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Research Article

Interpretation of 17-hydroxyprogesterone levels in early neonatal period by dissociation-enhanced lanthanide fluorescent immunoassay technique in a tertiary care centre

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ABSTRACT

Background: Mass screening for CAH is controversial worldwide because of the low positive predictive value. The cut off levels of 17 OHP in NBS are based on birth weight and or gestational age. Both RIAs and ELISAs have been almost replaced in most European countries by DELFIA. We used DELFIA technique. The aim and objective was to determine optimal cut off values of 17 OHP levels in early neonatal period based on gestational age and birth weight.
Methods: The study was conducted in the neonatal unit of RSRM Hospital Stanley Medical College as Prospective cross sectional study. All new borns with a gestational age of 34 weeks or more were included in the study. Sample was collected after getting informed consent after 48 hours of age till 7 days of age. 1695 babies who met the inclusion criteria were enrolled, the blood sample were collected by heel prick on filter paper. Neonates less than 34 weeks of gestational age, babies mothers who had received antenatal steroids, babies who had received blood transfusion prior to sampling and refusal of consent were excluded
Results: As the gestational age increases the mean 17 OHP values declines and plateaus at 37 weeks and beyond. Similarly analysis based on weight shows a decline in mean values of 17 OHP with increasing birth weight and plateaus from 2500 gm.
Conclusions: The study demonstrates clearly that there is linear trend in mean 17 OHP values in relationship to gestational age when compared to birth weight.

Keywords: 17 OHP mean values, Neonatal screening, DELFIA

INTRODUCTION

Congenital adrenal hyperplasia is one of the life threatening disorders in neonates. Neonatal screening program for congenital adrenal hyperplasia has been established in many countries.[1] An increased 17 OHP concentration in heel-prick blood is used to indicate patients at risk of having CAH. Screening for CAH using 17 OHP helps in preventing morbidity and mortality associated with hypoglycemia. Salt loss, dehydration and life threatening adrenal crisis.[1-7]

It is known that preterm newborns have higher 17 OHP concentrations in serum.[1,3] Therefore, cut off levels are based on birth weight (BW) or on gestational age (GA). In some of the countries the cut off values are based on birth weight and in other countries it is based of gestational age.[1] Very few studies have been performed to correlate 17 OHP values with gestational age and birth weight.[1,4]

For the determination of 17 OHP in dried filter paper, the auto-DELFIA neonatal 17 OHP assay (PerkinElmer Life and Analytical Sciences) was used.[2]

In our community data on 17 OHP values are not available to interpret the screening test based on either gestational age or birth weight. This study would help in finding the mean base line values that can be used to arrive at appropriate cut off values.

METHODS

This prospective cross sectional study was conducted in neonatal unit of RSRM lying in hospital-Stanley Medical College, Chennai, India from March to May 2012. The RSRM Lying in hospital is the obstetric and neonatal unit attached to government Stanley medical college hospital, Chennai in Tamilnadu which caters to the middle and lower income population with mother and neonatal care services.

All newborns with gestational age of 34 weeks or more delivered in the hospital were included in the study.

Exclusion criteria were neonates who were less than 34 weeks of gestational age, babies mothers who had received antenatal steroids, babies who had received blood transfusion prior to sampling and refusal of consent.

Sample was collected after getting informed consent after 48 hours of age till 7 days of age.

1695 babies who met the inclusion criteria were enrolled in the study from which sample was collected by proper aseptic precautions. The blood sample was collected by heel prick on to a filter paper and 17 OHP levels estimated by dissociation enhanced lanthanide fluorescent immunoassay (DELFIA).

The data of term, preterm, birth weight, 17 OHP values were tabulated analyzed and interpreted.

RESULTS

In our study total number of babies was1695-term 1588 and preterm 107 (Table 1). Term with birth weight more than 2500 gm babies were 1383. The mean 17 OHP value of this group is 3.9 nmmol/L and remaining 205 babies belong to Term with birth weight less than 2500 gms and their 17 OHP mean value was 5.3 nmmol/L concentration (Table 2 and Figure 1).

Among preterms 33 babies were more than 2500 g and their mean value is 7.9 nmmol/L and preterms less than 2500 g were 74 and their the mean value is 11.3 nmmol/L (Table 3).

Stratification analysis based on birth weight grouped under 4 categories 3000 gm and above were 670 and their mean 17 OHP value was 3.8 nmmol/L. 2500 to 2999 gm were 746 and their mean value was 4.1 nmmol/L. 2000 to 2499 gm were 236 and their mean value 5.8 nmmol/L. 1500 to 1999 gm were 43 and their mean value is 12.8 nmmol/L (Table 3, Figure 2).

Table 1: Gestational age-term/pre term distribution.

GA	Frequency	Percent
37 weeks and above	1588	93.7
<37 weeks	107	6.3
Total	1695	100.0

We infer there is 6.3% of preterm babies.

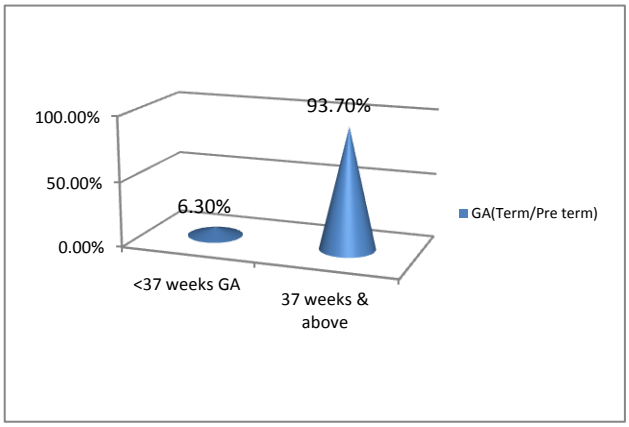

Figure 1: Gestational age-term/pre term distribution.

Table 2: Birth weight distribution.

Birth weight	Frequency	Percent
2500 gms and above	1416	83.5
<2500 gms	279	16.5
Total	1695	100.0

From above we infer that 16.5% are low birth weight babies.

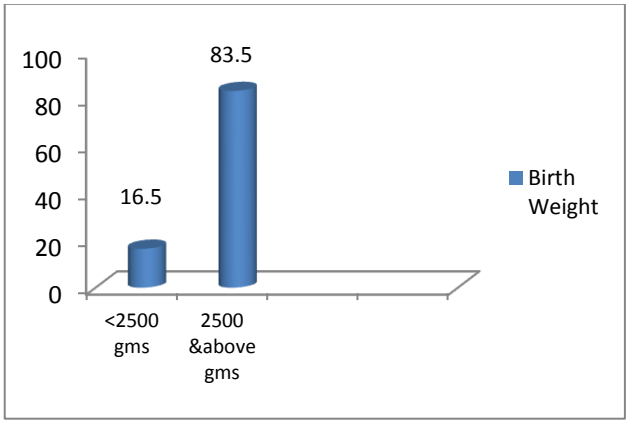

Figure 2: Birth weight.

Table 3: Group statistics.

Birth weight	N	Mean 17OHP (nmol/L)	Std. deviation	Std. error mean	P
Term <2500 gm	205	5.2584	4.61024	0.32199	0.000< 0.001 Significant.
Term >2500 gm	1383	3.8898	2.98703	0.08032	

This reveals that the 17 OHP mean values of low birth weight babies are increased than the normal birth weight babies in Term group. The statistical significance exists between both.

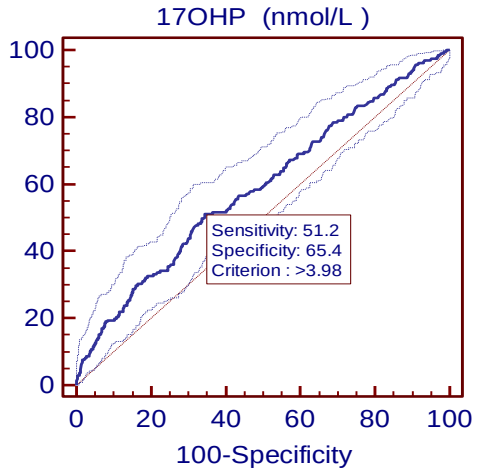

Figure 3: ROC curve.

Table 3A: ROC curve.

Variables	17OHP (nmol/L)
Classification variable	Birth weight
Sample size	1588
Term <2500 gm	205
Term >2500 gm	1383

Table 3 B: Area under the ROC curve (AUC).

Area under the ROC curve (AUC)	0.588655
Standard error[a]	0.0221
95% Confidence interval[b]	0.563992 to 0.612989
z statistic	4.020
Significance level P (Area=0.5)	0.0001

From Figure 3, Table 3A & Table 3 B, we infer that the optimal cut off criterion is >3.98 and sensitivity is 51.2, specificity is 65.4. The Area under curve is 0.588655. The ROC curve is statistically significant.

Table 4: 17 OHP (nmol/L).

					95% Confidence interval for mean	
Gestational age	N	Mean	Std. deviation	Std. error	Lower bound	Upper bound
34	33	16.7339	16.86944	2.93659	10.7523	22.7156
35	18	9.0244	8.17309	1.92642	4.9601	13.0888
36	56	6.8246	6.30557	0.84262	5.1360	8.5133
37	60	6.4628	6.09148	0.78641	4.8892	8.0364
38	336	4.2408	3.56860	0.19468	3.8579	4.6238
39	498	4.0402	3.00306	0.13457	3.7758	4.3046
40	689	3.8047	2.86281	0.10906	3.5905	4.0188
41	5	2.2820	1.27146	0.56862	.7033	3.8607
Total	1695	4.4569	4.56718	0.11093	4.2393	4.6744

From Figure 4 & Table 4, we infer that as a Gestational Age increases mean 17 OHP values declines significantly and plateau beyond 37 weeks.

From Table 5, Table 5A & Figure 5 & Figure 6 we infer that as birth weight increases, then 17 OHP mean values decreases significantly and plateau beyond 2500 grams.

Multiple comparison of this ANOVA, we further infer that the 17 OHP mean values of 1500-1999 grams and 2000-2499 grams are statistically significant. But, 2500-2999 grams and 3000 grams above there is no statistical significance.

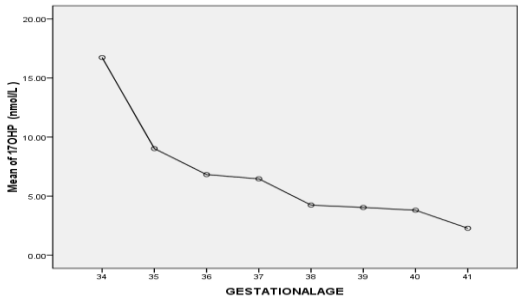

Figure 4: Means plots.

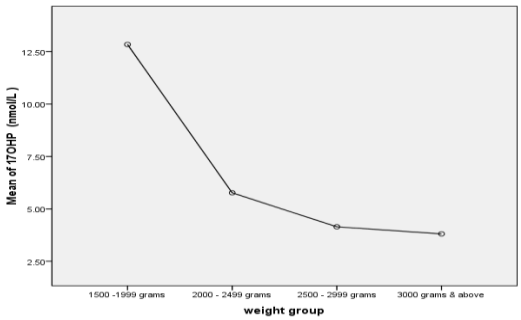

Figure 5: Means plots.

Table 5: Descriptives.

17OHP (nmol/L)	N	Mean	Std. deviation	Std. error
1500-1999 grams	43	12.8435	16.15572	2.46372
2000-2499 grams	236	5.7660	5.18815	.33772
2500-2999 grams	746	4.1433	3.22855	.11821
3000 grams & above	670	3.8066	3.11621	.12039
Total	1695	4.4569	4.56718	.11093

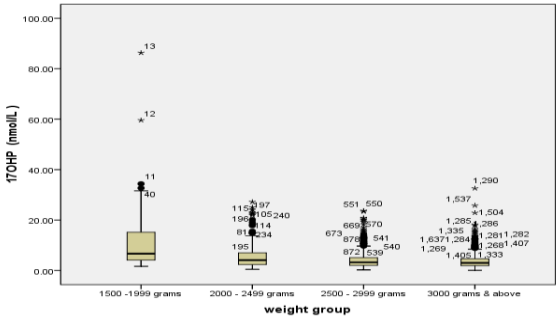

Figure 6: Box diagram.

Table 5A: Confidence interval for mean.

	95% Confidence interval for mean			
17 OHP (nmol/L)	Lower bound	Upper bound	Minimum	Maximum
1500-1999 grams	7.8715	17.8155	1.67	86.30
2000-2499 grams	5.1006	6.4313	0.50	27.20
2500-2999 grams	3.9113	4.3754	0.26	23.70
3000 grams & above	3.5702	4.0430	0.09	32.60
Total	4.2393	4.6744	0.09	86.30

Table 6: Group statistics.

Preterm	N	Mean 17OHP (nmol/L)	Std. deviation	Std. error mean
BW <2500 gm	74	11.2847	13.24129	1.53927
BW >2500 gm	33	7.9324	6.71905	1.16964

From Table 6 & Table 3, we infer that gestational age has got a greater influence on the biomarker (17 OHP), when compared to birth weight.

Table 7: 17 OHP (nmol/L)- preterm group.

					95% confidence interval for mean	
	N	Mean	Std. deviation	Std. error	Lower bound	Upper bound
34	33	16.7339	16.86944	2.93659	10.7523	22.7156
35	18	9.0244	8.17309	1.92642	4.9601	13.0888
36	56	6.8246	6.30557	0.84262	5.1360	8.5133
Total	107	10.2508	11.69597	1.13069	8.0091	12.4925

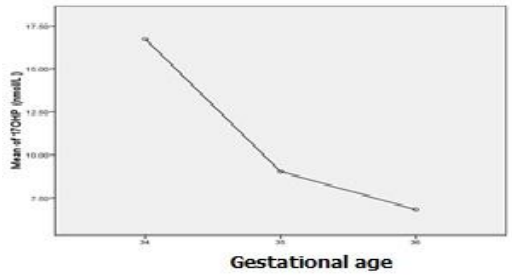

Figure 7: Means plot.

From Table 7 & Figure 7, we infer that among preterm group, as gestational age decreases, the mean 17 OHP values increases significantly.

Table 8: Correlation.

Variable Y	Baby weight (gms)
Variable X	17OHP (nmol/L)
Sample size	1483
Correlation coefficient r	-0.2467
Significance level	P<0.0001
95% Confidence interval for r	-0.2939 to -0.1983

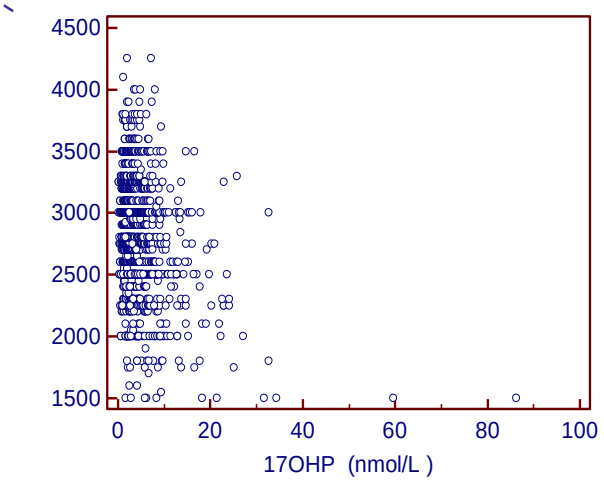

Figure 8: Scatter diagram.

Table 8A: Correlation.

Dependent Y	Baby weight (gms)
Independent X	17OHP (nmol/L)
Sample size	1483
Coefficient of determination R^2	0.06086
Residual standard deviation	426.2569

Table 8B: Regression equation.

y = 2888.3827 + -23.3908 x					
Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	2888.3827	15.2405	2858.4874 to 2918.2780	189.5199	<0.0001
Slope	-23.3908	2.3877	-28.0744 to -18.7071	-9.7964	<0.0001

Table 8C: Analysis of variance.

Source	DF	Sum of squares	Mean Square
Regression	1	17437080.05	17437080.05
Residual	1481	269090156.63	181694.91
F-ratio	95.97		
Significance level	P<0.001		

Table 9: Correlation.

Variable Y	Gestational age
Variable X	17OHP (nmol/L)
Sample size	1695
Correlation coefficient r	-0.3358
Significance level	P<0.0001
95% Confidence interval for r	-0.3774 to -0.2929

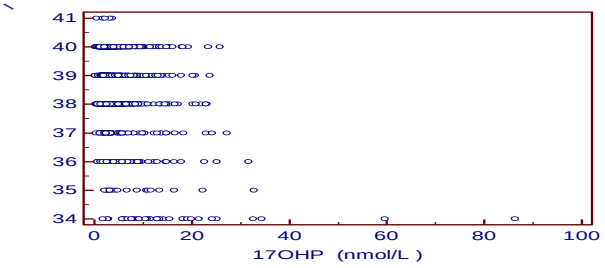

Figure 9: Scattered diagram.

Table 9A: Regression.

Dependent Y	Gestational age
Independent X	17OHP (nmol/L)
Sample size	1695
Coefficient of determination R^2	0.1128
Residual standard deviation	1.2299

Table 9B: Regression equation.

y = 39.3322 + -0.09598 x					
ParameterS	Coefficient	Std. Error	95% CI	t	P
Intercept	39.3322	0.04175	39.2503 to 39.4141	942.1630	<0.0001
Slope	-0.09598	0.006543	-0.1088 to -0.08315	-14.6699	<0.0001

Table 9C: Analysis of variance.

Source	DF	Sum of squares	Mean Square
Regression	1	325.5383	325.5383
Residual	1693	2560.9785	1.5127
F-ratio	215.2054		
Significance level	P<0.001		

Table 9D: Correlation.

Variable Y	Baby weight (gms)
Variable X	17OHP (nmol/L)
Sample size	1483
Correlation coefficient r	-0.2467
Significance level	P<0.0001
95% Confidence interval for r	-0.2939 to -0.1983

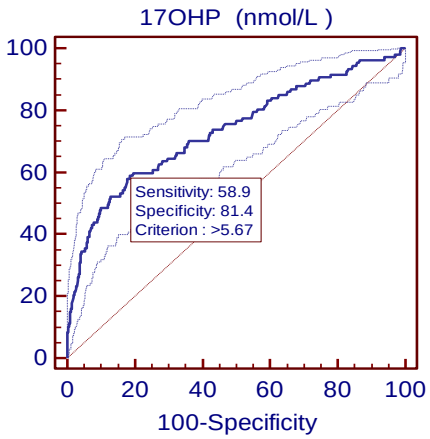

Figure 10: ROC curve.

Table 10: ROC curve.

Variable	17OHP (nmol/L)
Classification variable	GA
Sample size	1695
Positive group: GA = 1	107
Negative group: GA = 0	1588

Table 10A: Area under the ROC curve (AUC).

Area under the ROC curve (AUC)	0.733986
Standard error	0.0287
95% Confidence interval	0.712262 to 0.754897
z statistic	8.151
Significance level P (Area=0.5)	<0.0001

From Figure 8,9,10 & Table 8,9,10 we infer that optimal criterion value for gestational age beyond 37 weeks is >5.7. Sensitivity is 58.9, specificity is 81.4 and the area under curve is 0.733986. Good fit.

Figure 10 and Table 10, clearly shows, the 17 OHP value beyond 37 weeks GA, the area under curve is greater than 17 OHP of Low birth weight i.e 0.733986 >0.588. So, Gestational age beyond 37 week is a factor that influences the values of 17 OHP.

DISCUSSION

Our study results reveals that gestational age is a better predictor than birth weight for 17 OHP values. This correlates well with the study by Hetty J[1] et al. The 17 OHP values interpretations should be adjusted according to gestational age. It also correlates with study done by Laura Gruneiro de Papendieck[2].

Our study population consisted 6.3% preterm babies, 93.7% term babies and 16.5% were less than 2500 gm (Table 1 & 2).

The analysis reveals that 17 OHP mean values of low birth weight babies were increased than the normal birth weight babies in term group. A statistical significance exists between both.

From Figure 3, Table 3A & Table 3 B, we infer that the optimal cut off criterion is >3.9 and sensitivity is 51.2, specificity is 65.4. The area under curve is 0.588655. The ROC curve is statistically significant.

17 OHP values plotted against gestational age reveals that as a gestational age increases mean 17 OHP values declines significantly and plateau beyond 37 weeks (Table 4 & Figure 4).

17 OHP values plotted against birth weight reveals as birth weight increases, the 17 OHP mean values decreases significantly and plateaus beyond 2500 grams (Table 5, 5A & Figure 5 & 6).

Multiple comparison of this ANOVA, we further infer that the 17 OHP mean values of 1500-1999 grams and 2000-2499 grams are statistically significant. But, 2500-2999 grams and 3000 grams above there is no statistical significance.

When comparing term gestation with birth weight above 2500 gm to Preterms above 2500 gm we infer that gestational age has got a greater influence on the biomarker (17 OHP) (Table 6 & 3). Among preterm group, as gestational age decreases, the mean 17 OHP values increases significantly (Table 7 & Figure 7).

The 17 OHP value beyond 37 weeks of GA, the area under curve is greater than 17 OHP values of Low birth weight i.e 0.733986 >0.588. So, Gestational age beyond 37 week is a factor that influences the values of 17 OHP (Figure 10, 3 & Table 3 B).

The optimal criterion value for gestational age beyond 37 weeks is >5.7, Sensitivity is 58.9, specificity is 81.4 and the area under curve is 0.733986. Good fit (Figure 10).

CONCLUSION

Our study demonstrates clearly that there is relationship between mean 17 OHP values to gestational age and birth weight. As the gestational age increases the mean 17 OHP values declines and plateaus at 37 weeks and beyond. Similarly analysis based on birth weight shows a decline in mean values with increasing birth weight and the mean value plateaus beyond 2500 gm to 2999 gm.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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