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Cord blood neuroglobin in anoxo-ischemic encephalopathy syndrome

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Abstract

Anoxo-ischemic encephalopathy syndrome is frequent in neonates. This syndrome would induced anaerobic metabolism and the synthesis of neuroglobin, protein designated to protect neurons. Our aim was to investigate the relationship among parameters of diagnosis and prognosis of anoxo-ischemic encephalopathy and neuroglobin concentration in neonatal cord blood. This observational, prospective and case-control study took place from April 1, 2017 to April 30, 2018. It involved 51 newborns with anoxo-ischemic encephalopathy and 51 controls. After a clinical examination, we obtained 2 ml of umbilical venous blood sample for the determination of neuroglobin and lactates. Relationships between quantitative and qualitative variables were studied by the ANOVA test. Correlations were derived between lactate and neuroglobin. The threshold of significance was 5%. The amniotic fluid aspect was different between controls and patients (p=0.000). In the control group, the neuroglobin concentration was 3.6±1.2 ng/ml, compared to 4.1±0.8 ng/ml for anoxo-ischemic encephalopathy group (p=0.076). In addition, the lactate concentration was 1157.4±34.0 mg/dl in the control group compared to 1552.7 mg/dl for the anoxo-ischemic encephalopathy group (p=0.005). If the concentration of neuroglobin was related to the appearance of the amniotic fluid (p=0.0107), it was not, however, correlated with the lactate concentration (r=0.037, p=0.737). This study shows that the concentration of neuroglobin in cord blood does not differ between children with anoxo-ischemic encephalopathy and control ones. Therefore, this is not a valuable for the diagnosis of anoxo-ischemic encephalopathy syndrome.

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Introduction

Neuroglobin (Ngb) is a member of the globin's family (Xie and Yan, 2016;Baez*et al.,* 2016). His functions and its biochemical mechanisms are not fully understand to date (Yu*et al.,* 2012). However, due to its overexpression in case of hypoxia-ischemia (Ragy*et al.,* 2016; Van Leuven*et al.,* 2013; Li*et al.,* 2013; Jin*et al.,* 2010), most authors attribute to it neuroprotective functions, especially in cerebral affections. The association with cytochrome c, leading to the inhibition of caspase 9 stimulated by cytochrome c ferric, during ischemia and thus preventing cellular apoptosis (Tiwari *et al.,* 2018), would achieve this protection. This combination would lead to detoxification of oxygenated free radicals, reduction of apoptosis, anti-inflammatory activity and even tissue regeneration (Van Acker *et al.,* 2018). The phenomenon of hypoxia-ischemia is find in several other pathologies such as anoxo-ischemic encephalopathy that is due to a severe alteration of uteroplacental gas exchange occurring during the labor of delivery and reflecting a poor adaptation of the fetus to extra-uterine environment (Zupan Simunek, 2008; Boog, 2001). The anoxo-ischemic encephalopathy syndrome affects 2 to 4% of live births in the developed countries, while in Africa, the overall rate of anoxo-ischemic encephalopathy syndrome is 42% in hospitals (Okoko *et al.,* 2016). It is a public health problem in sub-Saharan Africa and a usualcase for hospitalization in neonatology services.

In Gabon, a study showed that anoxo-ischemic encephalopathy syndrome accounted for nearly 61.95% of neonatal admissions (Minko *et al.,* 2004). In low-income countries, such as ours, 90% of these newborns who survive will be at high risk of developing neurodevelopmental problems such as cerebral palsy, mental retardation, cognitive impairment and epilepsy (Boog, 2001). Hence the importance of diagnosis and its proper management. The diagnosis of acute fetal distress is based on evaluation of Apgar Virginia's clinical score, amniotic fluid appearance, pH measurement, and blood lactate assay (Xieet Yan, 2016). In addition to these parameters, we asked ourselves whether neuroglobin could be an element of this diagnosis. So we did this work to look for a variation of neuroglobin in case of acute fetal distress.

Materials and methods

Kind of the study

This is an observational, prospective and case-control study, that ran from April 1, 2017 to April 30, 2018 inclusive. Patient recruitment took place in the birth hall of the University Hospital Center of Libreville. The local ethics committee validated this study.

We realized it out according to Helsinki's ethical recommendations on the use of living beings (Association Médicale Mondiale, 2013). We informed parents about the work were informed of the work done and they signed an informed consent. We reassured them about data privacy.

Population used

The study involved two groups of newborns, the first consisted of 51 newborns with anoxo-ischemic encephalopathy syndrome, and the second included 51 controls. All newborns delivered on even days of the week from 9 am to 9 pm, were included. Newborns with chronic fetal distress, ultrasound abnormalities during pregnancy, or visible birth defects were not included. In the birth room, in addition to the usual care, each child had a clinical examination that began with the evaluation of the Apgar score at the fifth minute after an initial management carried out according to the case. At the end of the conditioning, we obtained 2 mL of venous umbilical blood to determine neuroglobin and lactate.

The main variables studied were the term of pregnancy, sex, weight, head circumference, brachial perimeter, thoracic perimeter, amniotic fluid aspect, Apgar score and plasma concentrations of neuroglobin and lactate. The term of the pregnancy was defined as weeks of amenorrhea (WA) stating that 7 SA +3 days corresponded to 7 SA and 7 SA + 4 days were equivalent to 8 SA. The amniotic fluid was clear, tinted, meconial or pea puree. The Apgar score

was assessed at the fifth minute of life (Table 1). The result was normal for a score greater than or equal to 7at the five minutes after the birth (Apgar, 1953).

Biologicals analysis

The neuroglobin was assayed by an ELISA technique, using an Elabscience® kit, according to the manufacturer's recommendations. Similarly, the blood lactate concentration was obtained using Randox® Lactate kits, following the manufacturer's procedures.

Statistical analysis

The data collected on a survey sheet were then entered into a Microsoft 2013® Excel file and analyzed using the Center for Diseases Control's EPI INFO 7® software. We obtained averages, proportions and standard deviations for the descriptive study. Relationships between quantitative and qualitative variables were studied by the Anova test. We used the simple correlation to look for relation between lactate and neuroglobin. The threshold of statistical significance was 5%.

Results

During the study period, 102 newborns with eligibility criteria were identified, including 51 newborns with anoxo-ischemic encephalopathy syndrome and 51 controls.

Table 1. Apgar score evaluation.

Criteria	0	1	2
Cardiac frequency	Absent	< 100 bpm	> 100 bpm
Respiration	Absent	Lowcry	Vigorous scream
Muscular tonus	Hypotonic	Bending of extremities	Flexion of the limbs
Cutaneous coloration	Whites/ blue	Cyanosis	Rose coloured
Reactivity	Nothing	Wince	Scream or active withdrawal

Female infants accounted for 45.1% (n=46/102). The amniotic fluid was clear in 42% of cases and tinted in 40.9% of cases. Its aspect was different between controls and patients (p=0.000). However, neither height nor weight showed a significant difference between these two groups (Table 2). The mean birth term was 39±2 WA and vaginal delivery was performed in 80.4% of cases. The average size of the children in the study was 49.3±2.6 cm, with an average weight of 3034±476.4 grams.

Table 2. Socio-epidemiological and anthropometric data of the working population.

Parameters studied	Control group	Patients group	p
Gender N (%)			0,326
- Female	22 (43,1)	27 (52,9)	
- Male	29 (56,9)	24 (47,1)	
Term of pregnancy (WA) (m± SD)	39,0±1,9	39,0±1,5	0,676
Presentation N (%)			0,477
- Cephalic	76 (77,6)	22 (22,4)	
- Seat	4 (100)	0	
Delivery modality N (%)			0,2271
- Lowway	62 (76,1)	20 (23,9)	
- Caesarean section	18 (88,2)	2 (11,8)	
Amniotic fluid aspect N (%)			0,0000
- Clear	24 (47,1)	13 (25,5)	
- Meconium	4 (7,8)	8 (15,7)	
- Peapuree	2 (3,9)	8 (15,7)	
- Tinted	21 (41,2)	22 (43,1)	
Height (cm) (m± SD)	49,3±2,7	49,6±1,9	0,9794
Weight (g) (m± SD)	3014,5±488,8	3104,7±436,0	0,5943
Cranial perimeter (cm) (m± SD)	32,7±1,7	33,0±1,5	0,6939
Thoracic perimeter (cm) (m± SD)	31,8±2,0	32,4±1,5	0,2299
Brachial perimeter (cm) (m± SD)	10,9±1,3	10,7±1,5	0,9245

N: Number; m±SD: medium ± standard deviation

The mean plasma concentration of Ngb (CmNgb) in the working population was 3.7±1.2 ng/ml [0.043 and 6] while that of lactate was 70±36.1 mg/ml. dl [13.6 - 228]. In the control group, the mean concentration of neuroglobin was 3.6±1.2 ng/ml, compared to 4.1±0.8 ng/ml for children with anoxo-ischemic encephalopathy syndrome (p=0.076). In addition, the average lactate concentration was 1157.4±34.0 mg/dl in the control group compared to 1552.7 mg/dl for the anoxo-ischemic encephalopathy group (p=0.005).

Table 3. Relation between the mean plasma concentration of Ngb and lactate with the parameters of the newborn in the birth room.

Parameters		Ngb (ng/ml)	p	Lactate (mg/dl)	p
Gender			0,3534		0,0513
-	Female	3,9 ± 1,9		79,0±43,1	
-	Male	3,6 ± 1,3		62,7±27,6	
Presentation			0,5726		0,9525
-	Cephalic	3,7 ± 1,1		70,1±36,4	
-	Seat	3,3 ± 1,5		66,2±32,7	
Delivery modality			0,0003		0,1720
-	Lowway	3,9 ± 0,9		72,2±37,6	
-	Caesarean section	2,8 ± 1,6		60,4±27,7	
Amnioticfluid			0, 0107		0,006
-	Clear	3,2 ± 1,3		60,1±36,8	
-	Meconium	4,4 ± 0,6		91,2±26,4	
-	Peapuree	3,6 ± 1,4		68,7±35,3	
-	Tinted	3,9 ± 0,7		75,1±35,6	

The children born vaginally had a mean Ngb concentration of 3.9±0.9 ng/ml whereas it was 2.8±1.6 ng/ml for children born by caesarean section (p=0.0003). The mean concentration of neuroglobin was related to the aspect of the amniotic fluid: 3.6±1.4 ng/ml if pea puree, 3.2±1.3 ng/ml if clear (p=0.0107) (Table 3). Nevertheless, neuroglobin was not correlated with the lactate concentration in the cord blood (r=0.037, p=0.737) (Fig. 1).

Discussion

The purpose of this work was to investigate the relationship between CNMb and the usual criteria for acute fetal distress. For this, we carried out a case-control and observational study, in which we determined neuroglobin in the cord blood of newborns with anoxo-ischemic encephalopathy syndrome and in controls, together with the lactate assay. We found that the concentration of neuroglobin was similar between two groups of newborns. Neuroglobin is a protein whose concentration rises in cases of hypoxia and acute cerebral ischemia (Ascenzi*et al.,* 2016), situations that are found in anoxo-ischemic encephalopathy syndrome. The lack of relationship between neuroglobin concentration and anoxo-ischemic encephalopathy syndrome demonstrated here might be because we obtained blood samples immediately at birth, in the cord blood, before the growing concentration of neuroglobin. In fact, Yang *et al.,* (2013) in a study of a protein similar to neuroglobin, cytoglobin, have showed that the elevation of the latter is progressive way, reaching a maximum at eighteen hours later. However, in the birth room, the resuscitation measures begin immediately, even after the result of the Apgar at the first minute, especially in the fifth minute, and usually continue beyond. As a result, these resuscitative measures, aimed at correcting maladaptation to ectopic life, may have also affected neuroglobin synthesis.

In contrast, children born by caesarean section had lower neuroglobin concentration than those born vaginally. This denotes the speed of decision-making. Indeed, children born by caesarean suffered of hypoxia for lesser time, because of the decision of the fast care. This is not necessarily true for children born vaginally, sometimes with misuse of the uterine test, any situation that may increase the duration and the intensity of hypoxia. This hypothesis is reinforced by the gradual increase in neuroglobin concentration with the aspect of the amniotic fluid, which is also a marker of fetal distress (Zupan Simunek, 2008; Boog, 2001). In this context, the slight decrease in the concentration of neuroglobin in the case of children having presented an amniotic pea puree liquid would to be due to the cellular necrosis of the brain cells of these children.

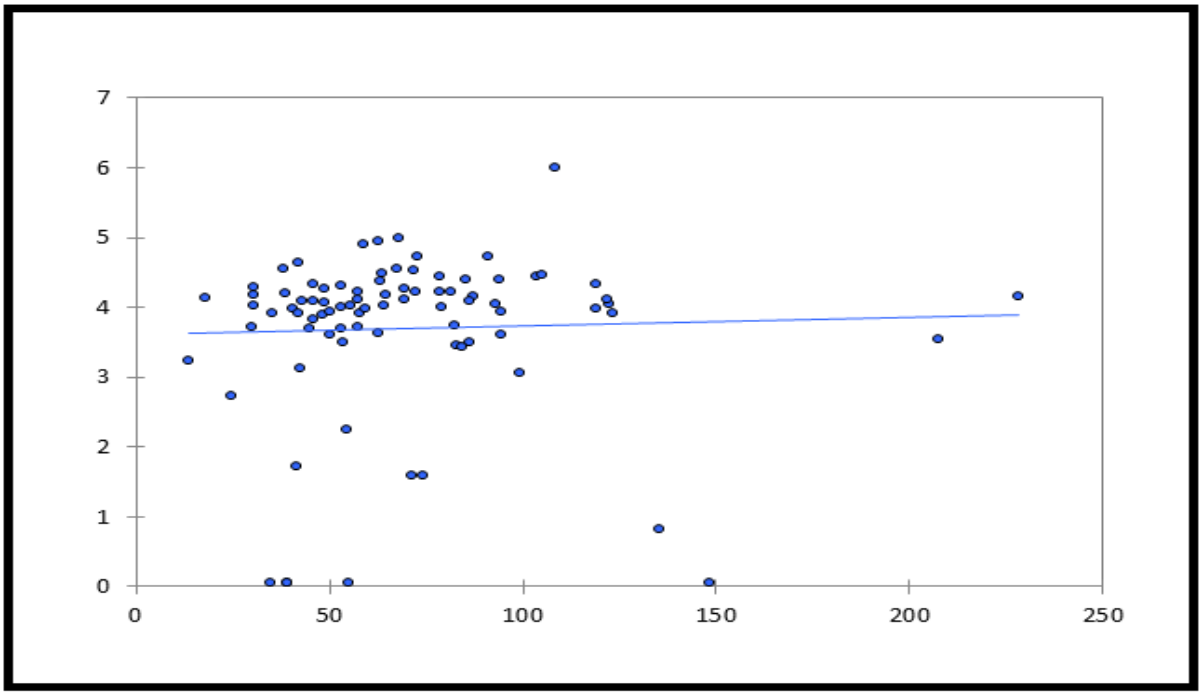

Fig. 1. Relationship between CmNgb and mean plasma lactate levels in neonates.

In addition, we did not find relationship between plasma concentration of neuroglobin and lactate concentration. We know that Lactate is a predictor of the fate of a newborn with hypoxia with at least the same efficacy as arterial blood pH (Gjerris *et al.,* 2008; Einikyte *et al.,* 2017). Therefore, if the concentration of neuroglobin is not associated with that of lactate, this assumes that cord blood neuroglobin is also unrelated to hypoxia-ischemia, which is responsible for acute fetal distress. Certainly in case of hypoxia-ischemia, one of the protective mechanisms is the reorganization of the cardiac blood flow towards the noble organs, in particular the brain (Chiang *et al.,* 2015), which can explain the modulation of neuroglobin production, whereas the rest of the tissues, switch to anaerobic metabolism, with lactate production. This lactate production is, moreover, independent of the weight of newborns (Zaigham *et al.,* 2018; Akerman *et al.,* 2018). Nevertheless, blood lactate retains its diagnostic value in this situation, including even lactate dehydrogenase, which is responsible for its production (Karlsson *et al.,* 2010). This is not the case for neuroglobin.

Conclusion

That study, whose results of which should be confirmed by the measurement of neuroglobin several hours after birth, shows that the concentration of neuroglobin in the cord blood doesn't differ between children with anoxo-ischemic encephalopathy syndrome and control children. Therefore, this

marker is not a valuable tool for the diagnosis of anoxo-ischemic encephalopathy syndrome.

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