Hypoxia-Inducible Factor in Cord Blood of Term and Preterm Newborns

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Abstract

Background: To study cord blood HIF-1α levels in newborns and to correlate them with co-morbidities and placenta histological features.

Methods: Arterial samples were collected in the delivery room from 39 full-term and 23 preterm infants. A blood gas was made and the sample was stored to detect HIF-1α. In 42 cases the placentas had macroscopic reporting and in 27/42 histology was performed.

Results: Overall, group HIF values are related to hemoglobin and hematocrit (Rho 0.42, p = 0.003 and Rho 0.44 and p = 0.002 respectively). Median HIF-1α was 481.9 pg/ml in term, 390.3 pg/ml in preterm. In term HIF-1α correlated with pH, hematocrit and hemoglobin. In preterm HIF-1α inversely correlated with pH and Base Excess, while showing a positive correlation with hematocrit. No relation was found among HIF-1α and the placental infants or thrombi as well as the choioamnionitis.

Conclusions: HIF-1α can be detected in the umbilical cord artery. A higher level identifies premature babies in a more unstable state at birth. Moreover, patients in a marked prenatal hypoxic state (higher HIF-1α) presented a higher hematocrit, in response to erythropoietin (EPO) synthesis up regulation. No correlation was found between HIF-1α and specific postnatal acute complications or placental lesions.

Keywords: Hypoxia inducible factor; Newborn; Preterm labor

Introduction

The Hypoxia Inducible Factor-1 (HIF-1) is a transcription [1] pivotal molecule regulating cellular homeostasis in response to hypoxia. It has been shown to up regulate various downstream genes – e.g. Erythropoietin (EPO) and Vascular Endothelial Growth Factor (VEGF) – and has been implicated in the pathogenesis of several diseases involving tissue hypoxia [2–5]. The role of HIF-1α in mediating both pro-death and pro-survival responses is dependent on the duration [6] and types of pathological stimuli [7] as well as on the cell type [8]. Moreover, during prenatal development, HIF participates in multiple activities that take place in a condition of so-called physiological hypoxia, and it is fundamental in several embryonic and fetal pathways [9]. The HIFs are produced throughout all aspects of normal and abnormal placental differentiation, growth and function; both during the first trimester at the physiologically low oxygen level, and during mid and late gestation, when adequate maternal blood supply and placental oxygenation occur. Furthermore, it can be up regulated in pathological pregnancies under placental hypoxia/ischemia, or inflammation oxidative dependent pathways, as shown in eclampsia [10,11]. An enhanced placental expression of HIF genes, including VEGF, was described in pregnancies under pre-eclampsia and Intrauterine Growth Restriction (IUGR) conditions [12], which may indicate an adaptive vascular response to chronic hypoxia at the feto-placental-maternal interface. Following preterm delivery, the transition to postnatal life with the sudden increase in oxygen levels interrupts many of these pathways [13]. It is very stimulating to assess the role of the oxidative stress and complicated pregnancies [14] as well as to find some correlated diagnostic and predictive metabolic markers of neonatal diseases [15].

While low fetal oxygen levels increase blood oxygen-carrying capacity (by up-regulating the genes in erythropoiesis and iron metabolism) postnatal oxygen administration is likely to play a role in promoting anemia in the preterm newborn (by suppressing HIF activity). HIF-1α expression increases in response to hypoxia and decreases rapidly by ubiquination in response to hyperoxia. The fetus exists in a physiologically hypoxic environment and HIF-1α has been previously shown to be involved in various aspects of both normal and abnormal prenatal development and adaptation to the extra uterine environment after birth. Indeed, high HIF-1α levels were found in neonatal pulmonary hypertension [16,17]. In extremely premature infants, the lung is suddenly exposed to an environment relatively rich in O2. This sudden dramatic, or also relative, shift in O2 levels alters the hypoxia-mediated signals necessary for normal lung development to the extent that even room air may interfere with normal lung architecture [18].

Since HIF-1α is such a sensitive and early marker for hypoxia, the ability to identify HIF-1α in the cord blood and correlate levels in a reproducible manner with various pathophysioologies in the neonate for hypoxic-ischemic injury, for instance, is a good albeit difficult endeavor. Very sparse [10] data is available in the literature on the normal cord blood values of HIF and we aim to explore the possibility of detecting HIF-1α in the cord blood in healthy newborns to assess normal values. Our secondary aim is to study HIF-1α both in term and preterm newborn infants and in the latter, to find co-morbidity.

Materials and Methods

Ethical Approval And Consent

Approvals from the ethics review boards of the Padova University Hospital were obtained before data collection (Prot. N. 3083/AO14). The principal investigator presented the study at hospital rounds so that maternity practitioners and nursing staff were informed about the study protocol and consent process. To invite participation in the study, Consent forms were signed by mothers or fathers in the maternity suites.

Study Population

Women were eligible to participate in the study if they were in active labor and were likely to deliver with vaginal birth, or were planning to give birth by cesarean section. Infants were not considered eligible in case of major congenital anomalies/ malformations, assuming that the normal adaptive patterns for HIF could be impaired in any of these conditions.

Data Collection

The Authors made this observational study from January 2014
to December 2015 at the Department of Women’s and Children’s Health at the Padova University Hospital, which is among the largest and busiest maternity hospitals in North East Italy, with approximately more than 3,000 births per year. Newborns were randomly enrolled at birth. The median umbilical cord clamping time at birth was less than 120 seconds (an intermediate clamping time with regards to Kc A et al. data [19]). After the umbilical cord was cut and clamped at the two ends, from 1 to 2 ml of arterial blood were withdrawn and blood gas and HIF-1α analysis performed.

HIF-1α Assessment

Samples were processed in the Lab of the Department of Surgery, Oncology and Gastroenterology, Section of Gastroenterology, Padova University Hospital, Padova, Italy. Five milliliters of peripheral blood were collected in vacutainer tubes containing Ethylenediaminetetraacetic Acid (EDTA) as an anticoagulant for plasma isolation and centrifuged (3,000×g, 15 min) at room temperature. The plasma present in the supernatants was carefully transferred into 2-ml microtubes and stored at −20 °C until use. To assess HIF-1α concentrations we used ELISA kit on microplates (MBS703254 MyBioSource San Diego, CA). The assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for HIF-1α was coated onto a microplate. The detection range was 62.5 pg/ml – 4000 pg/ml. Hemolysis was found in some samples which could interfere with the proper assessment of the HIF-1α, as a consequence the samples that showed the colour of ruptured red blood cells were excluded from the analysis.

Blood Gases Analysis

One ml of arterial cord blood was collected using a one ml syringe (Westmed, Tucson, Arizona, USA) and analyzed with a blood gas analyzer (Rapidlab 1265, Siemens Diagnostics, Erlangen, Germany).

Placentas

The weight of the placenta was recorded in the delivery room by midwives in all cases. Random cases of placentas were submitted to the Pathology Service and histopathology analysis was carried out according to guidelines [20]. Pathologists recorded the weight, diameters of the chorionic plate, length of the cord, and any macroscopically evident alterations. A minimum of five samples per case were then embedded in paraffin for histology [25].

Clinical Characteristics of the Newborns

Gestational Age (GA) of the newborn, Birth Weight (BW) and neonatal interventions immediately after birth were recorded. Data was collected at the time of the delivery, recorded on a designed data sheet. Newborns enrolled in the study were evaluated for Hypoxic-Ischemia Injury (HII) as characterized by clinical and laboratory evidence of acute or subacute brain injury due to asphyxia [21,22]. The following characteristics were obtained from the medical record of preterm patients: maternal clinical data, antenatal administration of steroids, type of delivery (vaginal or cesarean section, CS), BW, Apgar’ Score at 1st and 5th minutes, HII [23]; Respiratory Distress Syndrome (RDS) defined as the need for supplemental oxygen and/or ventilation support with the X-ray reticular pattern and O2 positive response to the instillation of surfactant [24,25]; Bronchopulmonary Dysplasia (BPD) defined as need for supplemental oxygen at 36 postmenstrual weeks [26–28], culture proven sepsis or clinically suspected sepsis; presence of Patent Ductus Arteriosus (PDA) requiring medical treatment only or ligation; Intraventricular Hemorrhage (IVH); Necrotizing Enterocolitis (NEC) and Retinopathy of Prematurity (ROP) [29].

Statistics

Median and range data is given, and showed in scatter plots graphs. Differences were analyzed performing the Wilcoxon test or Kruskal Wallis test among groups. Spearman’s correlations were assessed to evaluate the relations among the studied variables. χ2 test was made to compare the frequency of infarcts, thrombi and chorioamnionitis, as well as the placenta weight with the HIF-1α blood concentrations. A p ≤ 0.05 was considered statistically significant; overall statistics analysis was made using the Statistics 6 for Windows.

Results

The study group included 39 full term healthy newborns and 23 preterm newborns (Table 1). Median (range) HIF-1α plasma concentration was 481.9 (80.27-1885) pg/ml in the term infants group; 390.3 (67.10-1598.2) pg/ml in the preterm (Figure 1). There was no significant difference between the two groups. No relation was found among the values of HIF-1α and GA or BW, similarly they were not correlated to the pH and pO2 levels in the overall group. Conversely, HIF values were correlated to the hemoglobin and hematocrit values (Rho 0.42, p = 0.003 and Rho 0.44 and p = 0.002 respectively) (Figure 2). Four groups of GA were arbitrarily stratified among the studied newborns, i.e. from 25 to 28, and from 29 to 32, from 33 to 37 and more than 37-week gestation.

<table>
<thead>
<tr>
<th>Term newborns (n=39)</th>
<th>Preterm newborns (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>BW (g)</td>
<td>3300 (2790-4540)</td>
</tr>
<tr>
<td>GA (wk + d/7gg)</td>
<td>38±6 (37-40)</td>
</tr>
<tr>
<td>HIF-1α (pg/ml)</td>
<td>481.9 (60.27-1885.5)</td>
</tr>
</tbody>
</table>

Table 1: Clinical data of the studied groups.

Figure 1: Scatter plot of HIF-1α blood levels in term and preterm newborns with no differences between the median values.

Similarly, stratification was made based on BW, (< 1000 g, from 1000 to 1500, from 1501 to 2500 and > 2500 grams) and placental weight expressed with percentiles (< 25%, 25–75%, > 75%) [30]. No differences were found in the above groups as regards to the HIF-1α plasma concentrations (Figure 3).

**Blood Gases and Hematocrit/Hemoglobin Analysis**

Cord blood was used to perform gas analysis at birth; results were analyzed to find a possible correlation with HIF-1α levels. Blood gases and hematocrit/hemoglobin of the studied groups are summarized in Table 2.

**Table 2.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Term Group</th>
<th>Preterm Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median pH</td>
<td>7.31</td>
<td>7.34</td>
</tr>
<tr>
<td>Range pH</td>
<td>7.11-7.42</td>
<td>6.88-7.42</td>
</tr>
<tr>
<td>Median PO2</td>
<td>17.8</td>
<td>17.0</td>
</tr>
<tr>
<td>Range PO2</td>
<td>11.5-31.8</td>
<td>8.3-25.2</td>
</tr>
<tr>
<td>Median PCO2</td>
<td>51.3</td>
<td>50.2</td>
</tr>
<tr>
<td>Range PCO2</td>
<td>37-81.8</td>
<td>41.2-89.9</td>
</tr>
<tr>
<td>Median HCO3</td>
<td>26.1</td>
<td>25.4</td>
</tr>
<tr>
<td>Range HCO3</td>
<td>22.2-30.7</td>
<td>15.6-31.0</td>
</tr>
<tr>
<td>Median BE</td>
<td>-0.35</td>
<td>-0.25</td>
</tr>
<tr>
<td>Range BE</td>
<td>-4.1-4.1</td>
<td>-16.7-5.7</td>
</tr>
</tbody>
</table>

In the term group, median pH was 7.31 (range 7.11-7.42), PO2 was 17.8 (11.5-31.8) mmHg, PCO2 was 51.3 (37-81.8) mmHg, HCO3 was 26.1 (22.2-30.7) mEq/l and Base excess (BE) was -0.35 (-4.1-4.1) mEq/l. A significant correlation with HIF-1α was only found for pH values (Rho 0.36; p < 0.05). On the same samples, median hematocrit (HTC) and Hemoglobin (Hb) values were 44 (35-51) % and 14.9 (12-16.5) g/l respectively. Both positively correlated with HIF-1α (Rho 0.45, p < 0.05 and Rho 0.46, p < 0.05) (Figure 4).

In the preterm group, median pH was 7.34 (6.88-7.42), PO2 was 17 (8.3-25.2) mmHg, PCO2 was 50.2 (41.2-89.9) mmHg, HCO3 was 25.4 (15.6-31) mEq/l, BE was -0.25 (-16.7-5.7) mEq/l. Both pH and HIF-1α plasma concentrations (Figure 3).
embedded in paraffin for archive storage, while in the other 27 cases a microscopic report was also performed. The placenta weights were referred to the normal values for the GA, as quoted by Redline RW et al. [20]. In our series, 27 cases were > the 75th percentile and 4 cases < the 25th percentile. The histology results are reported in Table 1. Regarding HIF-1α values, we arbitrarily defined “low” HIF-1α values < 250 pg/ml and “high” values > 250 pg/ml. No correlation was found among HIF-1α concentrations and BE correlated with HIF-1α (Rho -0.49; p < 0.05; Rho -0.44, p < 0.05). HTC was 44 (32-68) % and Hb was 14.8 (11-19.9) g/l, the first correlated with HIF-1α (Rho 0.43, p < 0.05) (Figure 5).

**HIF-1α Cord Blood Values and Placenta Data**

Out of all the studied newborns (n. 62), 42 placentas from mothers were then submitted to the pathology service; in only 15 a macroscopic report was recorded and 4 samples of each case were

<table>
<thead>
<tr>
<th>Blood gases</th>
<th>Term newborns (n. 39) Median (range)</th>
<th>p</th>
<th>Rho</th>
<th>Preterm newborns (n. 23) Median (range)</th>
<th>p</th>
<th>Rho</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.31 (7.11-7.42)</td>
<td>0.03</td>
<td>0.36</td>
<td>7.34 (6.88-7.42)</td>
<td>0.02</td>
<td>-0.49</td>
</tr>
<tr>
<td>pO2 (mmHg)</td>
<td>17.1 (11.5-31.8)</td>
<td>NS</td>
<td>NS</td>
<td>17 (8.3-25.2)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>pCO2 (mmHg)</td>
<td>51.3 (37-81.8)</td>
<td>NS</td>
<td>NS</td>
<td>50.25 (41.2-89.9)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HCO3 (mEq/l)</td>
<td>26.15 (22.2-30.7)</td>
<td>NS</td>
<td>NS</td>
<td>25.4 (15.6-31)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BE (mEq/l)</td>
<td>-0.35 (-4.1-4.1)</td>
<td>NS</td>
<td>NS</td>
<td>-0.25 (-16.7-5.7)</td>
<td>0.03</td>
<td>-0.44</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>44 (35-51)</td>
<td>0.04</td>
<td>0.45</td>
<td>44 (32-68)</td>
<td>0.04</td>
<td>0.43</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>14.9 (12-16.5)</td>
<td>0.02</td>
<td>0.46</td>
<td>14.8 (11-19.9)</td>
<td>NS</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 3: Stratification of HIF-1α levels by GA (A), BW (B) and percentiles of the placental weight (C) with no differences found.

Figure 4: Spearman’s correlations between A) pH and HIF-1α blood levels (Rho = 0.36, p < 0.05); B) hematocrit (%) and HIF-1α (Rho = 0.45, p < 0.05); C) blood Hb (g/dl) and HIF-1α (Rho = 0.46, p < 0.05) in the term group.
Figure 5: Spearman’s correlations between A) pH and HIF-1α blood levels (Rho = -0.49, p < 0.05); B) Base Excess (mEq/l) and HIF-1α (Rho = -0.44, p < 0.05); C) hematocrit (%) and HIF-1α (Rho = 0.43, p < 0.05) in the preterm group.

placenta weight (Figure 3). Concerning histology, no correlation was found among HIF-1α blood cord values and the number of placental infarcts, thrombi or chorioamionitis.

Analysis of the HIF-1α Cord Blood Values and Morbidity

Thirteen of the 23 preterm infants developed RDS. Median (range) HIF-1α was 420.2 pg/ml (132.9–1598.2) in the RDS group, where HIF-1α in no RDS group was 292.1 (67.1–1470.1), with no significant difference.

Discussion

Even though HIF-1α is an intracellular factor regulated under the chromatin-binding cascade, it can be detected in peripheral blood, with some sparse data reported on the topic [16,17]. In the transition from fetal to newborn life the cord artery blood reflects the actual biochemical state of the newborn. First of all, we assessed the reliability of the HIF-1α ELISA analysis from the cord blood, and we proved that this method is suitable for the assessment.

Moreover, our study shows preliminary data of normalization of the HIF-1α blood levels in the cord blood at birth. It is worth noting that a difference between term and preterm newborns was not found and no relation with the GA was shown. This result may reflect a homogeneous or steady protein level that can be assumed during the last trimester in comparison to the high values reported in the first trimester of pregnancy [31,32]. During the fetal life HIF-1α reflects an adaptation to hypoxia from the first trimester towards the second and third ones [33–36]. This is concomitant with the rise in PO₂ from 2–3% to 8–10% after 10–12 weeks of gestation [32]. HIF-1α decreases according to the rise in PO₂, although most of the controlled pathways are supposed to be already triggered.

The blood pH in the term group presented a positive correlation with the values of the HIF-1α. It is noteworthy that none of the patients in our term group required resuscitation at birth and none of them developed a marked hypoxia-ischemia status. The fetal hypoxia for the babies at birth is considered to be different from vaginal delivery or CS [37], although here it is not assumed to be meaningful to sustain differences into the two term and preterm groups. Similarly, hypoxia and chronic placental insufficiency [38] are the most important causes of intrauterine growth restriction, IUGR, during the second and third trimester of pregnancy; despite this bias, IUGR babies (2 out of the 23 preterm infants with BW < 10° percentile) were included. Since alterations in placental implantation with fetal hypoxia requiring cardiovascular adaptation [39] are reported in IUGR newborns, they are assumed to be biased in the present studied field.

The group assessed here was designed to give us information about the testability and the normal range of HIF-1α, and not yet to detect a difference between healthy and sick term patients.

In the preterm group a poor pH or BE showed a negative correlation with HIF-1α. The Authors assume that a different distribution of the pH values, hypoxia and half-life of the HIF vs. HIF values explains the different trend line in the relation between HIF and pH in preterm vs. term newborns. Furthermore, by considering the transitional physiology at birth, the preterm infants show lower Apgar scores than the term newborns. The surfactant deficiency is another example of the co-morbidity of preterm infants in comparison to the term – related to the diseases of prematurity and the RDS. Acidemia is rising at the low gestational ages, assuming an impaired placental gas exchange under the preterm births [40]. The preterm newborn that is prematurely exposed to extra uterine life may present an acute or persistent acidosis that induces the preterm delivery. This increase in HIF-1α levels at birth could be justified by a hypoxic condition, in opposition to the normoxia, which is decreasing the HIF-1α levels in about 1 min [41] of half-life. It is likely that the respiratory mechanics and breathing may be partially dependent on the HIF-1α in the transition phase of extra uterine life, similarly to what has been demonstrated for the cardiomyocyte adaptation [42]. A significant correlation was found in the term and preterm group between HIF-1α levels and red blood cells volume that is related to the erythropoietin induced by HIF-1α [9]. The low oxygen level during fetal life explains the high level of hemoglobin in newborns in contrast to the further life phases. Assuming that in the premature infant some physiological conditions are suddenly broken down, this study has attempted to evaluate whether higher HIF-1α can identify some unstable neonatal conditions, like HII, RDS or early onset sepsis. No correlation has been found to date between these diseases and HIF-1α. Future investigations are needed to study the role of HIF-1α in preterm infant diseases. For instance,
as a consequence of premature delivery, a spontaneous or assisted breathing, with invasive or non-invasive mechanical ventilation, occurs that induces a non-physiological exposure to the oxygen in the room air. The lack of hypoxia and the enrichment of carefully given O2 can decrease HIF-1α in the lung and may compromise the normal lung development [43] in the acute phase of RDS or later when chronic respiratory disease has been established [18].

Here an association between RDS and HIF-1α has been suggested, taking into account the metabolic acidosis and hypoxia in the adaptation after delivery. To date it has been unpredictable to say whether HIF-1α could predict BPD development, nevertheless some interesting suggestions can be found in recent experimental animals models [18,44–47]. Limitations of the present study consist in the limited number of the cases; as a consequence, the enrollments of some biased cases were deemed mandatory. In addition, no patients with HII syndrome were suitable that could have been different in HIF-1α values from healthy newborns.

In conclusion, the regulatory role on the HIF-1α signaling from hypoxia or normoxia after birth merits further investigation. Our study was focused on the detection of HIF-1α at birth in the cord blood. HIF-1α was not predictive in all the preterm infants diseases. A higher HIF-1α level at birth is found in those preterm babies that undergo a more challenging perinatal phase, as proven by poor gas pH and BE. Further studies in a larger population will be needed to attain generalization of the reference values and to assess whether HIF-1α at birth could identify patients at risk of acute postnatal complications.

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Conflict of interest

Authors declared no conflict of interest.

References


