Gestational Hypertension and Their Impact on Activin A Concentration of the Human Milk

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Abstract

Introduction It is known that Preeclampsia affects the lactogenesis, but literature data on the effects of this syndrome on the neurobiomarkers composition and Activin A of Human Milk of the lactating mother are not available. The aim of this study is to investigate the effects of this gestational pathology on Activin A levels, a neurobiomarker known to play an important role in the development and protection of the central nervous system.

Methods The women recruited in the study were divided in two different study groups: preeclamptic or healthy women. All the breast milk samples were collected using the same procedure into sterile devices BPA-free. Activin A was quantified using an ELISA test. To investigate the effect of pathology in the Activin a concentration in the 3 phases, mixed linear model with unistructural covariance structure, mother as random effect, and fixed effects were performed.

Results Activin A was detected in all samples. There were no significant differences between Preeclamptic mother and Normotensive women. The only significant effect is related to the HM phase: in particular, it is significant the difference between colostrum and mature milk ($p<0.01$).

Conclusion There is not any significant difference in Activin A breast milk composition from hypertensive and normotensive women. This result allows us to affirm that breast milk beneficial properties are maintained even if gestational hypertension occurs.

1. Background

Preeclampsia (PE) is a common pregnancy complication that occurs in about 3–5% of pregnancies: it is a gestational hypertensive syndrome characterized by a complex disease with variable clinical manifestation [1]. In most cases, the symptoms occur after the 20th week of Gestational Age (GA); the earlier its onset, the more serious it is [2][3]. PE is one of the primary causes of fetal-maternal morbidity and mortality. In addition, infants born to preeclamptic mothers are at high risk for several disorders (not only endocrine, nutritional, and metabolic but also cognitive, due to neurodevelopmental impairments)[4] [5]. At the basis of PE there is an early functional alteration of unknown origin to the development of placental-vascularization[3]. Among the etiological hypotheses advanced we find an unbalance in the pro- and antiangiogenic factors, a reduced tolerance towards the child or the father, or insulin resistance[6].

An aspect of interest is the possible effect that PE may have on the mammary gland, and therefore on breast milk composition. In fact, it is well known that Human milk (HM) is a species-specific biological “dynamic” system, changing according to lactation phase and adapting its composition to different conditions such as GA, gestational pathologies and maternal diet[7]. Mother’s own milk is always considered the first choice for nutrition of all neonates, including preterm infants[8]. Several neonatal outcomes are improved, both in the short and the long-term[9][8], thanks to specific biological active components and immunomodulatory factors of the HM (i.e. hormones, immunoglobulins, lysozyme,

lactoferrin, saccharides, nucleotides growth factors, enzymes antioxidants and cellular components)[10]. Neurobiomarkers are important HM components and, between these, Activin A can play a relevant role as a growth factor[11][12]. Activin A is a dimeric protein belonging to the transforming growth factor beta (TGF-beta) superfamily and its receptors are widely distributed in the brain [13]. Studies in humans and animal model showed that Activin A can play a trophic and neuroprotective role on the Central Nervous System [14] [13].

Although it is known that PE affects the lactogenesis, literature data on effects of this syndrome on the neurobiomarkers composition and Activin A of HM of the lactating mother are not available.

Thus, the aim of this study is to integrate and to expand the available literature data by investigating the association between the composition of human milk and gestational hypertension, considering the variations of key biochemical markers in immature and mature milk in mothers having delivered term and preterm infants.

2. Materials And Methods

2.1 Setting and Population

The study protocol was approved by the local Ethic Committee of the Italian Association of Human Milk Donor Banks (AIBLUD). Mothers admitted into the study gave signed and informed consent. Newborns' mothers were recruited after delivery at "City of Health and Science Hospital of Turin, Neonatal Care Unit of the University, Italy.

The women recruited in the study were divided in the two different study groups: preeclamptic or healthy women, according to the PE definition (Artery blood pressure >140/90 mmHg after 20 weeks of gestational age and proteinuria >290 mg/l, possibly associated with headache, edema, scotomas and epigastralgia [1]). A minimum of 30 women for each group were recruited.

Criteria inclusion: PE diagnosed during the pregnancy; absence of diabetes mellitus and chorioamnionitis; absence of all CNS pathologies or psychiatric syndromes; no use of illicit drugs/alcohol during pregnancy; and a newborn without congenital anomalies or infection. Women mastitis or continuous use of medication were excluded. The control group was formed at the same time and made up of normotensive mothers who met the same inclusion criteria.

2.2 Collection human milk samples

According to standard criteria, we classify as "colostrum" the milk collected in the first three days after the delivery; "transition milk" the milk collected from the 8th day to the 14th day after the delivery and "mature milk" the milk collected after the 30th day[15]. All the breast milk samples were collected using the same procedure outlined below. Fresh milk samples were collected in the morning (between 9 a.m. and 12 a.m.) into sterile devices BPA-free. Milk was collected with standard extraction methods by emptying one breast completely by means of an electric breast pump (Medela Symphony, Baar,

Switzerland). A minimum of 10 ml of milk were collected and immediately frozen at -80°C before the analysis. Milk expression by the other breast was performed only in case it was not possible to obtain 10 ml from a single breast.

2.3 Activin A measurements

Activin A levels were determined using a specific ELISA test (ELH-ActivinA-1 Human Activin A ELISA) according to the manufacturer's instructions (RayBiotech, Inc.; USA). Investigators who performed the laboratory tests were blind to storage modalities. The assay detection limit is 15.00 pg/ml, the intra-assay CV is ≤ 5.0%, and the inter-assay CV is ≤ 10%. The assay is specific for Activin A, having been tested by the manufacturer for lack of cross-reactivity with other proteins of the Activin family.

2.4 Statistical methods

Birth weight was transformed in z-score using as reference INeS charts[16]. Newborns with a birth weight lower than the 10th or higher than the 90th centile were classified as Small for GA (SGA) or Large for GA (LGA) respectively. The continuous variables were summarized as mean (Standard Deviation) or median [Inter Quartile Range] according to their distribution, the categorical variables were summarized as absolute frequency (percent).

To investigate the distribution by HM phase and pathology of Activin A, specific box plot was done. Then Activin A concentration was normalized with the more appropriate Box-Cox transformation. To investigate the effect of pathology in the Activin A concentration in the 3 phases, mixed linear model with unistructural covariance structure, mother as random effect, and fixed effects were performed. The fixed effects were: hm phase, pathology, smoke, type of delivery, newborn GA and mother age (continuous), and the interaction between phase hm×pathology.

3. Results

Table 1 reports the basal characteristic of mothers and newborns included in this study. As expected, women with PE have a higher fraction of IUGR babies and a higher fraction of SGA babies.

Table 1. Basal characteristics of mothers and newborns

		No pathology	Hypertension
		N=46	N=39
Maternal characteristics			
Age (years)	median [IQR]	33.5 [31–37]	35 [31–38]
Italian	n (%)	35 (76.1)	31 (79.5)
Caesarian Section	n (%)	25 (54.4)	28 (71.8)
Weight gain (kg)	mean (SD)	10.9 (4.75)	10.4 (5.65)
Primipara	n (%)	29 (63.0)	25 (64.0)
Smoker	n (%)	6 (13.0)	2 (5.1)
Newborn characteristics			
Singleton	n (%)	38 (82.6)	36 (92.3)
IUGR	n (%)	2 (4.4)	16 (41.0)
GA (weeks)	median [IQR]	37 [31;39]	32 [29–35]
Girls	n (%)	19 (41.3)	19 (48.7)
Birth weight (g)	mean (SD)	2345 (1028)	1542 (720)
Birth weight (z-score)	mean (SD)	-0.21 (0.934)	-1.16 (0.810)
SGA	n (%)	6 (13.0)	19 (50.0)
LGA	n (%)	2 (4.4)	0 (0.0)

Figure 1 shows the box-plot Activin A distribution by HM phase and pathology (hypertension, no pathology). The variability in HM phase 1 (colostrum) is higher than in phase 2 (Transitional milk) and 3 (Mature milk). $\lambda = 0$ resulted the more appropriate Box-Cox transformation to normalized Activin A distribution. The mixed linear model resulted in no significant effect of pathology and phase hm×pathology. The only significant effect is related to the HM phase, in particular is significant the difference between phase HM 1 and 3 ($p<0.01$)

4. Discussion

Among the maternal organs affected by the Preeclampsia there are the mammary glands. As well as in the rest of the body, even at this level there could be changes in the endothelium and blood vessels: these would lead to a reduction in the development of the gland and changes in the mechanisms of production of milk[17]. It is also known that the children of preeclamptic mothers, exposed to intrauterine stress, may have special nutritional needs, in addition to a greater risk of complications[18][2]. In view of these

considerations, it is interesting to evaluate the potential differences between HM of PE women and normotensive women, in the different lactation phases.

Our study is the first that provide data on the association between PE and HM Activin A levels. Our results showed the absence of significant differences between the different groups. Considering the importance of HM nutrition, previous studies have focused their attention on PE lactating mothers. Data showed an alteration in the levels of several components: macronutrients (i.e. proteins, carbohydrates, lipids and energy metabolites) and pro- and anti-inflammatory cytokines, oxidative stress markers and antioxidant molecules[19][20][21][22][23][24][25]. Regarding neurotrophic factors, Dangat et al. examined the levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) [26][27]. At first, they evaluated the levels of milk neurotrophins only in colostrum and observed that milk NGF levels were similar, whereas milk BDNF levels were higher in the PE group as compared to controls[26]. In the second time, they extended the evaluation of these agents through the other phases of lactation and they found that the NGF concentrations at 1.5 and 3.5 months and BDNF levels at 1.5 months were lower in the PE group as compared to the control group [27].

However, our data show differences in Activin A concentration in the different lactation phases, with a significant decrease in levels from colostrum to mature milk, in mother having delivered also preterm and term of GA. Our results are in agreement with previous study that reported a similar decrease in Activin A levels from colostrum to mature milk of mother delivering at term GA[12]. In addition, this current study confirms the presence of Activin A also in HM of woman that delivered preterm[11] and our data do not show differences between term and preterm levels.

Anyway, these findings in Activin A levels herein reported warrants further consideration. In particular, Activin A probably acts in HM as a growth factor: previous studies demonstrated that Activin A plays a neurotrophic function in differentiations of many CNS target cell-types[13][28]. Moreover, It is useful in repairing neurotoxicity damage, *in vitro* and *in vivo researches*[29][30]. It seems to exert a role of CNS protection from antidepressant treatment side-effects[31].

It also performs, on the other hand, a biomarker function of damage, especially at the brain level, so it has also been suggested to use it as an early neonatal indicator of neurological insults, caused for example by asphyxia and intraventricular hemorrhage after birth[32]. In addition, a fairly large number of researchers have evaluated, in other types of samples (i.e blood, plasma, urine), the variation in the Activin A concentration in case of PE and many studies agree that there is a significant correlation between this pathology and Activin A blood levels [33].

Bearing in mind these considerations, the absence of differences in HM Activin A composition is an important finding: in fact, thanks to these data, it can be said that the beneficial properties of milk are maintained even in the event of the onset of PE. This is a very encouraging fact, especially considering the high vulnerability, already mentioned, of the children of hypertensive mothers.

Conclusions

There is not significant difference in Activin A breast milk composition from hypertensive and normotensive women. Our study confirms that the biological value of human milk associated with the Activin A content is maintained in preeclamptic mothers.

Declarations

Competing interests

The authors declare that they have no competing interests.

Financial competing interest

The authors declare that they have no financial competing interests.

Authors' contributions

All authors contributed to the conception and/or design of the study. SHL drafted the manuscript, and all of authors read and approved the final manuscript and contributed revising the manuscript critically for important intellectual content**.**

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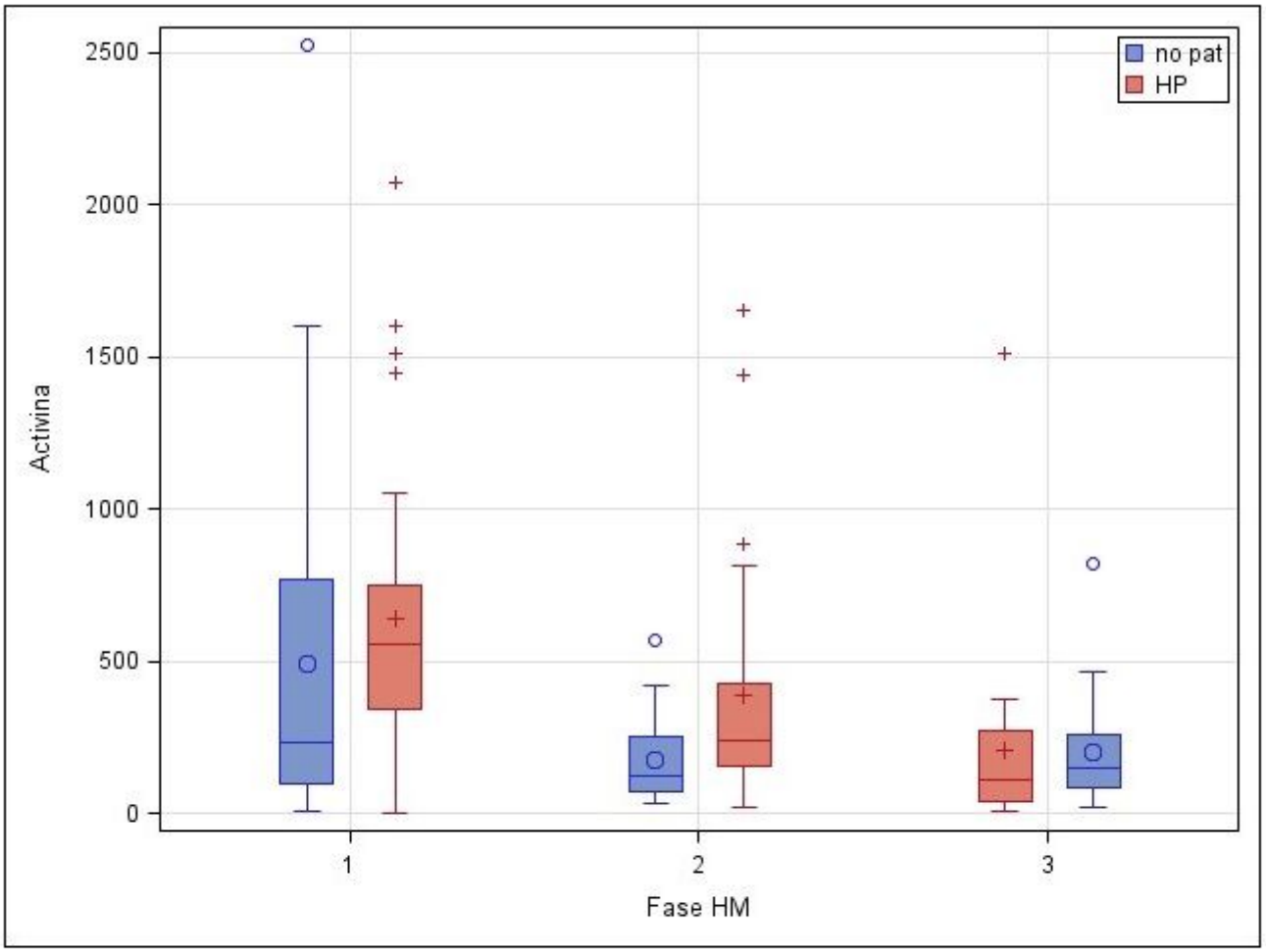
Figures



	n	Median [IQR]	n	Media [IQR]	n	Media [IQR]
No pat	30	232.47 [96.13-771.46]	24	122.47 [74.80-254.80]	25	147.46 [82.80-260.80]
HP	30	553.80 [340.13-751.46]	27	238.80 [152.80-428.13]	22	108.13 [37.46-274.8]0

Figure 1

Box-Plot of Activin by Phase HM and pathology. Median and [IQR] were reported for each Phases HM