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Harbingers of neonatal birth weight: The PON1 arylesterase and lactonase activities

[Düşük yeni doğan ağırlığı habercisi: PON1 arilesteraz ve laktonaz aktivitesi]*

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ABSTRACT

Objective: An important predictor for infant survival is birth weight. Normal fetal growth is related to various intrauterine factors. Low birth weight is thought to have relation with oxidative stress which plays an important role in reducing the birth weight. Among the paraoxonase family PON1 protects LDL and HDL from the lipid peroxidation. This is HDL associated enzyme having antioxidant property. We aimed to evaluate the arylesterase and lactonase activity of PON1 in cord blood in relation to birth weight. We hypothesized that cord blood PON1 arylesterase and lactonase activities will be compromised in neonates having low birth weight.

Methods: We included 80 neonates born in our hospital irrespective of mode of delivery as 40 cases and 40 controls. PON1 arylesterase and lactonase activity were measured using spectrophotometer.

Results: Serum arylesterase activity decreased significantly in low birth weight babies ($p<0.05$). Linear regression analysis ($R=0.595$) indicates significant correlation between arylesterase and birth weight. Serum lactonase activity of PON1 also gets reduced in low birth weight babies. Its linear regression analysis showed ($R=0.716$) suggesting significant correlation between lactonase and birth weight.

Conclusion: Reduced PON1 activity can be explained on the basis of ER stress and atherogenic changes in the placental circulation. Ours is the first study in cord blood paraoxonase activities in relation to birth weight. As the sample in our study is cord blood, it is essentially a noninvasive one. Further studies are needed in this direction to assess the effect of the oxidative stress on fetus through cord blood in its long term prospective.

Key Words: Paraoxonase1, arylesterase, lactonase, cord blood, low birth weight

Conflict of Interest: There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ÖZET

Amaç: Doğum ağırlığı bebek sağkalımı için önemli bir belirleyicidir. Normal fetal büyüme farklı birçok intraüterin faktöre bağlıdır. Doğum ağırlığının düşük olmasında önemli rolü olan oksidatif stresin düşük doğum ağırlığı ile ilişkili olduğu düşünülmektedir. Paraoksanoz ailesinden PON1 LDL ve HDL'yi lipid peroksidasyonundan korumaktadır. Bu HDL assosiye enzimin antioksidan aktivitesidir. Kordon kanında bulunan PON1in aril esteraz ve laktonaz aktivitelerinin düşük doğum ağırlığı ile ilişkisini değerlendirmeyi amaçladık. Hipotezimiz düşük doğum ağırlıklı yenidoğanda kordon kanı PON1 arilesteraz ve laktonaz aktivitesinin de düşük olacağıdır.

Metod: Doğum şeklinden bağımsız olarak hastanemizde doğan 80 yenidoğan çalışmaya 40 vakka 40 kontrol olarak kaydedilmiştir. PON1 arilesteraz ve laktonaz aktiviteleri spektrofotometrik olarak ölçülmüştür.

Sonuç: Serum Arılesteraz aktivitesi düşük ağırlıklı yenidoğanda belirgin olarak düşüktür ($p<0.05$). Lineer regrasyon analizi ($R=0.595$) arilestereaz aktivitesi ile düşük doğum arasında anlamlı korelasyon göstermektedir. PON1 için serum laktonaz aktiviteside düşük ağırlıklı yenidoğanda azalmaktadır. Lineer regrasyon analizi ($R=0.716$) laktonaz aktivitesi ve düşük doğum ağırlığı arasında anlamlı korelasyona işaret etmektedir.

Yorum: Düşük PON1 aktivitesi ER stresi ve plasental dolaşımdaki atoragenik değişiklikler ile açıklanabilir. Bizim çalışmamız kordon kanında paraoksonaz aktivitesi ve düşük doğum ağırlığına ilişkisine bakan ilk çalışmadır. Çalışmamızda kordon kanı kullanılmış olması bu çalışmayı non invazif kılmaktadır. Kordon kanı ile bu yönde ek çalışmalar ile oksidatif stresin fetus üzerindeki uzun süreli etkisi araştırılmalıdır.

Anahtar Kelimeler: Paraoksonaz 1, arilesteraz, laktonaz, kordon kanı, düşük doğum ağırlığı

Çıkar Çatışması: Bu makalede tartışılan materyel ile ilgili olarak hiçbir iktisadi kuruluş ile çıkar çatışması yoktur.

Introduction

The most important predictor for infant survival is birth weight. There is strong correlation between low birth weight and neonatal mortality as low birth weight is one of the precipitating causes for mortality in new born. However, its position in the causal pathway is unclear [1]. Normal fetal growth is related to maternal, fetal, placental and external factors along with genetic growth potential [2]. Impairment of one or more of these factors affects the fetal growth. Such intra-uterine events may have their effects over the birth weight [3]. Fetal growth is dependent on three different phases, namely cellular hyperplasia (up to 16 weeks gestation), combination of hyperplasia and hypertrophy (up until the third trimester) and cellular hypertrophy (mid-third trimester up until term) [4]. Oxidative stress is a major player in fetus of normotensive women having its role in IUGR and low birth weight [5, 6]. Literature reports studies revealing the effect maternal oxidative stress on fetal growth. The low birth weight is thought to have relation with oxidative stress which plays an important role in reducing the birth weight [7]. For this not only maternal but also fetal contribution to oxidative stress has to be taken into account.

The paraoxonase family has three members having genetic location at 7q21-q22. The three subtypes are PON1, PON2 and PON3 [8]. The enzyme came into focus with an idea that PON1 protects LDL and HDL from the lipid peroxidation [9-10]. This is high density lipoproteins (HDL) associated enzyme having antioxidant property which plays a vital role in prevention from micro-vascular complications due to oxidative stress and against various toxic chemicals [11, 12]. A 45 kD glycoprotein expressed in liver has arylesterase, lactonase and paraoxonase activity [13]. PON1 activity in the neonates shows an increasing trend till first year of life and then attains the plateau and again shows decrement in elderly/geriatric population [14].

In this study we evaluate the arylesterase (ARE), and lactonase (LACT) activity of PON1 in cord blood (CB) in relation to birth weight. We hypothesized that cord blood PON1 arylesterase and lactonase activities will be compromised in neonates having low birth weight.

Materials and Methods

This study has been approved by the Institutional Ethics Committee of SRTR Government Medical College, Ambajogai, Maharashtra, India. We included 80 neonates born in our hospital irrespective of mode of delivery. Those with congenital abnormality or maternal events like pre-eclampsia, chronic diseases, addictions like smoking/ tobacco chewing or any significant medical history were excluded. For all the birth weight was measured immediately after birth on digital scale. The neonates having birth weight < 2.5 kg were considered as cases and those having birth weight ≥ 2.5 kg were controls. Cord blood samples were collected immediately postpartum.

Cord blood samples obtained were centrifuged at 3000 rpm and serum samples separated were analyzed immediately. Serum PON1 Arylesterase activity was measured spectrophotometrically at 270 nm with 5 µl serum in 3 ml buffer-substrate solution containing 20 mmol Tris-HCl buffer and 4 mmol phenylacetate as substrate at pH 8.0 [15]. Activity expressed in kU/L based on extinction coefficient of phenol 1310 M^{-1} cm^{-1} at 270 nm, pH 8.0, and 25 ^{o}C. Serum PON1 Lactonase activity was measured spectrophotometrically at 270nm with 10µl serum 2ml buffer-substrate solution containing 50mmol Tris-HCl buffer and 1mmol dihydroxycoumarine as substrate at pH 8.0 [16]. Activity expressed in U/L based on difference in extinction coefficient of product and substrate 1295 M^{-1} cm^{-1} at 270 nm, pH 8.0, and 25^{o}C. Normality of the distribution of the arylesterase and lactonase activity is assessed by Shapiro-Wilk test. Two sample t-test is applied for hypothesis testing. We used the correlation and multivariate regression to assess the relationship between parameters. The results obtained were analyzed by using MYSTAT-12 software for Window

Results

The serum arylesterase activity was decreased significantly in low birth weight babies ($p<0.05$,). The results show that the arylesterase and lactonase singly can correlate well with birth weight and their combination even more so. The graph in Figure 1 shows the correlation between arylesterase and lactonase activity with birth weight. Linear regression analysis shows $R = 0.595$ indicating significant correlation between arylesterase and birth weight ($p < 0.001$). Similarly serum lactonase activity of PON1 also gets reduced in low birth weight babies. Its linear regression analysis showed $R = 0.716$ suggesting significant correlation between lactonase and birth weight ($p < 0.001$). Multivariate regression analysis gives value as 0.897 showing enhanced predictability by using both arylesterase and lactonase ($p < 0.001$). Table 1 shows statistically significant distribution of paraoxonase activities in cases and controls.

Discussion

To the best of our knowledge, ours is the first study done for the correlation between birth weights and cord blood arylesterase and lactonase activity. Plasma PON1 activity was found to be altered usually decreased in number of pathological conditions and diseases [17]. In this study we found that there is significant reduction in the PON1 arylesterase and lactonase activity in cord blood of low birth weight babies.

Some studies have shown that the level of PON1 in newborns is 3-4fold lower than the adults [18, 19]. But as per our knowledge no study has been conducted so far

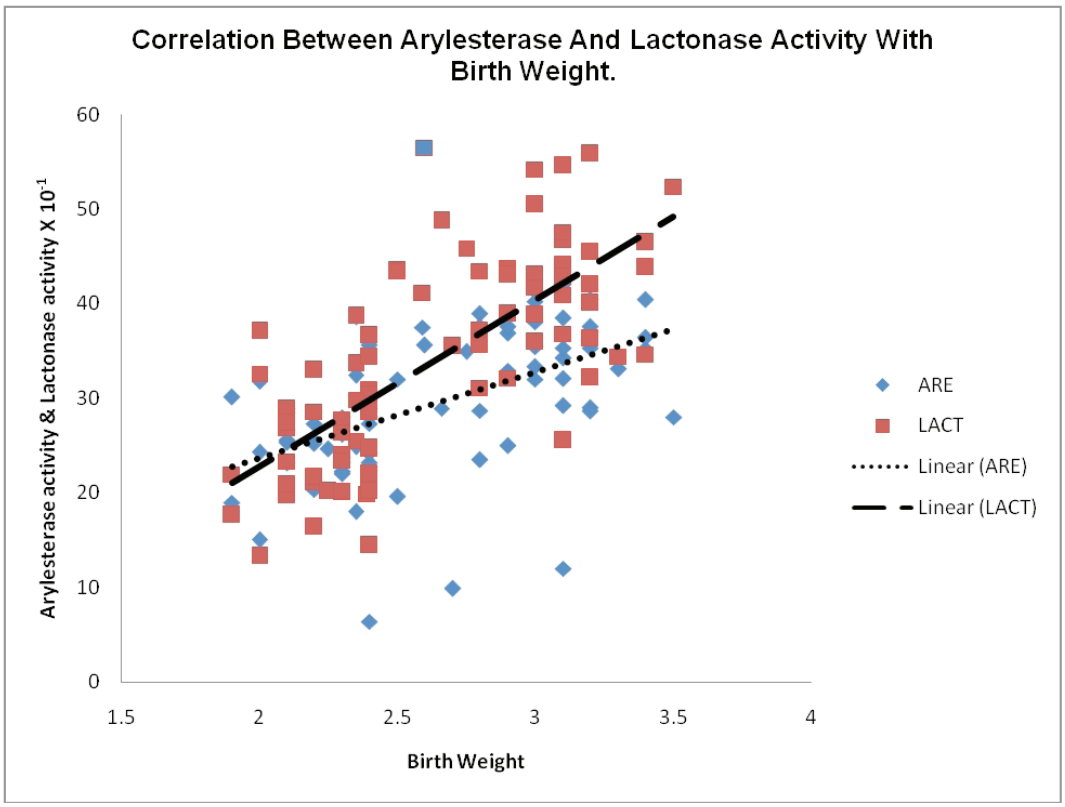

Figure 1: Correlation between arylesterase and lactonase activity with birth weight.

showing the relation between birth weight and cord blood arylesterase and lactonase activity. Physiological role of PON1 has not been fully known. It was demonstrated that it prevents LDL oxidation by hydrolyzing lipid peroxides and to have protective role against cellular damage [8, 20].

In a recent study it was reported that umbilical arterial lipids are more susceptible to peroxidation than umbilical venous lipids, indicating high oxidative stress in the fetal circulation irrespective of mode of delivery [21]. To balance such high level of oxidative stress in fetal circulation, PON1 is utilized. The low PON1 activity associated with low birth weight can be explained on this basis of oxidative stress. Various reactive oxygen species (ROS) in feto-maternal circulation lead to oxidative stress in fetus as well as mother. This invariably has the effect on fetal nourishment and development. Being an anti-oxidant, PON1 is involved in protection against the oxidative stress. PON1 may be inactivated by attack of hydroxyl radicals, direct oxidation by peroxides and/or alkylation by α, β-unsaturated aldehydes [17]. As an effect of oxidative stress, there is compromised fetal nourishment, affecting ultimately the birth weight.

One possible explanation for our finding of decreased PON1 arylesterase and lactonase activity in low birth weight is the susceptibility of the PON1 to get inactivated by oxidative damage. Literature reports the susceptibility is more so in case of lactonase as compared to arylesterase [22-25]. The endoplasmic reticulum stress is one of the sources of ROS [24]. It was demonstrated that normotensive IUGR is due to endoplasmic reticulum stress causing inhibition of protein synthesis [26]. Similar mechanism with oxidative stress has been reported in IUGR with pre-eclampsia.

Endoplasmic reticulum (ER) can be linked to oxidative stress through such protein misfolding which triggers ER stress response [27]. In ER molecular oxygen is terminal electron acceptor can lead to oxidative stress through the production of ROS and oxidized glutathione [28]. Another source of ROS is mitochondria where they originate during cellular metabolic processes as oxidative phosphorylation [29].

Similarly unfolded proteins may trigger the intracellular inflammatory signaling pathways causing oxidative stress and damage [30]. PON1 is an enzyme having antioxidant property. It comes into play to balance the oxidative stress that in excess may be harmful.

Another possible explanation can be atherogenic changes in the feto-maternal circulation limiting blood flow to fetus. Such compromised blood flow results in tissue hypoxia causes ER stress [31]. This triggers oxidative stress, producing NO and other ROS [32]. Placental

Table 1. Parameters of Cases and Controls

Parameter	Cases (n = 40) (mean ± SD)	Controls (n = 40) (mean ± SD)	p-value
Birth weight (Kg)	2.254 ± 0.773	3.039 ± 1.399	**< 0.001**
Arylesterase activity (KU/L)	26.795± 24.623	34.713 ± 33.146	**< 0.001**
Lactonase activity (U/L)	2.546 ± 3.057	4.265 ± 3.967	**< 0.001**

Results are expressed as mean ± SD

oxidative stress, which results from the ischemic injury, is being increasingly reported to be involved in the etiopathogenesis of IUGR and pre-eclampsia [33, 34]. The placenta may be the site of generation of the lipid peroxides [35]. Increased generation of ROS leads to increased lipid peroxidation.

There is reduced trophoblastic invasion in IUGR and small for gestational age babies. The result is deficient conversion of uterine spiral arteries. Role of this conversion is to remove the smooth muscles from the segment to allow an uninterrupted flow of blood to placenta and ultimately to the developing fetus [36]. This process requires a tight regulatory control and the coordinated actions of multiple cell types [37]. The spiral artery conversion is restricted to only decidual segment in small for gestational age newborns. Literature also reports the complete absence of such physiological changes throughout the entire length of some spiral arteries is seen in pre-eclampsia and SGA [38]. Deficient spiral artery conversion predisposes to placental malperfusion due to lipid-laden mononuclear cells forming intimal plaques [39]. Oxidative modifications of LDL in plaque can be prevented by HDL associated PON1. Also the anti-atherogenic properties of PON1 are all related to its lactonase activity [40]. This explains the decrease in PON1 activity.

Thus reduced activity of PON1 can be explained on the basis of ER stress and atherogenic changes in the placental circulation. As the sample in our study is cord blood, it is essentially a noninvasive one. Smaller sample size could be the limiting factor for our study. Also we have not looked for the perinatal outcome. Further studies are needed in this direction to assess the effect of the oxidative stress on fetus through cord blood in its long term prospective.

Conflict of Interest: There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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