbilical cord serum and breast milk.

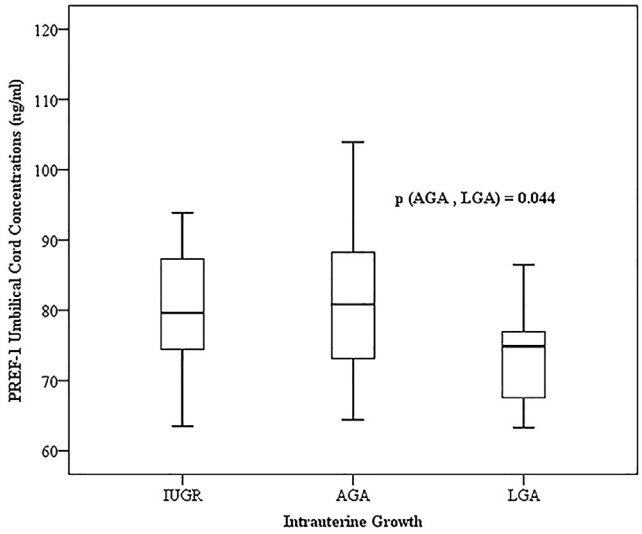

Fig. 2. Pref-1 umbilical cord concentrations in intrauterine growth restricted (IUGR), appropriate for gestational age (AGA) and large for gestational age (LGA) groups.

intrauterine growth groups (IUGR, AGA, LGA) (Fig. 3). Neonatal gender, mode of delivery and parity do not seem to affect breast milk Pref-1 concentrations in the whole study population or each group separately. Breast milk Pref-1 concentrations did not correlate with

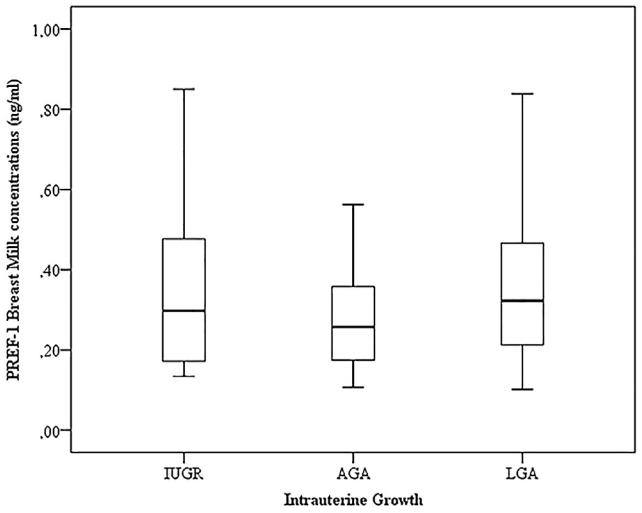

Fig. 3. Breast milk Pref-1 concentrations in intrauterine growth restricted (IUGR), appropriate for gestational age (AGA) and large for gestational age (LGA) groups.

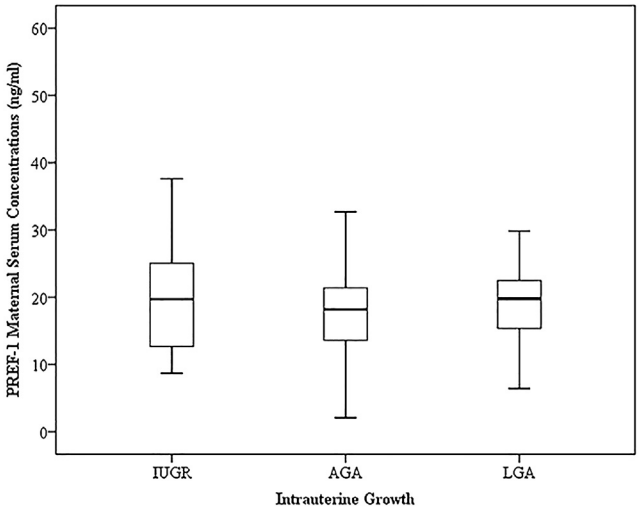

Fig. 4. Maternal serum Pref-1 concentrations in intrauterine growth restricted (IUGR), appropriate for gestational age (AGA) and large for gestational age (LGA) groups.

maternal age, BMI before and after gestation, gestational age, birth weight, body length, and customized centile.

Maternal serum Pref-1 concentrations did not differ between the

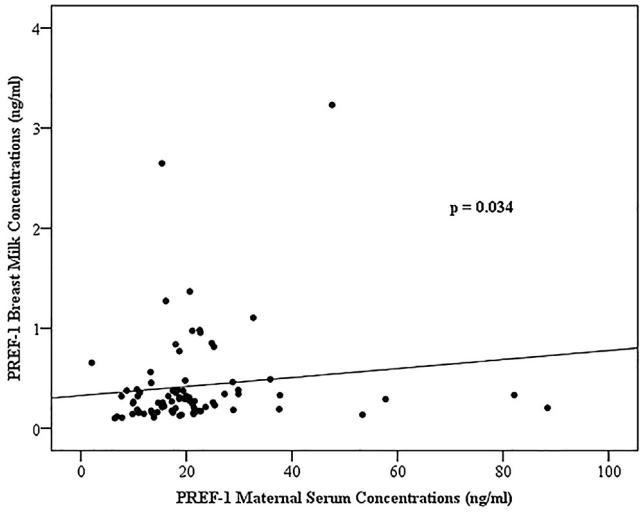

Fig. 5. Positive correlation between maternal serum and breast milk Pref-1 concentrations.

three intrauterine growth groups (IUGR, AGA, LGA) (Fig. 4). Similarly, maternal serum Pref-1 concentrations did not differ between genders, modes of delivery and parities, for each growth group (IUGR, AGA, LGA) separately or for the sample as a whole. Maternal Pref-1 serum concentrations did not correlate with maternal age, BMI before and after gestation, birth weight, body length, and customized centile.

Lastly, a weak positive correlation exists between maternal serum and breast milk Pref-1 concentrations (r = 0.238, p = 0.034) (Fig. 5) and a negative weak correlation between maternal serum and umbilical cord serum Pref-1 concentrations (r = -0.282, p = 0.013) (Fig. 6).

4. Discussion

The main results of this study indicate that maternal serum Pref-1 concentrations are significantly lower than umbilical cord ones, and breast milk concentrations are the lowest. Moreover, umbilical cord serum Pref-1 concentrations are significantly lower in the LGA than the AGA group and maternal serum Pref-1 concentrations positively correlate with maternal milk ones.

To our knowledge, this is the first time that breast milk Pref-1 concentrations have been determined, and according to our results, they are extremely low in comparison with umbilical cord and maternal

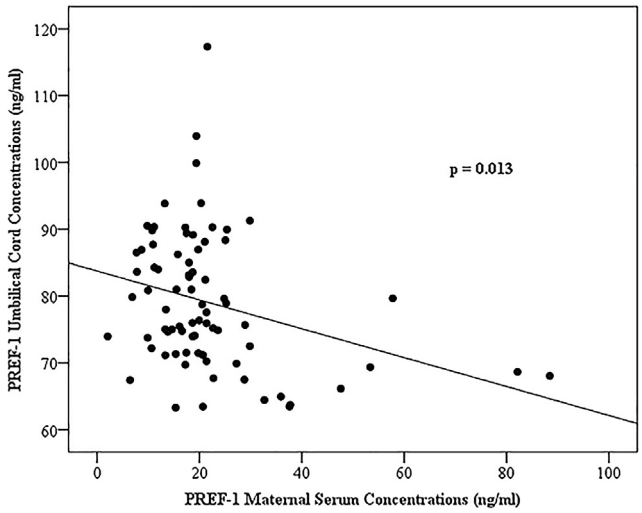

Fig. 6. Negative correlation between maternal serum and umbilical cord serum Pref-1 concentrations.

serum ones. Previous reports [19] documented a rapid decline of Pref-1 concentrations following delivery, and attributed this to the elimination of the placenta, which is speculated to contribute to maternal circulating Pref-1 concentrations. The positive correlation between maternal serum and milk concentrations may point to a transfer of the substance from the circulation to the breast and the determined very low milk concentrations may have resulted from considerably lower postpartum maternal ones.

The very low breast milk Pref-1 concentrations may contribute to the protective role of breast milk for the infant [20]. Published data claim that upregulated Pref-1 concentrations in early life are responsible for reduced adipocyte numbers, reduced lipid storage capacity and vulnerability to metabolic disease in adulthood [21]. Additionally, Pref-1 is considered to impair glucose control in vivo [14,15], its concentrations are higher in patients with type 2 DM when compared to controls [13] and positively correlate with markers of insulin resistance and triglycerides [22]. In this respect, Lee and collaborators [14] state that, Pref-1 by inhibiting in vivo adipogenesis and thus, by impairing adipocyte function, is implicated in metabolic disorders. However, the exact mechanisms linking Pref-1 to metabolic disease are not yet fully elucidated.

Our finding of significantly higher Pref-1 concentrations in the umbilical cord serum as compared to maternal serum is in accordance with the results by Schrey et al. [19] and de Zegher et al. [21], who demonstrated a respective 40-fold and 25-fold prevalence of Pref-1 in cord serum. This finding could possibly be associated with the reported role of Pref-1 in intrauterine development and growth [5], as well as with its fetal origin [20].

In line with our data, Li et al. [23] demonstrated decreased umbilical cord blood Pref-1 concentrations in pregnancies complicated by GDM, and consequently increased birth weight. Furthermore, this study showed the association of Pref-1 concentrations in umbilical blood with gestational age, in contrast to birthweight and maternal age. The same authors state that a previous small study from their group did not prove an influence of active labor on Pref-1 concentrations, thus the mode of delivery did not play a role, in accordance with our results.

However, the explanation for the lower Pref-1 concentrations in umbilical cord serum from pregnancies complicated by GDM and LGA fetuses/neonates, along with later health implications, is not clear-cut. Li et al. [23] referring to mice experiments [14], claim that Pref-1, by inhibiting adipogenesis, results to increased insulin sensitivity and improved glucose tolerance. Taken that glucose intolerance, insulin resistance and dyslipidemia promote metabolic disorders, they conclude that Pref-1, among other factors, mediates intrauterine hyperglycemic conditions with adulthood metabolic syndrome.

Another study [24], examining Pref-1 concentrations in pregnant women with GDM, found that Pref-1 serum concentrations do not differ from those of healthy controls. Similarly, our study, including women with LGA fetuses, among them those presenting with GDM, did not show a difference in Pref-1 concentrations, as compared to healthy women with AGA infants. Our current results do not show a correlation of maternal Pref-1 serum concentrations with maternal age, BMI before and after gestation, birth weight, body length, and customized centile, while in the above report by Wurst et al. [24], the cohort being age-, BMI-, and gestational-age matched, did not show significant differences between these parameters.

The IUGR group of our cohort did not demonstrate differences from the AGA one, concerning Pref-1 concentrations in umbilical cord, as well as maternal blood. In contrast, Schrey et al. [19] reported that maternal serum Pref-1 concentrations were downregulated in pre-eclamptic women -usually delivering IUGR neonates- when compared to healthy pregnant ones -expected to deliver AGA infants- and speculated that this result is linked to the antiangiogenic properties of Pref-1 [11]. In parallel, Diaz et al. [25] experimenting with placentas of SGA fetuses proved a reduction of Pref-1 expression and association with postnatal body weight, signaling its role in the regulation of

extrauterine development. Nevertheless, another research [26] reported elevated cord blood Pref-1 concentrations in cases of severe preeclampsia, when compared not only with normal ones, but also with cases of mild preeclampsia and pregnancy induced hypertension. Respectively, it should be mentioned that women in our cohort presented only with mild preeclampsia, as well as mild pregnancy induced hypertension. Additionally, the above authors found a negative correlation of Pref-1 concentrations with birth weight, emphasizing the implication of IUGR in the secretion of this adipokine. In accordance, the report by de Zegher et al. [21] verifies the high Pref-1 concentrations in SGA (with no obvious etiology) full-term (38–40 weeks) fetuses, as compared to AGA ones, stressing its contribution to diabetes susceptibility and metabolic disease in adulthood. According to Schrey et al. [19], the contradictory results in preeclampsia, probably implicating SGA/IUGR fetuses, may depend on separate mechanisms controlling circulating maternal and fetal Pref-1 concentrations. The same authors [19] report that Pref-1 serum concentrations during pregnancy positively correlated with gestational age at delivery and birth weight, and negatively associated with BMI. We were unable to show similar correlations.

The lack of a positive correlation between maternal and umbilical cord serum Pref-1 concentrations in our study may indicate the fetal (and not maternal or placental) origin of the substance, as previously assumed [21].

We should consider the following limitations of our study: First, we did not determine Pref-1 concentrations in maternal serum on the same day maternal milk was collected. We assume that maternal serum Pref-1 concentrations on day 3 to 4 postpartum, due to placental elimination, would have been lower than antepartum ones and possibly the correlation could have been stronger. Second, the number of included subjects is not very high. However, to the strengths of this study count the very well-defined IUGR, AGA and LGA groups, due to the use of customized centiles, and the collection of maternal serum, umbilical cord serum and breast milk from the same mother-infant dyads.

5. Conclusions

Our data suggest higher concentrations of Pref-1 in umbilical cord serum than in antepartum maternal serum, probably pointing to its fetal origin and role in fetal growth. Breast milk Pref-1 concentrations, being extremely low, and possibly implying offspring protection from metabolic disorders, positively correlate with maternal serum ones, conceivably suggesting a transfer of the substance from the circulation to the breast. Umbilical cord serum Pref-1 concentrations are lower in LGA fetuses/neonates, as compared to AGA ones, while the latter do not differ from the respective concentrations in IUGRs.

Declarations of interest

The authors state that they have no conflict of interest or financial support relevant to this article to disclose.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Despina D. Briana: Had responsibility for analysis/interpretation of data and principally writing the manuscript.

Aimilia-Eirini Papathanasiou: Participated in the development of the protocol, patient enrolment and acquisition of data.

Stavroula Gavrili: Had responsibility for patient enrollment and acquisition of data.

Sophia Georgantzi: Contributed to patient enrollment and acquisition of data.

Antonios Marmarinos: Contributed to the conduction of the laboratory measurements.

Christos Christou: Performed the statistical analysis of the data.

Konstantinos Voulgaris: Performed the statistical analysis of the data.

Dimitrios Gourgiotis: Participated in the analytical framework of the study and performed laboratory determinations.

Ariadne Malamitsi-Puchner: Had primary responsibility for protocol development, patient screening enrollment, analysis/interpretation of data, outcome assessment and critically reviewing the manuscript.

All authors critically revised the manuscript and approved the final version to be submitted.

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