Normal Ranges for Acute Phase Reactants (Interleukin-6, Tumour Necrosis Factor-alpha and C-reactive Protein) in Umbilical Cord Blood of Healthy Term Neonates at the Mount Hope Women's Hospital, Trinidad

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ABSTRACT

Objective: *To determine normal ranges for interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) in umbilical cord blood from healthy term neonates at the Mount Hope Women's Hospital (MHWH), Trinidad.*

Methods: *A prospective cohort study was conducted on healthy pregnant women admitted to the MHWH during the period October 1 to December 31, 2010. Women who were term with no signs of or risk factors for sepsis were recruited into the study after informed consent was obtained. Data were collected including maternal age, antenatal and perinatal history. Umbilical cord blood samples were collected and analysed for IL-6, TNF-α and CRP. Ethical approval was obtained from the Ethics Committee, Faculty of Medical Sciences, The University of the West Indies, Trinidad and Tobago.*

Results: *One hundred and sixty-two samples from healthy term neonates were analysed for IL-6 and TNF-α. One hundred and thirty-one samples were analysed for CRP due to one faulty kit. There were almost equal numbers of males (55%) and females (45%). Ninety per cent were > 2500 g at birth and 10% had low birthweight. Reference ranges of 0–16.4 pg/mL, 0–29.4 pg/mL and 0–12.4 mg/L were found for IL-6, TNF-α and CRP, respectively, with 95% confidence intervals (11.6, 21.5 pg/mL; 24.0, 33.1 and 8.4, 15.1, respectively).*

Conclusion: *The normal ranges for IL-6, TNF-α and CRP in umbilical cord blood for healthy term Trinidadian neonates were 0–16.4 pg/mL, 0–29.4 pg/mL and 0–12.4 mg/L, respectively.*

Keywords: Acute phase reactants, cord blood

Rangos Normales de los Reactantes de Fase Aguda (Interleucina 6, Factor Alfa de Necrosis Tumoral, y Proteína C Reactiva) en la Sangre del Cordón Umbilical de Recién Nacidos a Término Sanos en el Hospital de Mujeres Mount Hope de Trinidad

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RESUMEN

Objetivo: *Determinar los rangos normales de interleucina 6 (IL-6), factor alfa de necrosis tumoral (TNF-α), y proteína C reactiva (PCR) en la sangre del cordón umbilical de neonatos a término sanos en el Hospital de Mujeres "Mount Hope" (MHWH) de Trinidad*

Métodos: *Se realizó un estudio de cohorte prospectivo en mujeres embarazadas sanas ingresadas en el MHWH durante el período del 1ero. de octubre al 31 de diciembre de 2010. Las mujeres que estaban a término sin signos de sepsis o factores de riesgo de sepsis, fueron reclutadas para el estudio, tras obtenerse consentimiento informado. Los datos recogidos comprendían edad materna, historia prenatal y perinatal. Se recogieron y analizaron muestras de sangre para el estudio de TNF-α, IL-6 y la PCR. Se obtuvo aprobación ética de la Facultad de ciencias médicas, del Comité de ética de la Universidad de West Indies, Trinidad y Tobago.*

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Resultados: *Se analizaron muestras de ciento sesenta y dos de recién nacidos a término sanos para investigar la IL-6 y el TNF -α.Ciento treinta y una muestras fueron analizadas para investigar la PCR debido a un kit defectuoso. Hubo un número casi igual de varones (55%) y hembras (45%). El noventa por ciento pesó > 2500 g al nacer y 10% tuvo bajo peso al nacer. Se hallaron rangos de referencia de 0 – 16.4 pg/mL, 0 – 29.4 pg/mL y 0 – 12.4 mg/L para TNF -α, IL-6 y PCR, respectivamente, con intervalos de confianza del 95% (11.6, 21.5 pg/mL; 24.0, 33.1 y 8.4, 15.1, respectivamente).*
Conclusión: *Los rangos normales de TNF -α, IL-6 y PCR en la sangre del cordón umbilical de los neonatos trinitarios nacidos a término sanos, fueron 0 – 16.4 pg/mL, 0 – 29.4 pg/mL y 0 – 12.4 mg/L, respectivamente.*

Palabras claves: Reactantes de fase aguda, sangre del cordón

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INTRODUCTION

The 2010 World Health Organization statistics reported a neonatal mortality rate of 24 per 1000 live births for Trinidad and Tobago, seven per thousand for Barbados and nine per thousand for Jamaica. The neonatal mortality rate for Trinidad is six times that reported for the United States of America [USA] (four per thousand) and eight times that reported for the United Kingdom [three per thousand] (1). Neonatal sepsis has been cited as a leading cause of perinatal mortality (2). Neonatal sepsis was cited as being the fifth (7%) leading cause of death among children less than five years old in Trinidad and Tobago. The 7% mortality from neonatal sepsis in Trinidad is reported as the highest when compared to that of the USA (3%), United Kingdom (1%), Barbados (5%) and Jamaica [1%] (1).

Interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), C-reactive protein (CRP) and several other cytokines have been investigated for their role in the inflammatory process and in the prediction of early onset neonatal sepsis (2–15). Interleukin-6 and TNF-α have been identified as being responsible for the induction of the acute phase response and production of cytokines and CRP from hepatocytes (3). Interleukin-6 levels have been reported to rise early in infection, probably as a result of stimulation by TNF-α. The fetus is able to produce CRP and other acute phase reactants as early as four to five weeks of gestation (13). Paired mother and infant sampling showed that CRP does not cross the placenta (13). The 1998 Cancer Letters reported that TNF-α does not cross rat placenta. Bell *et al* (16) reported an incidence of 6.7/1000 live births in culture proven sepsis in Jamaican neonates. A neonatal sepsis rate of 10/1000 live births was reported by Ali (17) at the Mount Hope Women's Hospital (MHWH), Trinidad. A literature search did not reveal any study reporting values for acute phase reactants in umbilical cord blood in Trinidad or the Caribbean region. The aim of this study was to determine ranges of IL-6, TNF-α and CRP in healthy term neonates.

SUBJECTS AND METHODS

Ethical approval was obtained from the Ethics Committee, Faculty of Medical Sciences, The University of the West Indies, St Augustine, Trinidad and Tobago. A prospective cohort study was conducted among healthy term neonates delivered at the MHWH. Convenience sampling was used and women from whom informed consent was obtained were recruited on admission to the delivery suite at the MHWH during the period October 1 to December 31, 2010. All women at term (≥ 37 weeks gestation), with no identifiable risk factors for neonatal sepsis were eligible. Women with risk factors for sepsis: preterm labour and delivery (< 37 weeks gestation), prolonged rupture of membranes (> 24 hours), maternal pyrexia > 38 °C, elevated maternal white blood cell count (> 15) and chorioamnionitis were excluded from the study. Baseline data on maternal demographics, relevant antenatal history (parity, blood group and rhesus, HIV and VDRL status and co-morbidities), perinatal history (period of gestation, time to rupture of membranes, mode of delivery and nature of liquor), and neonatal data (gender, APGAR scores at one and five minutes, and birthweight) were collected on a data collection sheet.

Umbilical cord blood was collected within five minutes of delivery from the fetal side of the cord in an EDTA vacutainer and plain vacutainer. A complete blood count was performed on the EDTA sample. The clotted samples were centrifuged, sera were separated using disposable pipettes and stored in coded microsample tubes at -70 °C. Human enzyme-linked immunosorbent assay (ELISA) test kits (Anogen, a division of Yes Biotech Laboratories Limited, Ontario, Canada) were used for analysis of IL-6, TNF-α and CRP according to the manufacturer's instructions (18–20). The ELISA kits apply a technique of quantitative sandwich immunoassay. The optical density of the standards in each kit was used to plot standard curves for each test kit using Microsoft Excel and a formula generated to calculate respective concentrations of acute phase reactants from the absorbances obtained.

Data analysis was performed using Stata version 11.0. All test results were considered significant at the p value ≤ 5% level. The distributions were characterized by a marked rightward skew and Tukey's outlier filter was used to adjust the population range for outliers. Reference range lower limit was taken as the 5th centile and upper limit as the 95th centile of the adjusted population.

RESULTS

A total of 162 cord blood samples were collected. Results for IL-6 and TNF-α were obtained for all 162 samples. Unfortunately, due to one faulty CRP test kit, only 131 results were obtained for CRP. Baseline characteristics of the sample population are shown in Table 1. Most mothers (66%) were in the 19–30-year age group, only 37% were primigravida, 39% were nulliparous and 43% had one or more miscarriages. Ninety-two per cent were born *via* normal spontaneous vaginal delivery and 88% had clear liquor at birth. There was a male predominance (male, 55% to female, 45%) and the majority of neonates (90%) were appropriate for gestational age, with 10% being low birthweight.

Table 1: Baseline characteristics of the sample population: n = 162

Characteristic	Category	% of women (n)
Maternal age (years)	≤ 18	6 (10)
	19–30	66 (107)
	> 31	28 (44)
Live births	0	39 (63)
	1	37 (60)
	2	10 (17)
	≥ 3	14 (22)
Miscarriages	0	57 (92)
	1	31 (51)
	2	8 (13)
	≥ 3	4 (6)
Mode of delivery	NSVD	92 (149)
	Assisted delivery (forceps)	1 (2)
	Elective C-section	1 (1)
	Emergency C-section	6 (10)
Liquor nature	Clear	88 (142)
	MSL Grade 1	8 (13)
	MSL Grade 3	2 (3)
	Offensive	2 (4)
Neonatal gender	Male	55 (89)
	Female	45 (73)
1-minute APGAR score	8–10	87 (141)
	5–7	13 (21)
5-minute APGAR score	8–10	99 (161)
	5–7	1 (1)
Birthweight (grams)	> 2500	90 (146)
	2001–2500	9 (14)
	1501–2000	1 (2)

NSVD = normal spontaneous vaginal delivery
MSL = meconium-stained liquor

A low one-minute APGAR score (< 5) was associated with higher cord blood IL-6 (p = 0.0280). There was no statistically significant association between cord blood IL-6 values and maternal age, mode of delivery, meconium staining of liquor, gravidity, number of live births, number of miscarriages, gender of the neonate or birthweight.

Assisted delivery (Caesarean section and forceps) and low birthweight were associated with elevated cord blood TNF-α (p = 0.0014 and p = 0.024, respectively). There was no statistically significant association between cord blood TNF-α values and maternal age, meconium staining of liquor, number of live births, number of miscarriages, gravidity, gender of the neonate and one-minute APGAR score.

There was no statistically significant association between cord blood CRP values and maternal age, number of live births, number of miscarriages and gravidity, rhesus groups, mode of delivery, meconium staining of liquor, birthweight, gender of neonate or one-minute APGAR score. Table 2 shows the calculated ranges together with 95% CIs for the upper and lower limit of the ranges. The upper limit cut-off values for IL-6, TNF-α and CRP were 16.4 pg/mL, 29.4 pg/mL and 12.4 mg/L, respectively. The mean values for IL-6, TNF-α and CRP were 6.0 pg/mL, 5.4 pg/mL and 6.5 mg/L, respectively (Table 2).

DISCUSSION

In order to interpret responses in IL-6, TNF-α and CRP to infectious and non-infectious conditions, it is first necessary to determine a reference range for these substances in healthy Trinidadian neonates.

Analysis of the results revealed a normal range of 0–16.4 pg/mL for IL-6 (95% CIs). This range of values is similar though slightly lower than those of 20 pg/mL and 31

Table 2: Ranges for acute phase reactants in umbilical cord blood, mean values and 95% confidence intervals (CI)

Acute phase reactant	Reference range		95% CI of lower limit	95% CI of upper limit	% considered as outliers*	n	Mean value
	Lower limit (5^{th} centile)	Upper limit (95^{th} centile)					
IL–6 pg/mL	0	16.4	0.0, 0.0	11.6, 21.5	15.4%	162	6.0
TNF-α pg/mL	0	29.4	0.0, 0.0	24.0, 33.1	6.8%	162	5.4
CRP mg/L	0	12.4	0.0, 0.0	8.43, 15.1	7.6%	131	6.5

IL-6 = interleukin-6, TNF-α = tumour necrosis factor-alpha, CRP = C-reactive protein

Table 3: Comparison of cut-off values for IL-6, TNF-α and CRP used in various studies and the sample size used in analysis

Study	IL-6 cut-off (pg/mL)	TNF-α cut-off (pg/mL)	CRP cut-off (mg/L)	No. of controls
Present study	**16.43**	**29.4**	**12.4**	**162 (131 for CRP)**
Santana Reyes *et al*, 2003 (10)	30	3.5	1.52	20
Kowalik *et al*, 2003 (11)	–	15	–	36
Fida *et al*, 2006 (12)	11.43	29.86	4.02	16
Arsenescu *et al*, 2011 (14)	–	61.5	–	40
Khassawneh *et al*, 2007 (2)	19.9	–	1.0	54
Celik *et al*, 2010 (15)	24.65	–	4.82	50
Kocabas *et al*, 2007 (6)	3.6	7.5	10	29

IL-6 = interleukin-6, TNF-α = tumour necrosis factor-alpha, CRP = C-reactive protein

pg/mL found by Onal *et al* (8) and Ng *et al* (9), respectively. A Jordanian study (2) used 18.2 pg/mL as the cut-off for sepsis, with a sensitivity of 87% and specificity of 50%. These previous studies used a sample size of 50 patients or less which may account for the possible over-estimation found. This study evaluated at least three times this number (n = 162).

A range of 0–29.4 pg/mL was found for TNF-α in this study. The range of cut-off values varied in previous studies from 3.5 pg/mL to 61.5 pg/mL, as illustrated in Table 3. Fida *et al* reported a value of 29.86 pg/mL (12) which was closest to our cut-off of 29.4 pg/mL. The sample size was 40 in Arsenescu *et al* (14) and included both full-term and preterm neonates whom they deemed healthy. Our study included only healthy term neonates as prematurity in itself is a risk factor for sepsis.

Unfortunately, one of the CRP kits purchased was faulty so values for only 131 samples were analysed. Results showed a normal range of 0–12.4 mg/L for CRP (95% CIs) in the umbilical cord blood of healthy term neonates who were discharged with no clinical signs of sepsis. In the Neonatal Intensive Care Unit, MHWH, a cut-off of 10 mg/L for CRP is routinely used as a marker for sepsis. Table 3 illustrates the varying range of CRP from as low as 1.0 mg/L (2). This present study has analysed at least three times the number of healthy non-septic neonates as previous studies.

One drawback of this study was that the samples were not pre-aliquotted into three separate aliquots and thus underwent two freeze/thaw cycles which may have resulted in degradation of acute phase reactants. It is hoped that by establishing reference ranges these may be used in future studies on neonatal sepsis.

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