RESEARCH PROTOCOL: PART 1

Project Summary

Rationale: Dietary modification and reduction of daily fluid intake constitute the conventional therapeutic measures for toddlers' diarrhea. Compliance with these measures can be challenging for the child. Using pharmacotherapy may be an attractive option given the published report showing that proton-pump inhibitors or H2-receptor blockers can effectively control the frequent bowel motion and post-prandial urgency associated with this functional bowel disorder.

Objective: To determine the effectiveness of a short course of daily oral ranitidine in the treatment of toddlers' diarrhea

Methods: A parallel-group randomized controlled trial (RCT) in which each participant is to be randomly assigned to three groups after meeting the eligibility criteria. The three groups of participants will consist of a group to be given daily oral ranitidine (3mg/kg) for 10 days (oral ranitidine group- ORG), the group that will receive probiotics for 10 days (PBG), and the control group that will be given a placebo (vitamin C- 50mg daily) for the same duration.

Populations: 40 participants who met the following eligibility criteria-(1) Age range of 1-3 years (2) Duration of diarrhea lasting 3 weeks or more (3) Historical evidence of the characteristic stooling pattern (3) Absence of pyrexia and signs of dehydration (4) Normal anthropometry and (5) Normal findings on stool analysis, microscopy, and culture.

Time frame: Three years

Expected outcomes: Stool frequency and consistency recorded on the 5^{th} and 10^{th} day, and follow-up documentation 30 days after the end of interventions. Normalization of stool frequency and consistency is expected on the selected days.

General information

Protocol Title: Short course of daily oral ranitidine as a novel treatment for toddler's diarrhea: a double-blind randomized-controlled clinical trial.

Protocol identifying number/date: ISRCTN, ISRCTN10783996/ 8th April 2016

Name and address of sponsor/funder: Self-funded

Name and title of investigators:

1. Samuel N Uwaezuoke (Associate Prof). Department of Pediatrics, University of Nigeria Teaching Hospital, Ituku-Ozalla Enugu, Nigeria. +2348033248108. Responsible for application for ethics approval and registration of trial protocol
2. Ikenna N Ndu (Associate Prof). Department of Pediatrics, Enugu State University Teaching Hospital, Park Lane Enugu, Nigeria. +2348170861591. Responsible for application for ethics approval and registration of trial protocol
3. Chizoma I Eneh (Dr.). Department of Pediatrics, Enugu State University Teaching Hospital, Park Lane Enugu, Nigeria. +234 8036435357. Responsible for drawing the trial plan
4. Chikere A Anusiem (Prof.). Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria. +2348063717200. Responsible for ensuring compliance with trial timelines
5. Adaeze C Ayuk (Associate Prof). Department of Pediatrics, University of Nigeria Teaching Hospital, Ituku-Ozalla Enugu, Nigeria. +2348036754123. Responsible for budget planning.

Name and address of clinical laboratory/institution involved in research: Medical laboratory section of the Restoration Medical Centre (Children's Clinic). No 10 Nnaji-Nwede Street, Achara Layout, Enugu, Nigeria.

Rationale & background information

Toddlers' diarrhea or chronic non-specific diarrhea in childhood is a common cause of persistent loose stools in under-five children (Kneepkens & Hoekstra, 1996; Mascarenhas, 1997). According to Mascarenhas (1997), toddlers' diarrhea is defined as chronic diarrhea lasting more than 3 weeks in a toddler who has normal anthropometric parameters. In addition, the absence of systemic symptoms such as vomiting and fever, signs such as dehydration, as well as normal findings on stool examination will further confirm the diagnosis. The age ranges of affected children are between 6 and 40 months (Fleisher, 2000) or between 1 and 2 years (Mascarenhas, 1997). Children with chronic non-specific diarrhea have a characteristic stooling pattern (Mascarenhas, 1997; Davidson, 1987); the essential features include passage of foul-smelling, watery, or 'mushy' stools (containing food remnants) during the day which alternates with normal stool consistency and frequency at night.

The illness is currently grouped among the functional gastrointestinal disorders of childhood and has these synonyms namely 'functional diarrhea' and 'irritable colon of childhood' (Rasquin-Weber et al, 1999). Most authors now believe it is a gut motility disorder modulated by dietary factors and excessive fluid intake (Kneepkens & Hoekstra, 1996; Green & Ghisan, 1983; Treem, 1993; Hoekstra, 1998; Dennison, 1996). According to Kneepkens & Hoekstra (1996) and Dennison (1996), low-fat diets and the consumption of fruit juices especially juices high in sorbitol and those with a high fructose: glucose ratio, have been implicated. Thus, dietary modification and reduction of daily fluid intake have been suggested as effective therapeutic measures, and remain the current conventional modality of treatment. Other workers have reported that some probiotics such as *Lactobacillus rhamnosus* and *Saccharomyces boulardii* may also be effective in the resolution of symptoms (Guarino et al, 2009; Roggero et al, 1990).

Interestingly, some researchers have noted that inhibition of gastric secretion with proton-pump inhibitors or H_2-receptor blockers effectively controls the frequent bowel motion and post-prandial urgency associated with functional diarrhea or irritable bowel syndrome, probably by suppressing the gastro-colic reflex (Dave & Rubin, 1999). This finding may open a new vista for the use of these pharmacologic agents in the treatment of toddlers' diarrhea.

Ranitidine (an H_2-receptor antagonist or blocker) is currently licensed in children for the treatment of peptic ulcers. We hypothesize that based on the pharmacologic action of suppressing the gastro-colic reflex, the drug can be a novel treatment for toddlers' diarrhea. Moreover, its safety profile in children makes it an attractive therapeutic option for this disorder.

Although dietary modification and fluid restriction constitute the current standard modality of managing toddlers' diarrhea, strict compliance with dietary measures by caregivers may not be optimal, prompting the desirability of a safe pharmacologic option that can easily be adhered to.

Secondly, despite the benign nature of the disorder, self-advised medications by caregivers and misdiagnosis by physicians in developing countries, where childhood diarrhea remains a major public health problem, may lead to unnecessary laboratory investigations and misapplication of drugs, especially antibiotics. Anecdotal evidence suggests that toddlers' diarrhea is wrongly managed as one of the infective diarrheas by physicians leading to huge treatment costs, and the promotion of antibiotic resistance.

As a novel alternative treatment option, ranitidine given orally for a short duration of 10 days will not only ensure prompt resolution of diarrhea but also ensure better compliance by caregivers. In addition, the treatment cost will be more affordable while the side effects for children at this phase of life are very minimal or non-existent.

Study goals and objectives

Study goal: To establish an alternative therapeutic strategy for toddlers' diarrhea using pharmacotherapy

Primary objective: To determine the effectiveness of a short course of daily oral ranitidine in the treatment of toddlers' diarrhea.

Secondary objective: To compare the effectiveness of oral ranitidine with the probiotic (*Lactobacillus rhamnosus*) in the treatment of toddlers' diarrhea

Study design

A parallel-group randomized controlled trial (RCT)

Methodology

Setting: Pediatric Out-patient Clinic at the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla Enugu; and Restoration Medical Centre (Children's Clinic), #10 Nnaji-Nwede Street, Achara Layout, Enugu

Participants' recruitment: Sequential enrolment of 40 participants who met the following eligibility criteria:

(i) Age range of 1-3 years (ii) Duration of diarrhea lasting 3 weeks or more (iii) Historical evidence of the characteristic stooling pattern (iv) Absence of pyrexia and signs of dehydration (v) Normal anthropometry and (vi) Normal findings on stool analysis, microscopy and culture.

Study design: The design is a parallel-group randomized controlled trial (RCT) in which each participant is to be randomly assigned to three groups after meeting the eligibility criteria. For the randomization procedure, we will use permuted-block randomization The three groups of subjects will consist of a group to be given daily oral ranitidine (3mg/kg) for 10 days (oral ranitidine group-ORG), the group that will receive probiotic for 10 days (PBG) and the control group that will be given a placebo (vitamin C- 50mg daily) for the same duration.

Twenty participants will be assigned to each group- ORG, PBG, and PG. A proforma will be used to record the bio-data, anthropometry of participants, and findings of their stool analysis and stool microscopy/culture.

Intervention: In the ORG- participants will be given oral ranitidine (3mg/kg/day) once daily for 10 days; in the PBG, they will receive probiotics in appropriate doses for 10 days; while in the PG,

the participants will be given vitamin C (50 mg) daily for the same duration. We will ensure allocation concealment by using sequentially numbered, opaque, sealed envelopes (SNOSE) containing the medications. In a double-blind fashion, each enrolled participant will be given color-coded envelopes containing ranitidine, the probiotic, or vitamin C tablets unknown to both the caregivers and outcome assessors.

Follow-up/outcome measures: Stool frequency and consistency will be recorded on days 5 and 10. Any adverse drug reactions will be noted on these follow-up days. There will also be follow-up documentation of stool frequency and consistency 30 days after the end of interventions. Failure of participants to present for documentation of the study outcomes on any of the days will be the qualification for dropping out.

Safety considerations: Follow-up on adverse drug reactions (especially adverse reactions related to ranitidine) will be extended up to 60 days after the end of the intervention. Clinical monitoring of participants for adverse reactions to probiotics will be ensured. Provision will be made for hospitalization and appropriate intervention

Data management and statistical analysis: Data entry and analysis will be done using the Statistical Package for Social Sciences (SPSS) version 25 for Windows. Data will be scrutinized for incorrect information and will be cleaned periodically. The Student's t-test will be used to determine any statistically significant differences in the mean daily stool frequencies of the three intervention groups on the follow-up days. ORG and PBG will be used to report the study effect size. Two standardized measures (Cohen's d and estimation of risk difference) will be used. A 95% confidence interval will be assumed while a p-value < 0.05 will be adopted as the level of statistical significance.

For sample size determination, a predetermined table with the desired statistical power of 0.95 and a Cohen's d (effect size) value of 0.8 will be used, approximating a sample size of 42 (To give room for attrition, 40 will be the final sample size). The value of 0.8 will be chosen to correspond to a large effect size in the predetermined table, indicating the likelihood of a stronger effect.

Study expected outcomes: Normalization of stool frequency and consistency will be the expected outcome of the intervention with oral ranitidine. If no adverse reactions are recorded during and after the study, the intervention may become a paradigm shift in the treatment of toddlers' diarrhea. Clinicians may be more inclined to use this novel pharmacologic approach than the conventional non-pharmacologic methods.

Publication policy/target: We aim to disseminate the findings by publishing them in a reputable international journal within a year of completing the study. SNU will take the lead in the publication. All the investigators will be co-authors.

Duration of research: A proposed period of three years will be allocated for the project implementation and publication of findings. The proposed details include monthly enrollment of eligible participants for 6 months, allocation of participants to intervention groups within a month, follow-up of participants and weekly documentation of outcomes for 6 months, and data analysis for 12 months/manuscript drafting for publication for another 12 months.

Anticipated problems: Seamless participants' enrollment may be affected in the government-run tertiary hospital setting chosen as the study site because of clientele attendance and administrative

bottlenecks. A plan B privately-run health facility will be adopted as a study site to facilitate participants' enrollment.

Project management: Each investigator will assume the roles and responsibilities mentioned against his/her name:

1. Samuel N Uwaezuoke - Responsible for application for ethics approval and registration of trial protocol
2. Ikenna N Ndu -Responsible for application for ethics approval and registration of trial protocol
3. Chizoma I Eneh -Responsible for drawing the trial plan
4. Chikere A Anusiem -Responsible for ensuring compliance with trial timelines and quality assurance
5. Adaeze C Ayuk - Responsible for budget planning.

Ethics: Informed consent will be obtained from the caregivers before enrollment. Ethical approval for the study will be obtained from the Health Research Ethics Committee of UNTH Ituku-Ozalla, Enugu. The ethical concern will be anticipated in the use of oral ranitidine which is presently licensed for the treatment of peptic ulcers in children. However, its safety profile in children will be considered a strong point for its administration in the study participants. Anticipation for idiosyncratic adverse drug reactions will be part of their clinical monitoring.

Informed consent form: A copy of the informed consent form meant for the participants' caregivers is presented as follows in the English Language:

Caregivers' consent form

This study is to determine the effectiveness of using the drug- ranitidine- in treating toddlers' diarrhea (which your child is presenting with). The nature of the interventions will be duly explained to you. We wish to enroll your child in any of the intervention groups.

Thank you.

Study investigator (on behalf of co-investigators)

I …… hereby consent to the enrollment of my child into the study after due explanation to me about the nature of the study and the interventions. Any adverse outcome of the medication to be given to my child has also been explained to me.

Signed……

Date……..

Flow chart for the study

CONSORT 2010 Flow Diagram

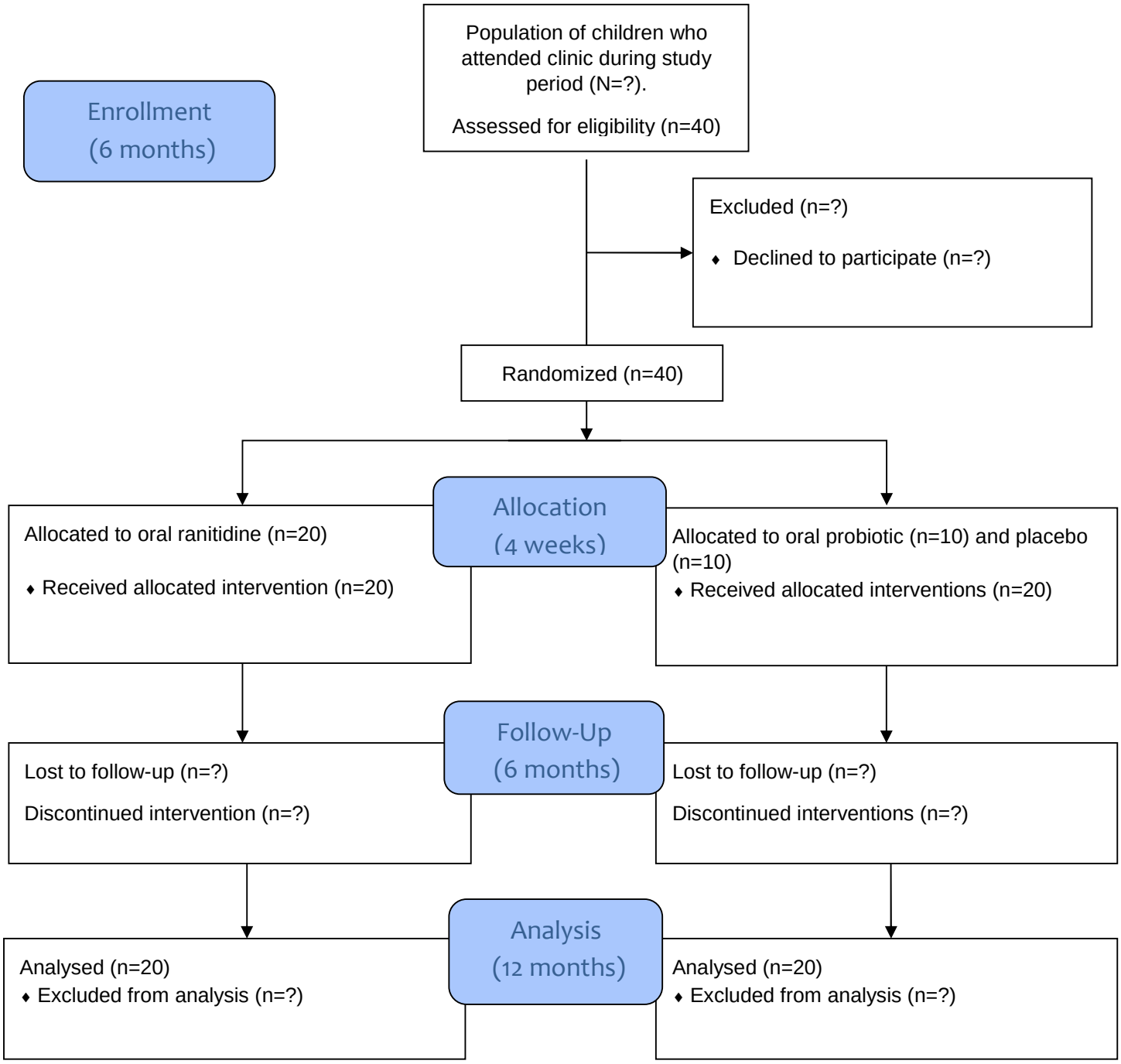

Figure 1: Participants' flow diagram showing their assignment, the interventions, and the analysis

References

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2. Davidson M (1987). Functional problems associated with colonic dysfunction: the irritable bowel syndrome. *Pediatr Ann* ;16:776-795
3. Dennison BA (1996). Fruit juice consumption by infants and children: a review. *J Am Coll Nutr*;15:4-11
4. Fleisher GR. Diarrhea. In: Fleisher GR, Ludwig S (Eds). *Textbook of Pediatric Emergency Medicine*, 4th edn. Philadelphia, PA; Lippincott Williams & Wilkins, 2000;204
5. Green HL, Ghisan FK (1983). Excessive fluid intake as a cause of chronic diarrhea in young children. *J Pediatr* ;102:836-840
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8. Kneepkens CM, Hoekstra JH (1996). Chronic non-specific diarrhea of childhood: pathophysiology and management. *Pediatr Clin North Am*;43:375-390
9. Mascarenhas MR. Toddlers' diarrhoea. In: Schwartz MW(Ed), *The Five-minute Pediatric Consult*. Philadelphia, PA: Lippincott Williams & Wilkins, 1997;756-757
10. Rasquin-Weber A, Hyman PE, Cucchiara S et al (1999). Chronic functional gastrointestinal disorders. *Gut*;45:60-68
11. Roggero P, Volpe C, Ceccatelli MP, Lambri A, Giuliani MG, Donattini T, Garavaglia MC, De Vincentiis A (1990). Crystalline lactulose and oral preparations of micro-organisms for the treatment of chronic aspecific diarrhea in children: a controlled clinical study. *Minerva Pediatr* ; 42(4): 147-150
12. Treem WR (1992). Chronic non-specific diarrhea of childhood. *Clin Pediatr*;31:413

RESEARCH PROTOCOL: PART 2

Budget: Research will be self-funded by the investigators. A budget template will be prepared as follows based on the local currency (Naira):

DESCRIPTION OF ITEM	EXPECTED FROM			TOTAL
	FUNDING BODY	INSTITUTION	OTHERS (INVESTIGATORS)	
1.0 Personnel Costs				
1.1 Principal Investigator				
1.2 Team Members(4)				
1.3 Technical Support				
1.4 Others (Please specify)				
Sub-Total (Not >20% of budget)				
2.0 Equipment (List & Specify)				
2.1 Laboratory consumables for stool analysis/stool microscopy & culture				
2.2 Microscope				
Sub-Total (Not > 25% of budget)				
3.0 Medications **3.1 Probiotic sachets** **3.2 Ranitidine tablets (150 mg)-5x10** **3.3 Vitamin C orange-flavored tablets (1 tin of 500 tablets)**			**210, 000**	
Sub-Total			**210,000**	**210,000**
4.0 Data Collection & Analysis				
4.1 Research Assistants			**50,000**	
4.2 Technical Assistants			**20,000**	
4.3 Data Analysis			**30,000**	
Sub-Total			**100,000**	**100,000**
5.0 Dissemination				
5.1Publication in a reputable journal			**500,000**	
Sub-Total			**500,000**	**500,000**
6. Others/Miscellaneous (Specify)				
6.11Internet subscription/access			**15,000**	
Sub-Total			**15,000**	**15,000**
GRAND TOTAL				**825,000**

Curriculum vitae of the investigators:

Principal investigator: Samuel N Uwaezuoke

A. BIODATA

1. NAME: Samuel Nkachukwu Uwaezuoke
2. DATE OF BIRTH: 4TH MARCH 1966
3. SEX: MALE
4. MARITAL STATUS: Married

B. EDUCATIONAL QUALIFICATIONS

1. MB, BS (NIG)- 1988
2. FWACP (PAED)- 1999
3. DIP. TH – 2006

C. PROFESSIONAL EXPERIENCE

1. INTERNSHIP- UNIVERSITY OF NIGERIA TEACHING HOSPITAL ENUGU (1988-89)
2. NATIONAL YOUTH SERVICE PROGRAM- STATE HOUSE ANNEX CLINIC PORT-HARCOURT (1989-1990)
3. POST-GRADUATE RESIDENCY TRAINING IN PAEDIATRICS- UNIVERSITY OF NIGERIA TEACHING HOSPITAL ENUGU/WEST AFRICAN POST-GRADUATE MEDICAL COLLEGE (1991-1999)
4. PRINCIPAL RESEARCH FELLOW- INSTITUTE OF CHILD HEALTH, UNIVERSITY OF NIGERIA TEACHING HOSPITAL ENUGU (1999-2003)
5. ASSISTANT CHIEF RESEARCH FELLOW- INSTITUTE OF CHILD HEALTH, UNIVERSITY OF NIGERIA TEACHING HOSPITAL ENUGU (2003-2005)

D. UNIVERSITY CAREER

1. LECTURER 1 IN PAEDIATRICS- COLLEGE OF MEDICINE, UNIVERSITY OF NIGERIA (2005-2009)
2. SENIOR LECTURER IN PAEDIATRICS- COLLEGE OF MEDICINE, UNIVERSITY OF NIGERIA (2009-2015)
3. ASSOCIATE PROFESSOR IN PAEDIATRICS- COLLEGE OF MEDICINE, UNIVERSITY OF NIGERIA (2015-DATE)

E. NUMBER OF SCIENTIFIC PUBLICATIONS IN PEER-REVIEWED LOCAL AND INTERNATIONAL JOURNALS: 112

F. PRESENTATION/PARTICIPATION IN CONFERENCES AND WORKSHOPS: 40

G. RESEARCH INTERESTS: CHILDHOOD NEPHROTIC SYNDROME; URINARY TRACT INFECTION IN CHILDREN; CHILDHOOD DIARRHEA; PNEUMONIA

H. POST-GRADUATE SUPERVISIONS: 6 FELLOWSHIP DISSERTATIONS

Co-investigator: Ikenna Kingsley Ndu

A. BIO-DATA

1. DATE OF BIRTH: 31st May, 1970.

2. SEX: MALE

B. MARITAL STATUS: MARRIED

C. **EDUCATIONAL QUALIFICATIONS:** Received medical education at the College of Medicine, University of Nigeria from where he graduated in 1995 with MB; BS degrees. He subsequently had his residency training at the University of Nigeria Teaching Hospital from where he obtained his Fellowship of the West African College of Physicians in Pediatrics in 2013

D. PROFESSIONAL CAREER: He is currently a Consultant Paediatrician and Head, Children Emergency Unit, Department of Paediatrics at the Enugu State University of Science and Technology Teaching Hospital and an Associate Professor of Paediatrics with the College of Medicine, Enugu State University of Science and Technology.

E. SCIENTIFIC PUBLICATIONS: over 100 academic publications in local and international journals.

Co-investigator: Chizoma Ihuarula Eneh

A. SEX: Female
B. MARITAL STATUS: Married
C. CURRENT POSITION: SENIOR LECTURER PAEDIATRICS DEPARTMENT ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY (ESUT)

CONSULTANT PAEDIATRICIAN AND HEAD OF PAEDIATRIC HAEMATOLOGY /ONCOLOGY UNIT DEPARTMENT OF ENUGU STATE UNIVERSITY TEACHING HOSPITAL PARKLANE (ESUTHP).

D. QUALIFICATIONS: MB;BS (NIG), FMCPaed (NATIONAL POSTGRADUATE MEDICAL COLLEGE OF NIGERIA)