Original Research Article

Utilizing the Bilirubin-Albumin Ratio as a Predictive Marker for Bilirubin-Induced Neurologic Dysfunction: A Comparative Analysis in Two Referral Hospitals in Abeokuta

ABSTRACT

AIM: We aim to assess the predictive value of Bilirubin Albumin Ratio (BAR) for evaluating bilirubin-induced neurologic dysfunction (BIND).Bilirubin-induced neurologic dysfunctionis a major cause of morbidity in neonates with highest burden in Sub-Saharan Africa and other resource-limited settings. Bilirubin Albumin Ratio (BAR) has been purported to correlate better with BIND than total serum bilirubin (TSB) which is the conventional tool used in evaluating babies with severe hyperbilirubinemia. There is however paucity of studies utilizing BAR in evaluating BIND.

Study design: The study design wascross-sectional.

Place and Duration of study: Neonatal Units of two referral hospitals; Federal Medical Centre Abeokuta and Sacred Heart Hospital Abeokuta in Ogun State, Nigeria between October 2019 – May 2020.

Methods: We included 84 late preterm and term babies with severe hyperbilirubinemiain the first 14 days of life. Babies were grouped into those with and without BIND using the modified BIND score, their sera was assessed for total serum bilirubin, and albumin and the BAR was calculated by dividing the plasma

bilirubin level by serum albumin and findings were compared between the two groups.

Results: Bilirubin-induced neurologic dysfunction was present in 67.1% of babies with severe hyperbilirubinemia with a male: female ratio of 1.8:1. The mean BAR for babies with and without BIND were 7.77 ± 1.76 and 5.43 ± 1.27 mg/g respectively. Babies with BIND had a significantly higher BAR than those without BIND ($p < .001$). Using the receiver operating characteristic curve analysis, the cut-off value for BAR was 6.46 mg/g with sensitivity, and specificity of 84.2% and 81.5% respectively.

Conclusion: The prevalence of BIND among babies with severe hyperbilirubinemia is high and BAR has a high predictive value in evaluating babies at risk of developing BIND.

Keywords: ("Bilirubin-induced Neurological Dysfunction", "Bilirubin-Albumin Ratio", Hyperbilirubinaemia, Late preterm, Term)

1. INTRODUCTION

Hyperbilirubinemia is a common cause of morbidity and mortality in the newborn period affecting about 60 percent of term and 80 percent of preterm neonates within the first week of life [1–3]. It is generally benign for most neonates but the level of the unconjugated bilirubin fraction can rise to levels severe enough to damage the developing brain, causing Bilirubin-induced neurologic dysfunction (BIND) with long-lasting permanent neuronal damage [4,5]. The burden of severe neonatal jaundice in the African region is high (667.8 per 10,000 live births) compared to America and Europe (4.4 and 3.7 cases per 10,000 live births respectively) [6]. In North America and Europe, the estimated incidence of kernicterus the chronic state of BIND ranges between 0.4 and 2.7 cases per 100,000 live births in neonates born at ≥ 35 weeks of gestation [7]. Even though bilirubin encephalopathy is

preventable, sporadic cases still occur in developed countries but remain endemic in many developing countries including Nigeria [8].

There are basically three phases of BIND manifestation; early phase characterized by lethargy,hypotonia, poor suck; the intermediate phase by hypertonia, high pitched cry, irritability and theadvanced phase presents with seizures, coma, profound retrocolis, opisthotonus and death. Hence prompt diagnosis are needful before the onset of these symptoms. However, diagnosis of neonatal hyperbilirubinemia has been based primarily on Total Serum Bilirubin (TSB), a function of the intravascular concentration of bilirubin that is bound to albumin [4,8,9] which does not always correlate with BIND [10,11]. Free bilirubin, a measure of unconjugated fraction of bilirubin not bound to albumin or other plasma proteins, is a better indicator of neurotoxicity of bilirubin than TSB but its use is limited because laboratory facilities for its measurement is not readily available [12,13].

Bilirubin-Albumin ratio (BAR) has been proposed as a surrogate measure for assessing free bilirubin [3,10,12–15] and has been recommended in evaluating severe hyperbilirubinemia even among late preterm neonates [16]. The present study is aimed at evaluating the utility of BAR in determining the severity of neonatal hyperbilirubinemia as studies on BAR are limited and none has been published in Nigeria.

2. METHODOLOGY

2.1 Study design, site, and criteria for inclusion

The study was a hospital-based observational, cross-sectional design conducted among late preterm and term neonates with severe hyperbilirubinemia admitted into level II Special Care Neonatal Units of two referral hospitals; Federal Medical Centre Abeokuta (FMCA)-Hospital A and Sacred Heart Hospital Abeokuta (SHH)- Hospital B, Ogun State, Nigeria between

October 2019 – May 2020. Late preterm (≥35 weeks) and term neonates aged 0 to 14 days who were admitted consecutively during the study period with severe neonatal hyperbilirubinemia defined as a TSB ≥ 20mg/dl or neonates who required an exchange blood transfusion (EBT) at a lower serum bilirubin level (TSB ≥ 12mg/dl in the first 24 hours of life or ≥ 15mg/dl in less than 72hours) whose parent gave written informed consent were included in the study [17].

Neonates excluded were those with conjugated hyperbilirubinemia defined as direct bilirubin ≥ 20% of the TSB [10]and unconjugated fraction less than 20mg/dl, those who had had double volume EBT prior to presentation which may alter serum albumin and bilirubin levels, severe perinatal asphyxia defined as persistently low APGAR score at 5 minutes and or clinical features of hypoxic ischemic encephalopathy (HIE), on prior use of medications that are known to compete with serum bilirubin for albumin like ceftriaxone and ibuprofen [18-19]. Also excluded were those with congenital anomalies like hydrocephalus and spinal bifida as the neurologic abnormalities from these conditions may mimic Bilirubin-induced neurologic dysfunction.

2.2 Sample size

The sample size was determined using the Fisher's formula [20]:

N $= \frac{z^2\, p\,(1-p)}{d^2}$ Where N = sample size, Z = standard normal variation (1.96 at 5% type 1 error) , P = prevalence of bilirubin encephalopathy estimated to be 14.8%[2] and d = margin of error (5%)

N = $\frac{1.96^2 \times 0.148\,(1-0.148)}{0.05^2}$ = 194

Adjusting for finite population of babies with severe neonatal hyperbilirubinaemia (SNH) whose population were less than 10,000,

$n = \sqrt{\frac{N}{1+\frac{N-1}{N1}}}$ (n is the minimum sample size). Where N_1 is estimated population size = 144, the combined number of neonates with severe hyperbilirubinemia who had a double volume EBT in 2018 from both centres.

$n = \sqrt{\frac{194}{1+\frac{194-1}{144}}}$ = **84**

The calculated sample size was divided between the two study sites by the proportion of neonates with SNH who had double-volume EBT carried out in 2018. Eighty-three and 61 babies had severe hyperbilirubinemia in hospitals A and B respectively; that is a total of 144. The sample population was distributed as follows;

Hospital A: $\sqrt{\frac{83\times84}{144}}$ = **48** and Hospital B: $\sqrt{\frac{61\times84}{144}}$ = **36**

2.3 Research ethics

Ethicalapproval for the study was obtained from the Research and Ethics Committee of FMCA (NREC/08/10-2015) and SHH (SHH/EC/EA/01/12/18). Written informed consent was obtained from the parents/guardians of all subjects recruited for the study.

The proforma documented information on the subject's bio-data, maternal bio-data, clinical manifestations, and laboratory investigation results. This was pretested on nine subjects (10% of the sample size) and deficiencies observed were corrected before the full commencement of the study. Neonates enrolled for the pre-test were not included in the main study.

2.4 Procedure

Consecutive neonates seen at both hospitals during the study period who met the inclusion criteria were enrolled until 48 and 36 subjects were enrolled from hospitals A and B respectively. Estimated gestational age was determined by using mother's last menstrual period and/or early ultrasound scan. Each subject was then examined and grouped into neonates with and without BIND and those with BIND were further categorized into early, intermediate and advanced. Socioeconomic class of each neonate's parents was

determined by the Ogunlesi socio-economic status classification [21] using the parental highest level of education and occupation. Following standard precautions, 4ml of venous blood from each subject aseptically and injected it into lithium heparin bottles. Each sample collected was placed in a small dark paper box to prevent the sample from light rays which can metabolize bilirubin and results in a falsely low serum bilirubin level. Within 30 minutes of sample collection, each sample was spun in a bench centrifuge (model 80-2) at 10,000 revolutions per minute for five minutes to separate the plasma from blood cells. To avoid extracting red cells, the plasma was carefully removed with a fine-bore pipette and transferred to a plain bottle labelled with each neonate's serial number. Subsequently, each sample was stored in the refrigerator at $2^{o}C$ to $8^{o}C$ within three hours of sample collection . The TSB (mg/dl) and serum albumin (g/dl) were entered in the proforma. Bilirubin-Albumin ratios (mg/g) were calculated by dividing the plasma bilirubin level by serum albumin and the results also entered in the proforma.

Conventional blue light phototherapy with a wavelength of about 450nm was commenced for all neonates with severe hyperbilirubinemia while awaiting double volume exchange blood transfusion. Neurologic status was assessed daily using the M-BIND scoring chart (which was previously used at admission to determine the presence and severity of BIND) until discharge. The outcomes of treatment including discharge with or without BIND, mortality, discharge against medical advice (DAMA) and referral were entered in the proforma.

2.4.1 Laboratory methods:

2.4.1.1 Total serum bilirubin: TheTSB was determined using the Jendrassik-Groft method [22] while the conjugated bilirubin was determined by using normal saline in place of caffein benzoate with the resultant colour change from pink to blue-green complex intensity which is directly proportional to bilirubin concentration determined by measuring the increase in absorbance at wavelengths of 578nm for total bilirubin and 546nm for conjugated bilirubin using a spectrophotometer. Total serum bilirubin and conjugated bilirubin were calculated as

follows: **TSB concentration (mg/dl)** = Optical density of test – Optical density of reagent blank x 29 while

2.4.1.2 Direct bilirubin concentration(mg/dl): Direct bilirubin concentration = Optical density of test – Optical density of sample blank x 16

2.4.1.3 Serum albumin assay [23]: This was determined using the bromocresol-green dye to produce a colour change which is proportional to the albumin concentration in the serum and **Albumin concentration (g/dl)** = $\frac{absorbance\ of\ sample}{absorbance\ of\ standard}$ X 3

Hypoalbuminemia was defined as serum albumin < 3g/dl [24].

In addition to serum bilirubin and albumin, other samples routinely used in the evaluation of babies with neonatal jaundice at both centres were taken; and included samples for packed cell volume (PCV), mother's blood group, baby's blood group, reticulocyte count, direct Coombs test (DCT), G6PD assay and blood cultures – using thioglycollate broth.

2.5 Data analysis

Subjects were grouped into two; those with and without BIND. The data obtained from the proforma were collated and analysed using the Statistical Package for Social Sciences (SPSS) software version 22.0. Frequency distribution tables generated from computed variables and numerical data (weight, age, serum bilirubin, serum albumin, BAR) were summarized using mean ± standard deviation. Independent t-test was used to compare these while Chi square was used to compare categorical variables (sex, social class, place of delivery) between both groups. Univariate and multivariate logistic regression were utilized to identify independent risk factors for development of BIND while Receiver Operating Characteristics (ROC) curve was used to determine cut off values for bilirubin-albumin ratio. Using Euclidian's index method [25-27], the cut-off value corresponds to the point on the ROC curve nearest to the (0, 1) point on the y-axis. Statistical significance was defined as p-value < 0.05.

3. RESULTS AND DISCUSSION

3.1 Results

Of the 84 babies enrolled for the study, 57 (67.9%) had BIND at admission while 27 (32.1%) babies did not. Seventy-three (86.9%) of the 84 babies were referred to the study facilities (out-born) and 57 (78.1%) of them had BIND at admission. None of the 11 inborn babies developed BIND. The mean age of the 84 babies with SNH at admission was 5.00 ± 2.07 days with majority of them 69 (82.1%) presenting to the hospital after 72 hours of life. The mean ages of babies with and without BIND were 5.58 ± 1.76 and 3.77 ± 2.15 days respectively **(t = 4.080, df = 82, p < .001)** and BIND was significantly higher in babies who presented after 72hrs of life than those that presented earlier **(χ^2= 24.889, df = 1, p < .001).** There were more male neonates, 54 (64.3%), than females 30 (35.7%) in the study population with a male to female ratio of 1.8:1. Bilirubin-induced neurologic dysfunction was present in 42 (77.8%) of the male subjects and was significantly higher than 15 (50%) of the female neonates with BIND **(χ^2 = 6.823, df = 1, p < .01) [Table I].** Bilirubin-induced neurologic dysfunction was present at an advanced phase in 37 (64.9%) of 57 babies at admission, 13 (22.8%) babies were admitted at the intermediate phase of BIND while seven (12.3%) babies were seen at the early phase.

Table I: Sociodemographic characteristics of babies with and without BIND

Characteristics	**BIND**	**No BIND**	**df**	**χ^2**	**p-value**
	n = 57	**n = 24**			
	n (%)	**n (%)**			
Age group(days)					
< 3	2 (3.5)	13 (48.1)	1	24.889	**< .001***
≥ 3	55 (96.5)	14 (51.9)			
Sex					
Male	15 (26.3)	15 (55.6)	1	6.823	**.009**

Female	42 (73.7)	12 (44.4)			
Weight (grams)					
< 2500	28 (49.1)	11 (40.7)	1	0.518	.47
≥ 2500	29 (50.9)	16 (59.3)			
Social class					
High	5 (8.8)	4 (14.8)	2	28.690	**.013***
Middle	7 (12.3)	10 (37.0)			
Low	45 (78.9)	13 (48.1)			

***fisher's exact test applied**

The mean value of serum albumin of the 84 subjects was 3.95 ± 0.37 g/dl with a range of values from 2.00 g/dl to 4.70 g/dl. The mean serum albumin values for babies with and without BIND were 3.91 ± 0.41 and 4.01 ± 0.26 g/dl respectively; the difference in the serum albumin in babies with and without BIND was not statistically significant. Two (2.4%) of the 84 babies had hypoalbuminemia and both babies developed BIND; 55 (67.1%) of the remaining 82 babies who had normal serum albumin developed BIND. Total serum bilirubin values of the 84 babies ranged from 12.04 mg/dl to 55.20 mg/dl with a mean value of 27.51 ± 7.45 mg/dl. Babies with BIND, 57 (67.9 %) of 84 had significantly higher TSB levels (30.29 ± 7.07 mg/dl) than those without BIND (21.63 ± 4.31 mg/dl) with a **p-value < .001 (Table II)**.

Table II: Comparison of laboratory findings in babies with and without BIND

Laboratory parameters	SNH with BIND Mean ± SD	SNH without BIND Mean ± SD	t- test	p- value
TSB (mg/dl)	30.29 ± 7.07	21.63 ± 4.31	5.857	**< .001**
Serum albumin (g/dl)	3.91 ± 0.41	4.01 ± 0.26	1.164	.25
BAR (mg/g)	7.77 ± 1.76	5.43 ± 1.27	6.175	**< .001**
PCV (%)	36.26 ± 9.26	40.26 ± 7.75	1.941	.06

The area under the ROC curve (AUC) of TSB for predicting BIND was 0.862 with a **p-value < 0.001**. Using the Euclidian's index method,[25–27] where the cut-off value corresponds to the point on the ROC curve nearest to the (0, 1) point on the y-axis. After identifying this point, the co-ordinates on the y and x-axis were determined; the optimal cut-off value of TSB for predicting BIND (25.1 mg/dl) was derived from a table of TSB cut-offs and their co-ordinates (**Figure 1)**.

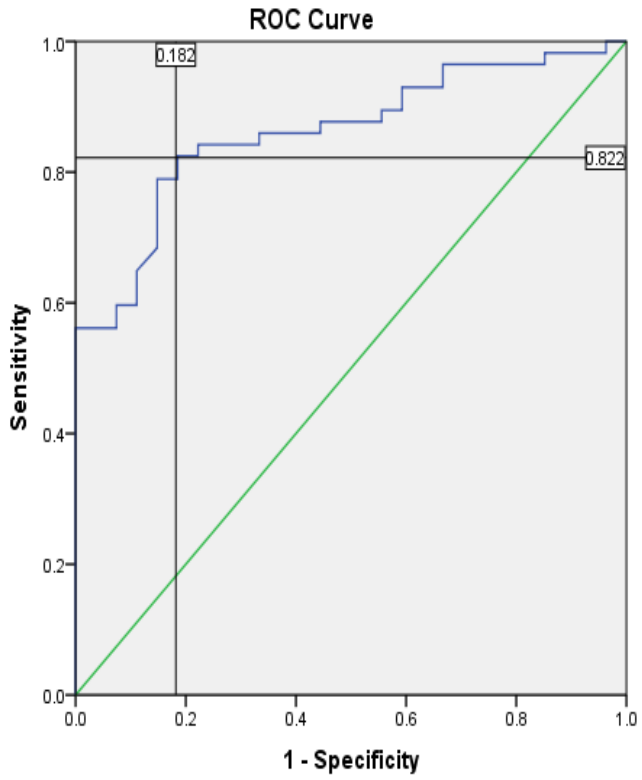

Figure 1: ROC curve for TSB in determining BIND

Bilirubin-albumin ratio of the 84 babies ranged from 2.90 mg/g to 12.80 mg/g with a mean of 7.02 ± 1.95 mg/g. Babies with BIND had a significantly higher BAR (7.77 ± 1.76 mg/g) than those without BIND (5.43 ± 1.27mg/g). The area under the ROC curve (AUC) for BAR in determining the presence or absence of BIND was 0.861 with a **p-value of .001**. Using the Euclidian's index method, the optimal cut-off point for BAR was estimated to be 6.46 mg/g (**Figure 2**).

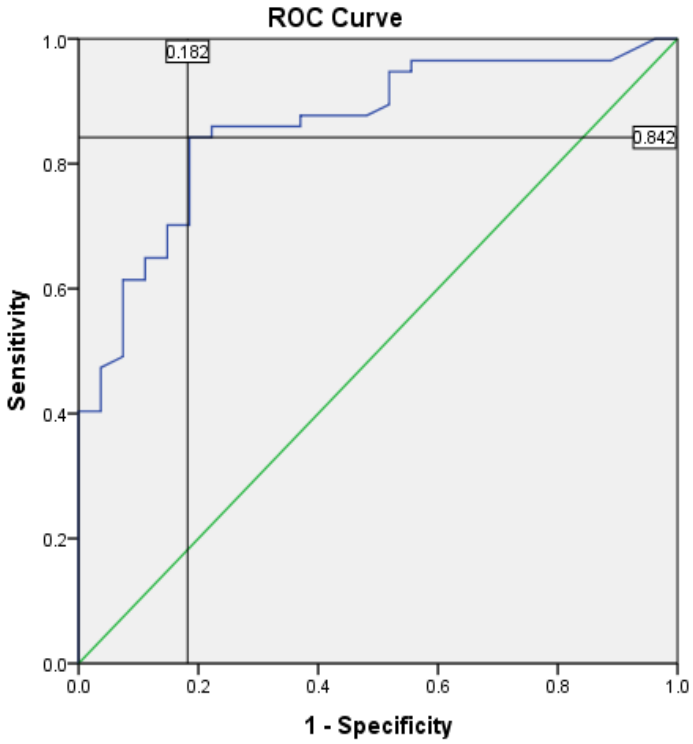

Figure 2: ROC curve of BAR for predicting BIND

Using these cut-offs, 52 (61.9%) babies had a TSB ≥ 25.1 mg/dl while 32 (38.1%) babies had a TSB < 25.1 mg/dl. Forty-seven (90.4%) babies with TSB ≥ 25.1 mg/dl had BIND and this was significantly higher than 10 (31.3%) babies who had BIND at a TSB level < 25.1 mg/dl. The sensitivity (ability of TSB to correctly identify babies with BIND) was 82.5% while the specificity (the ability of TSB to correctly identify babies who did not have BIND) was 81.5%. On the other hand, 53 (63.1%) babies had BAR values above the cut-off point while 31 (36.9%) had values below it. Forty-eight (90.6%) of the 53 babies with BAR ≥ 6.46 mg/g had BIND and is significantly higher than nine (29.0%) of the 31 babies who had BIND at a BAR < 6.46. The sensitivity of BAR in predicting BIND was 84.2% while the specificity was 81.5%; the positive predictive value (the probability that a baby identified as having BIND actually had BIND) was 90.6% while the negative predictive value (the probability that a baby identified as not having BIND using BAR did not have BIND) was 71.0% (**Table III**).

Table III.Test of validity of TSB and BAR for predicting BIND

TSB (mg/dl)	BIND N (%)	No BIND N (%)	Total
≥ 25.1	47 (82.5)	5 (18.5)	**52**
< 25.1	10 (17.5)	22 (81.5)	**32**
Sensitivity = 82.5%	**Positive predictive value = 90.4%**		
Specificity = 81.5%	**Negative predictive value = 68.8%**		

BAR (mg/g)	BIND N (%)	No BIND N (%)	Total
≥ 6.46	48 (84.2)	5 (18.5)	**53**
< 6.46	9 (15.8)	22 (81.5)	**31**
Sensitivity = 84.2%	**Positive predictive value = 90.6%**		
Specificity = 81.5%	**Negative predictive value = 71.0%**		

3.2 Discussion

In the present study, the mean BAR in babies with and without BIND were comparable with findings from studies by Habibi *et al.* [28] in north-western region of Iran and Mosallam *et al.* [29] in Egypt but lower than the mean BAR reported by Ardakani*et al. [*12] from northern part of Iran and Wang *et al.* [30] in China. The lower BAR documented in the present study when compared with reports by Ardakani*et al.* [12] and Wang *et al.* [30] may be attributable to the higher serum albumin levels found in the current study which will ultimately reduce the BAR.

The mean serum albumin levels of babies without BIND in the present study were comparable with the mean serum albumin levels reported by Habibi *et al. [*28] in Iran but higher than the values documented by Wang *et al.* [30] in China, Mosallam *et al. [*29] in Egypt and Ardakani*et al.* [12] in Iran. This variation may be due to differences in genetic composition of babies from different geographical location as there have been documented lower serum albumin levels in newborn babies from other studies in Asia[31] and Egypt[32]. Wang *et al. [*30] in China reported a serum albumin levels of 3.19 ± 2.26 g/dl in well babies and 2.11 ± 0.39 g/dl in babies with sepsis in a study to determine the relationship between serum albumin and infection in neonates.

Likewise, a lower serum albumin level was documented in a study on serum albumin concentration and clinical disorders by gestational ages in newborn babies by Lee *et al. [*33] in South Korea. In the same vein, Khairy *et al. [*32] and Mashad *et al. [*34] in Egypt reported cord albumin levels of 3.30 ± 0.50 g/dl and 2.76 ± 0.71 g/dl respectively which were lower than the cord albumin levels of 4.49 ± 1.12 g/dl documented by Abdurrahman *et al. [*35] in Nigeria. These findings corroborate earlier remarks that genetic variation may be responsible for the higher serum albumin values documented in the present study despite the presence of hyperbilirubinemia [3,12,28-30]. The cut off value of BAR in predicting BIND identified by the ROC analysis in the current study is comparable with BAR cut-off points of 6.68 mg/g and 7 mg/g reported by Mosallam *et al. [*29] and Patel *et al. [*3] respectively. Other studies

(Habibi *et al.* [28] and Ardakani*et al.* [12]) however have documented a higher BAR cut-off of 8 mg/g. It is noteworthy that there are variabilities in the reported validity of BAR in previous studies. At a BAR cut-off of 8 mg/g, Ardakani*et al.* [12] documented a sensitivity of 100 percent and specificity of 94 percent. Contrary to this, Habibi *et al.* [28] (with the same cut-off of 8 mg/g) reported a low sensitivity (40 percent) with a specificity of 100 percent but had an improvement in sensitivity of up to 100 percent when the cut-off was reduced to 5.6 mg/g. The reason for the low sensitivity reported by Habibi *et al.* [28] may be as a result of the study being longitudinal with some of the babies lost to follow-up.

Up to date, there is still no consensus as to which indicator better predicts BIND between TSB and BAR. Hence, it is imperative to do so. In the present study, BAR and TSB had the same specificity; but BAR had a higher sensitivity and negative predictive value (with comparable specificity and positive predictive value) and the overall appears to be a better predictor of BIND. Ardakani*et al.* [12] in a study on the utility of BAR in predicting BIND reported that BAR and TSB were both good predictors of BIND although the specificity was higher with BAR. This was reiterated by Iskander *et al.* [13] who documented that TSB and BAR were both strong predictors of BIND and that at same sensitivities, their specificities were similar. On the other hand, Habibi *et al.* [28] found that TSB was a better predictor of BIND than BAR although BAR was more specific; it was however reported that combining both indicators improved the prediction of BIND.

4. CONCLUSION

The present study highlighted the usefulness of BAR in predicting BIND as it has both high sensitivity and specificity for the prediction of BIND and should be routinely used in evaluating babies with severe hyperbilirubinemia. Also based on findings from the present study, it is suggested that using BAR in conjunction with TSB may help reduce the number of EBT from vigintiphobia that had increased the frequency of EBT at a TSB of 20mg/dl in resource-poor settings [36].

CONSENT (WHEREEVER APPLICABLE)

All Authors hereby declare that written informed consent was obtained from the parents/guardians of all subjects recruited for the study.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

All authors hereby declare that the proposal for this study has been examined and approved by the appropriate ethics committee of the two hospitals where the study was conducted (NREC/08/10-2015 and SHH/EC/EA/01/12/18) and that the study has been performed per the ethical standards laid down in the 1964 Declaration of Helsinki."

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