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# Total and Free Plasma Bilirubin and Clinical Outcomes in Severe Hyperbilirubinemia

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# *Total and Free Plasma Bilirubin and Clinical Outcomes in Severe Hyperbilirubinemia*

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**Background:** Acute bilirubin encephalopathy (ABE) and kernicterus spectrum disorder (KSD) have become relatively uncommon in high income
countries but remain a major cause of morbidity and mortality in low- and middle-income countries

**Objective:** To better understand the relationship between free (Bf) and total (TB) bilirubin levels and the development of ABE and KSD we followed infants born in three large tertiary centers in northern and central Nigeria (Jos, Kano and Zaria).

## Study Design:

- Prospective Cohort Study
- Inclusion Criteria
  - TB ≥ 15 mg/dL
- Exclusion Criteria
  - Active infection
  - Hypoxic ischemic encephalopathy
  - Other metabolic developmental abnormalities

## Sample and Data Collection:

- Blood Samples – for DNA extraction
- Serum Samples – For TB and Bf measurement
- Data
  - General demographics
  - G6PD deficiency status
  - Rh disease
  - ABO incompatibility
  - Pregnancy history
- Neurological Assessment at 1, 3, and 6 months
  - Modified Barry-Albright Dystonia + Spasticity Scale
  - Clinical Adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS)
- Bilirubin Measurements
  - TB measurement (Total and direct) measured on centrifugation separated serum by the BR2 Bilirubin Stat-Analyzer using the Malloy-Evelyn diazo method at time of collection.
  - Bf and TB measurement measured at Children's Mercy Hospital via the Peroxidase Method (Ahlfors 2000)(Jacobsen and Wennberg 1974)

## Results:

- 67 infants enrolled in initial 3 month period
- Peak TB range: 15 – 62.6 mg/dL (256.5 – 1,071 umol/L)
- Bf range: 2.34 – 33.54 nM
- Pearson Correlation of 19 Bf / TB samples did not show a significant correlation ($R^2$ = 0.094)

## Conclusions

- [TB] is a poor predictor of toxic [Bf] in these samples
- Hemolysis is a more serious issue than previously anticipated and will require more careful sample collection

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**Free bilirubin measurements** in 3 Nigerian Hospitals over a 3-month period **show poor correlation with total bilirubin.** However hemolysis proved to be a significant problem.

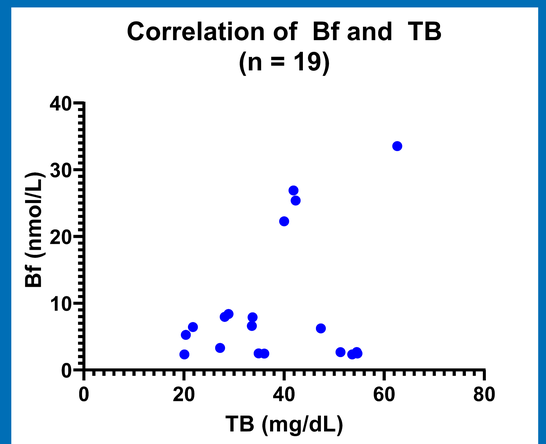



Figure 1: Non-Hemolyzed samples with reliable Bf readings. Bf and TB readings from CMH. n = 18

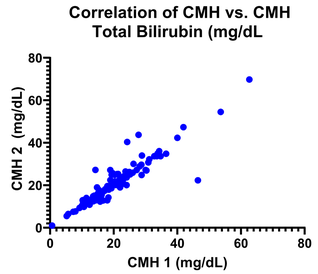
| | |
|---|---|
| Pearson r | 0.307 |
| $R^2$ | 0.094 |
| P value (two-tailed) | 0.201 |
| P value summary | ns |

| Test Site | Hemolyzed* | Total | Percent Hemolyzed |
|---|---|---|---|
| Zaria | 25 | 27 | 93% |
| Jos | 49 | 64 | 77% |
| Kano | 32 | 49 | 65% |
| **Total** | **106** | **140** | **76%** |

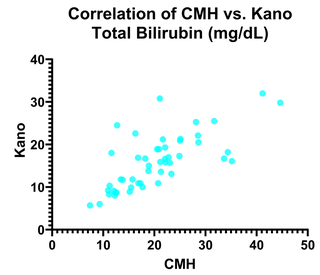
Table 1: Samples were categorized as hemolyzed if the hemoglobin signal exceeded 10% of the bilirubin signal ($A_{578}$ / $A_{460}$ < 10%)

Scan the Code for access to information about this poster's design methodology

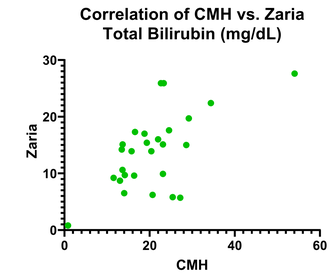
## Measurement Reproducibility



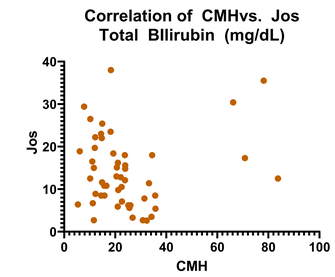

| | |
|---|---|
| Pearson r | 0.923 |
| $R^2$ | 0.851 |
| P value (two-tailed) | <0.0001 |
| P value summary | **** |




| | |
|---|---|
| Pearson r | 0.714 |
| $R^2$ | 0.509 |
| P value (two-tailed) | <0.0001 |
| P value summary | **** |




| | |
|---|---|
| Pearson r | 0.623 |
| $R^2$ | 0.3881 |
| P value (two-tailed) | 0.0005 |
| P value summary | *** |




| | |
|---|---|
| Pearson r | 0.119 |
| $R^2$ | 0.014 |
| P value (two-tailed) | 0.405 |
| P value summary | ns |

## Next Steps:

- Implement new protocols for improved sample collection and minimization of hemolysis
- Expand the sample size and continue with follow up of patients to identify correlation of Bf with severity of KSD

## Acknowledgements:

- Research team members in Nigeria involved in consenting, sample collection, storage, and shipping
- Charles Ahlfors for training on TB and Bf testing
- Josh Wheatley for performing TB and Bf testing

## References:

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