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Cord Blood Procalcitonin Levels of Normal Neonates Born At a Tertiary Health Care Center

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ABSTRACT – Procalcitonin(PCT), a precursor of hormone of calcitonin, is a pro-inflammatory marker released in response to inflammatory stimuli, especially bacterial sepsis. There are many studiesregarding the use of PCT as a marker for sepsis. However, there are very limited studies in India related to PCT levels in cord blood of septic as well as healthy neonates. The current studyis to find the level of cord blood PCT in healthy neonates.

AIM: To know the Cord blood pro-calcitonin concentration in healthy neonates born to mothers with no risk factors for development of early onsetneonatal sepsisandto study the influence of various maternal factors like parity, mode of delivery and gender of the baby on pro-calcitonin levels.

METHODS: One hundred and thirty-two randomly selected healthy Indianwomen in late pregnancy were included in the study. Pro-calcitonin levels were measured in the umbilical cord blood collected at birthby ELISA. The Umbilical cord blood PCTmean, and median concentration were calculated.

RESULTS: The mean umbilical cord blood PCT of 132 neonates was found to be 2.09ng/ml (0.05-3.45ng/ml) andmedian was 0.8ng/ml.

CONCLUSION: Cord blood PCT levels in normal neonates was found to be higher than that observed in published literature. The factors like parity and mode of delivery in the mother; the birthweight and gender of neonate did not have an influence on the PCT concentration in normal neonates although they are known to affect the PCT levels in neonates at risk of development of EONS.

I. INTRODUCTION:

The term sepsis was derived from Greek word "σηψις" first mentioned in poems of Homer about 2700 years ago and means "Decomposition" or "Putrefaction".[(1)]When pathogenic organismsenter blood stream and cause a devastating systemic inflammatory response(SIRS)[(2)] in neonate within the initial 28days[(3)] of birth it is termed as neonatal sepsis[(2)]. If sepsis sets in within initial 72hours of life[(4)], it is termed as Early onset neonatal sepsis(EONS) and it is termed Late onset sepsis (LOS) if the sepsis sets in after 72hours of birth. [(3)(5)(6)]

Sepsis is found to be commonest cause of neonatal mortality in developing countries and accounts to 30 - 50%[(7)]of neonatal deaths every year.[(8)(9)(10)]The incidence of neonatal sepsis was 30 per 1000 live births according to the National Neonatal Perinatal Database report of 2002-2003[(11)].Further, of all neonatal deaths reported in India, 19% is contributed by neonatal sepsis[(11)]

Blood culture from peripheral venous bloodis considered as the gold standard for diagnosis of sepsis[(12)(13)]. However, in neonates blood culture was found to have a low sensitivity due to inadequate volume of blood collected by painful and difficult phlebotomy of peripheral veins[(12)], intermittent bacteraemia or use of prophylactic antibiotics before blood was obtained for culture[(12)(13)(14)]. This warrants for an alternative diagnostic test that can be used as a biomarker for sepsis.

BIOCHEMISTRY OF PROCALCITONIN:

Existence of Pro-calcitonin, a 116-amino acid precursor of the hormone calcitonin was suggested by Moya et al[(15)] in 1975 in chicken. It belongs to the calcitonin (CT) superfamily of peptides and has a molecular weight (MW) of about 13kDa[(16)]. It is divided into three sections i.e. the amino terminus of the PCT region, immature CT, and CT carboxyl-terminus peptide-1 (CCP-1, also called katacalcin). In healthy individuals, pre-pro-calcitonin (the precursor of Calcitonin from CALA-1 gene, located on chromosome-11) following post-transcriptional modification produces Calcitonin that is stored in the Para-follicular C cells of Thyroid gland. Following endocrine stimulation, the stored calcitonin is released into circulation that is involved in calcium and phosphorus homeostasis[(15)].In healthy subjects, the PCT concentration in blood is as low as 0.05ng/ml.[(15)(17)]

During bacterial sepsis PCT is produced by alternate pathway by non-endocrine cells under

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direct influence of bacterial endotoxins/Lipo-polysaccharide (LPS) or indirectly by various inflammatory mediators like Interleukin-6 (IL-6) or Tumour necrosis factor alpha (TNF-α)[17]. These elevated levels of PCThave been tested as a predictor for sepsis[18] over recent years.

There have been few studies conducted on the Umbilical cord blood PCT as a biomarker of EONS. Umbilical cord bloodPCT concentration may be affected by the mode of delivery, and gender of the neonate. Itmay also be affected by the parity status of the mother.However, there are very few studies thatprovide umbilical cord blood PCT concentrations in healthy neonates born to mothers without any risk factors for development of EONS in India.

Hence, the present study was undertaken to find the cord blood PCT concentration in neonates born to mothers with no risk factors for development of EONS and to know the effects of various factors like gender of the neonate, mode of delivery and the parity status of the mother on Cord blood PCT levels. These finding will help in knowing the normal levels of PCT that will be useful in interpreting the significance of raised cord blood PCT values in the diagnosis of sepsis.

II. MATERIALS AND METHODS:

Present study was undertaken in the department of Microbiology of a tertiary health care centre in Maharashtra. Study was commenced after receiving clearance from the Institutional ethics committee. All theparticipating mothers were given prior information about the nature of the study and the sampleswere collected after consent.

One hundred and thirty-two randomly selectedpregnant women with no risk factors for development of EONS were selected. Cord blood was collected immediately after delivery with all standard aseptic precautions. Cord was clamped at 2 sites and cut in between immediately after delivery of neonate.Length of cord was selected on the maternal end of the cord to draw blood. Area was painted with isopropyl alcohol[3][12]and allowed to dry. By a sterile needle, 5ml of blood was drawn from the umbilicalartery/vein and collected into red top plain bulb. Blood was allowed to clot. Following centrifugation, serum was separated and stored -20^{o}C until further processing.

Cord blood serum PCT was estimated by commercially available human PCT sandwich ELISA kit (Elabscience Biotechnology Inc. USA). Test was performed as per manufacturers' instructions. Serum was diluted with the diluent provided in the kitin the ratio 1:10.The optical density (OD) was measured by spectrophotometry at a wavelength of 450 nm ± 2 nm. OD value was calculated as per kit instruction. Final concentration of PCT was then calculated.

III. RESULTS AND OBSERVATIONS:

TABLE 1: Showingthe distribution of neonates based on the birth weight, parity status of the mother, mode of delivery and gender of the neonate

Birth weight in grams	Total samples	Primi-gravida	Multi-gravida	Normal delivery	LSCS delivery	Male	Female
1501-2000 VLBW	2	2	0	2	0	2	0
2001-2500 LBW	52	27	25	48	4	26	26
>2500	78	33	45	69	9	39	39
Total	132	62	70	119	13	67	65

In above table it is observed that most of the neonates had a birth weight of >2500gms, which is considered to be a healthy weight. The number of primi-parous mother and multi-parous mothers was almost same. Majority of neonates were born by a normal vaginal delivery. It is also observed that number of male neonates is almost equal to that of female neonates.

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TABLE 2: Showing the comparison of mean cord blood PCT of neonates with respect to gender, mode of delivery, parity status of the mother and the birthweight of the neonate

Maternal group	Total samples	Male	Female	Normal deliver	LSCS delivery	Primi-gravida	Multi-gravida	Birth weight in grams
No.	132	67	65	119	13	62	70	132
Mean PCT value (ng/ml)	2.09	1.62	2.45	2.15	1.52	1.79	2.35	2668.1

In the above table it is observed that the mean cord blood PCT of all the neonates included in this study was 2.09ng/ml. Mean cord blood PCT concentration was also calculated for various parameters like gender of neonate, parity status of the mother and mode of delivery.

TABLE 3: Shows the comparison of mean cord blood PCT of neonates with respect to gender of the neonatewith P value

Groups	Females	Males	Unpaired t-test	P value
Mean PCT	2.45	1.65	1.478	0.142
Sd	3.752	2.262		

In above table the mean cord blood PCT was compared between the male and female neonates. A difference can be observed in the mean PCT concentrations of cord blood of males and female neonates. However, as per the P values calculated, this is not statistically significant.

TABLE 4: Showing the comparison of mean cord blood PCT of neonates with respect to mode of delivery and their P value

Groups	Normal	Caesarean	Unpaired t-test	P value
Mean PCT	2.15	1.52	0.691	0.491
Sd	3.233	1.827		

In above table the mean cord blood PCT of neonates born normally was compared to those born by caesarean section. It was observed that the mean PCT concentrations of cord blood of neonates born by normal vaginal delivery was higher than those born by caesarean section. However, as per the P values calculated, it was not found to be statistically significant.

TABLE 5: Shows the comparison of mean cord blood PCT of neonates with respect to parity of the mother and their P value

Groups	Primi-gravida	Multi-gravida	Unpaired t-test	P value
Mean PCT	1.79	2.35	1.022	0.309
Sd	2.535	3.563		

In above table the mean cord blood PCT of neonates born to primi-gravida mothers was compared to those born to multi-gravida mothers. It was observed that the mean PCT concentrations of cord blood of neonates born to multi-gravida mothers was higher than those born to primi-gravida mothers. However, as per the P values calculated, it was not found to be statistically significant.

IV. DISCUSSION:

Early onset neonatal sepsis is a clinical syndrome that leads to systemic inflammatory response in the neonates[(2)] within the initial few hours of life[(17)]. It is a leading cause of neonatal morbidity and mortality inIndia. Many perinatal risk factors seen in the mother predispose neonates

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to sepsis, which can be prevented if these factors aredetected and treated on time.

In neonates, the signs and symptoms of sepsis are non-specific[7][19]. Any delay in initiation of appropriate treatment can be detrimental, hence, laboratory investigations are required for the early diagnosis of neonatal sepsis.

Culture isthe gold standardfor diagnosing sepsis[20]which usually takes 48 to 72 hours[7]. The most commonly used sample for culture in neonates in peripheral venous blood. In clinical practice, very frequently, signs and symptomsof sepsis manifest themselves in the absence of a positive culture[17]. Culture has poor sensitivity and specificity and becomes unreliable. In neonates the inadequate volume of peripheral venous blood collected for culture is the main reason for this low sensitivity and specificity[12][13][14][21]. Other causes can be intermittent bacteraemia or administration of antibiotics before collection of blood for culture[22].

Blood flowing in the umbilical cord is neonatal blood[23]. There are studies that show that the cord blood can be easily be substituted for infant blood for routine sepsis evaluations of neonates[21]. It can be collected easily in adequate volume when compared to peripheral venous blood and that to without any pain to the neonate[13][21].

Procalcitoninis a valuable marker of bacterial sepsis in adults[24][25]**.** Review of literaturereveal studies that use peripheral venous blood in neonates to determine PCT levels[25][26]but there are hardly any studies on cord blood PCT levels in Indiato give a cut-off level of cord blood PCT in Indian population

Present study was undertaken to know the cut-off level for cord blood PCT with respect to gender of the neonate, mode of delivery and the parity status of the mother and whether these factors affected the mean cord blood PCT.

In the present study, there were 50.75% (67/132) male neonates, and 49.25%(65/132) neonates were female. The number of males and female neonates was almost equal.

As observed in table 1 most of the neonates had birth weight above 2000grams. The mean cord blood PCT values observed in this study was 2.09ng/ml(0.05-3.45ng/ml) and the calculated median cord blood PCT was 0.8ng/ml

In a study by StrankZbyneket al., 2016,[27] it was observed that the mean cord blood PCT was 0.23ng/ml (± 0.1ng/ml) which was lower than that observed in this study.

In another study by Kordek et al. 2003,[16]the median cord blood PCT in healthy neonates was found to be 0.79ng/ml (0.58-1.62ng/ml) which was also found to be same as that observed in this study.

A study conducted by Johramet al. 2011[19] it showed that the median cord blood PCT was 0.16ng/ml which was lower than that was observed in this study.

However, the higher mean values observed in present study compared to other studies mentioned above may be due to small sample size in present study or the method of testing.

In this study it was observed that the mean cord blood PCT of male neonates was 1.65 ng/ml and it was 2.45ng/mlin female neonates. P value by unpaired t-test was 0.142.

The mean cord blood PCT of neonates delivered normally was 2.15 ng/ml and it was 1.52ng/ml in neonates delivered by Caesarean section. P value by unpaired t-test was 0.491.

Also, the mean cord blood PCT in neonates born to primi-parous mothers was 1.79 ng/ml and those of multiparous mothers was 2.35 ng/ml. P value by unpaired t-test was 0.309.

However, we can note that according to the P values calculated, these differences in Mean cord blood PCT concentrations with respected to the gender, parity status of mother or mode of delivery was not statistically significant.

TABLE 6: Shows the comparison of mean and median cord blood PCT concentrations in present study to other studies

Study parameters	**Present Study**	ZbynekStrank et al. 2016	Kordek A et al. 2003	Johram N et al. 2011
Mean cord blood PCT	2.09ng/ml	0.23ng/ml	-	-
Median cord blood PCT	0.8ng/ml	-	0.79ng/ml	0.16ng/ml
Mean PCT in male neonates	1.65ng/ml	-	-	-
Mean PCT in female	2.45ng/ml	-	-	-

neonates				
Mean PCT in neonates delivered normally	2.15ng/ml	-	-	-
Mean PCT in neonates delivered by caesarean	1.52ng/ml	-	-	-
Mean PCT in neonates born to primi parous mothers	1.79ng/ml	-	-	-
Mean PCT in neonates born to multi parous mothers	2.35ng/ml	-	-	-

Most of the above-mentioned studies do not mention the effect of gender, mode of delivery or parity status of the mother on the cord blood PCT in healthy neonates born to mothers with no risk factors for development of EONS. However, it was observed that the difference in mean cord blood PCT with respect to gender of neonate, mode of delivery or parity status was not statistically significant.

V. CONCLUSION

The present study shows that, the mode of delivery, gender of neonates or the parity status of the mother with no risk factors for development of EONS had no effect on cord blood PCT concentration of healthy neonates. Unless we know the range of normal PCT concentration in cord blood of healthy neonates, interpretation of cord blood PCT in clinically suspected cases of EONS will be difficult.However, the higher mean values observed in present study may be due to small sample size compared to other studies or the method of testing.

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