

BIOCHEMICAL PREDICTORS OF ENTEROCOLITIS IN CHILDREN WITH COLORECTAL ANOMALIES POST COLOSTOMY AT UNIVERSITY COLLEGE HOSPITAL, IBADAN

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Background: A large proportion of patients with preoperative enterocolitis still have enterocolitis persisting even after surgery while others resolve thereafter. Some researchers have studied Calprotectin, C-reactive protein (CRP), Blood and Plasma viscosity as markers of inflammation, hence, the choice of their use.

The aim of the study is to determine the sensitivity and reliability of Calprotectin, C-reactive protein (CRP), Blood and Plasma viscosity as biochemical predictors of enterocolitis in children with colorectal anomaly post-surgery at University College Hospital Ibadan.

Patients and Methods: This is an observational analytic study of 32 patients with either Hirschsprung's disease or Anorectal malformation carried out over a year period. The demographic data of the patients, clinical condition and the preoperative and postoperative readings of the biochemical analytes were recorded in a chart. Statistical analysis were carried out using SPSS version 23 and test for statistical association done.

Results: The incidence of Hirschsprung associated enterocolitis is 12.5% and for Ano rectal malformation 6.3%. Gender difference was not statistically significant even with the observed clinical difference. Plasma viscosity and blood viscosity correlate positively with each other. C-reactive Protein and Calprotectin did not predict enterocolitis in this study and the Sensitivity of blood viscosity at T1 and T2 is as low as 66% with a Positive Predictive Value of 25 %.

Conclusion: The incidence of Enterocolitis associated with Hirschsprung's disease and Anorectal malformation is 19 %. Calprotectin and C-reactive protein did not predict enterocolitis in these patients. The outcome of care was satisfactory in over 90 % of the patients.

Keywords: Biochemical predictors, Children, Enterocolitis, Ibadan

INTRODUCTION

A retrospective study of one hundred and thirty children at the University College Hospital, Ibadan by Ogundoyin *et al.*,¹ on the pattern and outcome of childhood intestinal obstruction showed that anorectal malformations (22.4%) and Hirschsprung's disease (13.9%) were among the most common causes of congenital intestinal obstruction.

Hirschsprung's disease-associated enterocolitis (HAEC) in neonates occur in about 16% of patients and presents with greater likelihood of progression to septic shock and toxic mega colon in the older children especially after surgery.²⁻⁴

In the case of a large recto urethral fistula, the patient frequently passes urine into the colon. A more distal colostomy allows urine to escape through the distal stoma without significant absorption, however, with

a more proximal colostomy; the urine remains in the colon and is absorbed, with increased possibility of sepsis. Also patients with ARM presenting late will have prolonged distention of the rectal pouch and may produce an irreversible hypo motility disorder, leading to severe constipation later in life with possibility of associated enterocolitis. Post Posterior Sagittal Anorectoplasty (PSARP) stricture, though rare may precipitate recurrent constipation and possible enterocolitis.⁵

Mortality due mainly to late recognition and treatment has been reported to be between 1% and 5% of the children undergoing operation for HD.⁶ Early clinical and radiological signs of enterocolitis are often non-specific, hence, the need for accurate prediction of enterocolitis at an early stage to obviate this delay in diagnosis and its unfavourable sequelae. Several

researches have studied Calprotectin, C-reactive protein (CRP) and Plasma viscosity as markers of inflammation.

CRP when used alone may not be able to accurately predict intestinal inflammation, combination with calprotectin increases the yield and gives a better diagnostic accuracy. As specificity increased when adding calprotectin measurement, false positive test results decreased dramatically.⁷

Several studies have also highlighted the usefulness of plasma and blood viscosity as markers of inflammation in that it has a high sensitivity, high negative predictive value, though low specificity and fewer false negative results.^{8,9}

This study seeks to determine the incidence of enterocolitis in patients with HD and ARM, determine the validity and reliability of these biomarkers and also determine the outcome of treatment of patients with HAEC and ARM associated enterocolitis.

PATIENTS AND METHODS

This study was conducted in the Division of Paediatric Surgery, Department of Surgery, University College Hospital (UCH) Ibadan between January 2017 and December 2017. Thirty two consecutive patients aged first day of life to 15 years with HD or ARM presenting with intestinal obstruction and scheduled for surgery were prospectively enrolled over a year period.

Enterocolitis is defined by the presence or persistence of fever, passage of frequent watery stool and biochemical evidence of leukocytosis in the setting of intestinal obstruction due to Hirschsprung's disease (HD) or Ano rectal malformation (ARM).

It occurs either pre operatively or post operatively. Eight milliliters of venous blood samples were taken from the patients on three different occasions, at diagnosis of HD or ARM and or enterocolitis (preoperatively) while the second and third samples were taken on the second- and fourth-day post-surgery respectively.

The blood samples were centrifuged immediately, and sera stored at -80°C until batch analysis for Calprotectin and C-reactive protein assay while Plasma and blood viscosity were carried out within 48 hours of collection. NSAIDs were omitted from routine analgesia as part of intra and peri-operative analgesia by the anesthesiologist because of the association with enteropathy. Children on NSAIDs within the last three weeks prior to sample collection; Patients with focus

of sepsis such as acute otitis media, tonsillitis, rheumatoid arthritis, respiratory infections et cetera; Patients who have had colostomy or pull-through surgery before presentation were all excluded from participating in the study as they can also elaborate some level of plasma Calprotectin.

The data was collected and entered in a proforma which was used for analysis.

Statistical analyses were carried out using SPSS version 22 and descriptive data were represented in tables and test for statistical association done using Students t-test, ANOVA and regression analysis for the outcome variables such as length of hospital stay, serum levels of bio markers and the presence or absence of post operative enterocolitis were carried out. Ethical approval was received from the joint UI/ UCH Ethical committee.

RESULTS

A total of 32 patients with colorectal anomaly were recruited over the one-year period.

Majority (63%) of the patients are males with, (M: F 1.9 :1) and Median age of (6.5 days). Table 1.

Table 1: Social-demographic characteristics

Variables	Frequency (%)
Age; Median (Range); days	6.50 (1.00 – 356.00)
Sex	
Male	20 (62.5%)
Female	12 (37.5%)
Ethnic Group	
Yoruba	23 (71.9%)
Igbo	7 (21.9%)
Hausa	2 (6.3%)
Parents' Level of Education	
Primary	9 (28.1%)
Secondary	17 (53.1%)
Tertiary	6 (18.8%)

Gender difference was not statistically significant even with the observed clinical difference.

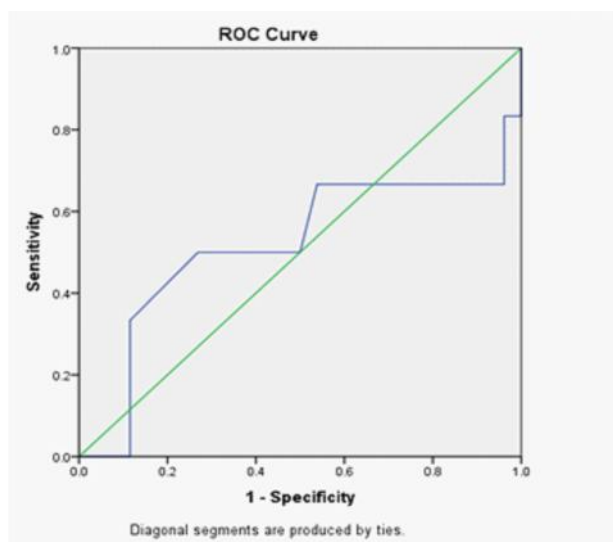
Abdominal Distension and Constipation accounted for the commonest presentation (28%). About 90% of patients seen presented with abdominal distension. Table 2.

Half of the patients seen had Ano rectal anomalies. A total of 94% of the patients with ARM seen had fistula with Recto urethral type being the commonest.

Table 2: Clinical presentations of the patients

Variables	Frequency (%)
Abdominal Distension with normal anus	6 (18.8%)
Abdominal Distension with absent anus	8 (25.0%)
Abdominal Distension with failure to thrive	10 (31.3%)
Abnormal Anal opening with Abdominal Distension	3 (9.4%)
Abnormal Anal opening without Abdominal Distension	5 (15.6%)

Preoperative enterocolitis was seen in six patients giving a prevalence of 19%. A higher proportion of patients, 4 (67%) had Hirschsprung associated enterocolitis (HAEC) while two were associated with anorectal malformations. The mean difference in the bio-makers were compared to presence or absence of preoperative enterocolitis. $F=1.884$; $P > 0.05$.



Area under the curve	p-value
0.66	0.23

Figure 1: Receiver operator curve for blood viscosity at T2 and preoperative enterocolitis

Majority of the patients (34%) had surgical intervention within two days of presentation. About two-third of patients had Colostomy, predominantly Devine colostomy (88%) while one patient died prior to surgical intervention. The modal duration of surgery is 60 minutes with a median of 65 minutes.

Two of the patients were discharged against medical advice. Two mortalities were recorded, one died before colostomy could be established and was censored while the other died after surgery.

Elevated levels of analytes were seen at T1, T2 and T3 respectively in the Table above. This observed difference was statistically significant at day two for all analytes ($P < 0.05$). However, Calprotectin and CRP did not predict enterocolitis at day 1, 2 and 4 post surgery. Table 3. Plasma viscosity and blood viscosity correlated positively with each other.

Table 3: Elevated levels of analytes

Variables	Frequency of Elevated Analytes at		
	T1	T2	T3
CRP	7	1	6
Calprotectin	3	3	5
Blood Viscosity	0	3	8
Plasma Viscosity	2	7	10

Table 4: Predictors of preoperative enterocolitis using linear regression

Analyte	Standardized Coefficient	Significance	
	Beta	t-value	p-value
SERUM CREATIVE PROTEIN T1	-0.054	-0.306	0.762
SERUM CREATIVE PROTEIN T2	0.427	2.008	0.054
SERUM CREATIVE PROTEIN T3	-0.093	-0.440	0.663
SERUM CALPROTECTIN T1	-0.339	-1.560	0.130
SERUM CALPROTECTIN T2	0.450	1.626	0.115
SERUM CALPROTECTIN T3	-0.114	-0.468	0.643
BLOOD VISCOSITY T1	-0.396	-2.104	0.044
BLOOD VISCOSITY T2	0.455	2.184	0.037
BLOOD VISCOSITY T3	-0.146	-0.735	0.469
PLASMA VISCOSITY T1	-0.364	-1.916	0.066
PLASMA VISCOSITY T2	0.383	1.533	0.137
PLASMA VISCOSITY T3	-0.164	-0.694	0.493

Above table shows that serum C-reactive protein and Calprotectin did not predict preoperative enterocolitis, ARM or Hirschsprung's disease ($p>0.05$). Table 4.

Blood viscosity T1 and T2 were found to be a significant predictor of preoperative enterocolitis ($p<0.05$), although the area under the curve was not significant which suggest that blood viscosity T1 and T2 are not sensitive or specific for preoperative enterocolitis. Calprotectin was not affected by gender ($p>0.05$). Figure 1.

Elevated levels of analytes were seen at T2 in the graph. These observed difference was statistically significant at day 2 for all analyte ($P<0.05$). Blood viscosity at T1 was also found to be significant ($p=0.04$). However, the area under the curve has poor discrimination of blood viscosity at T1 and T2 with a sensitivity of 66 %, specificity for enterocolitis of 54 %. The Positive Predictive Value (PPV) was as low as 25 % however, the Negative Predictive Value was 88 %.

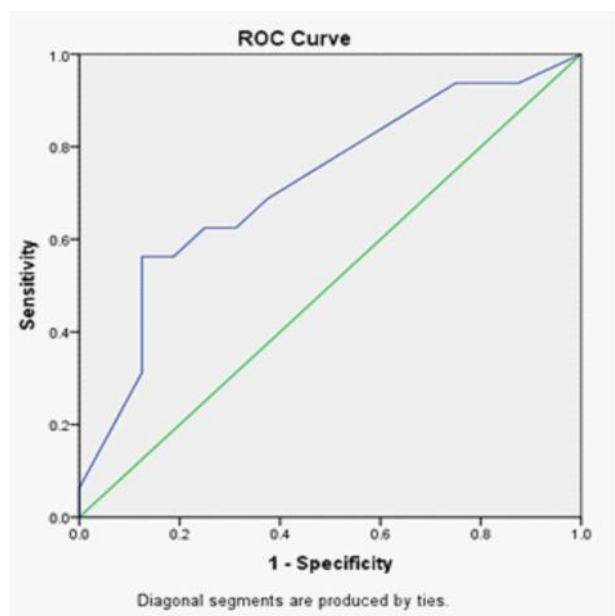


Figure 2: Receiver operator curve for plasma viscosity at T1, T3 and ARM

Elevated levels of analytes were seen at T3 in the graph below, However Calprotectin and CRP did not predict enterocolitis at day four post-surgery.

Elevated levels of plasma viscosity were also seen at T1; However, Calprotectin and CRP did not predict enterocolitis at day one. Figure 2.

DISCUSSION

Anorectal malformations are common paediatric surgical problem with reported incidence as high as 1 in 3,500 and the outcome of management of especially the high type of ARM remained poor globally owing to high risk of overwhelming sepsis which results in rapid deterioration in clinical parameters.⁵

Authors have reported that HAEC may be associated with female gender.^{10, 11} This was different from our finding of four males and two females who had HAEC however, this observed association was not statistically significant.

HD is heritable with 2.8% to 12% of patients with a family history of HD^{12, 13} and development of HAEC may also be influenced by heritable factors. We only found a patient whose sibling was managed for HAEC prior to surgery.

Pastor and colleagues in 2009,¹⁴ have shown that some children have a predisposition towards development of HAEC, and that one episode of HAEC may increase the risk of future HAEC regardless of therapy. Similarly, Reding *et al.*,¹⁵ reported twelve cases of pre-operative HAEC, four of which re-developed HAEC in the post-operative period. None of the six patients who had enterocolitis in this study re-developed HAEC. This may not be unrelated to the short follow up period.

The enterocolitis associated with Hirschsprung's disease has not been clearly characterized even though the major presenting features observed by Elhalaby *et al.*¹⁶ in 1995 were majorly abdominal distension (83%), explosive diarrhoea (69%), vomiting (51%) and fever (34%). About 90% of patients evaluated in this study presented with abdominal distension which is similar to that found in Elhalaby's study.

Prompt diagnosis of sepsis is crucial to establishing treatment and modifying the prognosis of diseases in Paediatric patients. In sub-Saharan Africa, there is paucity of long-term evaluations of these patients, as majority of the cases are lost to follow-up.

ARM and HD are common causes of intestinal obstruction in infants with its attendant morbidities in sub-Saharan Africa. Enterocolitis is a frequent and life-threatening complication associated with HD, although, rarer with ARM but still remains a major cause of significant morbidity and mortality especially after surgery for HD. Hirschsprung's disease-associated enterocolitis in neonates occurred in about 16% of patients³ and postoperative enterocolitis is as high as 35% in a report by Singh *et al.*¹⁷ and these have a greater

likelihood of progression to toxic mega colon leading to shock. Bode and Gbobo¹⁸ at Lagos University Teaching Hospital in 2001 reported two of the patients (3%) out of 33 that died from Hirschsprung's disease associated enterocolitis and sepsis in infancy. A mortality of 6.3% was recorded in this review which was related to multiple co morbidities the neonate had and late presentation.

Childhood colostomy is often indicated for obstructive congenital lesions of the colon and anorectum as well as a few acquired ones.^{3,18-20} Twenty eight patients (90%) out of the patient studied had colostomy. Three had Anoplasty, Anal transposition and a mini PSARP.

The outcome of surgery was satisfactory in 28 of the patients who did not need to stay longer than five days on admission with babies discharged home alive. C-Reactive protein is commonly used but the concentration increases rather slowly in the initial phase of the inflammatory response to pathogens, and its sensitivity is insufficient hence the combination with Calprotectin.

Calprotectin was superior to CRP in the detection of anastomotic leakage, by both earlier (POD 2 to 3 versus POD 4) and more accurate detection. Though, CRP when used alone may not be able to accurately predict intestinal inflammation, combination with calprotectin increases the yield and gives a better diagnostic accuracy. As specificity increased when adding calprotectin measurement, false positive test results decreased dramatically.⁷ In this review, serum level of Calprotectin and CRP did not predict enterocolitis at days 1 (Preoperative), 2 and 4 post surgery. Some individuals do however; have labile calprotectin concentrations with greater day-to-day variation.²¹

The mean difference in the bio-makers compared to presence or absence of preoperative enterocolitis was not statistically significant. The data obtained also showed that Calprotectin was not influenced by age or gender.

Plasma viscosity and blood viscosity correlated positively with each other.

A test of association between analytes and preoperative enterocolitis showed an area under the curve with poor discrimination of blood viscosity at T2 with a very low PPV. This is not unrelated to the low prevalence of the preclinical disease, even while using a test with high sensitivity and specificity.

It can only be speculated why Calprotectin is a better marker for inflammation than CRP. As part of the

innate immune response, neutrophils provide a first line of defense against bacterial infection and are recruited to sites of infection or inflammatory stimuli within minutes, with a peak by 24 to 48 hours.^{22,23} In this study we found an elevated levels of analytes at T1, T2 and T3 respectively and these observed difference was statistically significant at day two for all analytes which coincided with the peak of neutrophil degranulation.

LIMITATION OF STUDY

The small sample size was noted over this one-year study period; however, this is not unrelated to the low incidence of both diseases. A collaborative study will be a way out in future studies.

CONCLUSION

The prevalence of hirschsprung associated enterocolitis is 19% and the sensitivity of blood viscosity at T1 and T2 is 66% and a positive predictive value as low as 25%.

In the context of fast-track surgery, an inflammatory marker with a high Positive and Negative predictive value could allow safe and early diagnosis, discharge from hospital and resultant reduction in morbidities.

A multi-centred study with longer follow up in future studies to further test the validity of these bio markers and establish the relationship between the segment of the disease in HD and the development of enterocolitis is recommended.

Author's Contribution

OOO: Concept, design, definition of intellectual content, literature search, clinical studies, manuscript preparation, manuscript editing and manuscript review.

DIO: Concept, design, definition of intellectual content, literature search, clinical studies, manuscript preparation, manuscript editing and manuscript review

IKE: Design, definition of intellectual content, literature search, clinical studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review.

Declaration of Consent

The authors certify that they have obtained all appropriate patient consent forms from their care givers. In the form, the care givers have given their consent for images and other clinical information to be reported in the journal. They understand that the names of their wards will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Consent of Interest

We declare that we do not have conflict of interest either financially or otherwise in the course of carrying out this study. The study was majorly funded by the authors with a support research grant by the management of the University College Hospital, Ibadan, Nigeria.

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