Micronutrient Status as Predictors of Low Birth Weight and Pre-Term Delivery in Women Attending Antenatal Care in Challenge, Lusaka, Zambia. A Proposal for Research

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Abstract: *Low birth weight is a significant public health problem. Every year, about 16% of infants are born with a birth weight less than 2,500 gms, with 95% from the developing countries [1]. Micronutrient supplementation of Ferrous sulphate and Folic acid for all pregnant women attending antenatal care in Zambia's healthcare facilities is being practised without estimating their serum concentrations. Available information states that excessive Iron stores are associated with higher risk of metabolic disorders such as hypertension, metabolic syndrome and cardiovascular disease [2-5]. There might be a need for supplementation of other micronutrients in our setting apart from the ones currently being given. Based on current evidence, it is essential to determine the levels of micronutrients among pregnant women to inform policy and practice in Zambia. This study will estimate serum concentrations of Iron, Folic acid, Selenium and Calcium among pregnant women receiving antenatal care in Chilenje, Lusaka district of Zambia. The study will be a Cross-sectional design; quantitative data will be collected at three-time points during the first, second and third trimester of pregnancy. A sample of 226 women attending antenatal care for the first time at Chilenje hospital will be considered for interviews and blood will be obtained for laboratory analysis. The hospital was chosen after random sampling and participants after systematic sampling. The women will have their nutritional status determined by getting the Mid-Upper Arm Circumference, and weight gain assessed during pregnancy. Ethical clearance will be obtained from the University of Zambia Biomedical Research Ethics Committee and the National Health Research Authority. Participants will be allowed to consent before participating in the study. A structured interview schedule will be used to obtain data on demographic characteristics and dietary intake. The analysis will be conducted using STATA version 13 and GraphPad Prism version 5.01. Descriptive statistics will be used to analyse demographic data and inferential statistics to determine whether levels of iron, Zinc, selenium and calcium have a relationship with low birth weight and preterm delivery. Appropriate recommendations will be made after analysing the data.*
Keywords: micronutrients, antenatal, Low birth weight, preterm delivery

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I. Introduction

Micronutrients, vitamins and minerals obtained from the diet are essential for biologic activity, and their status in pregnancy and other populations vary worldwide [6]. Low-income and middle countries encompass both undernutrition with overweight and obesity [7, 8] with Africa and Asia still having women and children with a low Body-Mass Index. Black further states that deficiency of micronutrients such as Zinc can result in deaths while lack of Iron can lead to stunting. These can contribute to children not reaching their developmental potential. Zinc deficiency is prevalent in pregnant women [9], and so there is need to identify those who might be lacking it. On the other hand, Calcium supplementation is likely to reduce the risk of developing hypertensive disorders in pregnancy [10] which plays a significant role in pre-term births. Deficiencies of Selenium in pregnant women can lead to abortions, impairment of the nervous and immune system of the unborn child and low birth weight, intrauterine growth restriction and increased mortality and morbidity [11-13].

Intake of micronutrients is vital during the pre- and peri-conceptional period to reduce the risk of Low Birth Weight and preterm deliveries [14-16]. Studies suggest that adequate intake of mineral supplements before and during pregnancy is associated with a reduced risk of delivering babies who are of low birth weight and preterm deliveries [17]. When multiple micronutrients are lacking in a pregnant woman, this is likely to lead to poor pregnancy outcome. This problem is prevalent in Africa, probably because of the high poverty levels and low levels of education. This study will estimate serum concentrations of Iron, Folic acid, Selenium and Calcium among pregnant women receiving antenatal care in Chilenje, Lusaka district of Zambia.

II. Materials and Methods

Study Design

The study will be a Cross-sectional design; data will be collected at three-time points during the first, second and third trimester. Pregnant women will be recruited as they come for antenatal care. A sample of 226 women aged 15 to 49 years attending antenatal care for the first time at Chilenje hospital will be considered for interviews and blood obtained for laboratory analysis.

Study Design: Cross-sectional study design

Study Location: Chilenje District Level I Hospital in Lusaka, Zambia.

Study Duration: December 2017 to June 2018

Sample Size: 223 pregnant women

Sample size calculation:

$$n = \frac{z^2 p(1-p)}{d^2}$$

= [(1.96)^2 x 0.14(1-0.14)]/(0.05)^2

N = size of sample randomly selected from the population
P = prevalence of Iron deficiency in women (Khana, 2013)
e = Precision (i.e. maximum acceptable difference between sample and population) (5%)
Z = confidence level (1.960 for 95% confidence level
 = **178**
To account for a 20% non-response, the sample size will increase to **223**

Subjects and selection method: the study population will be drawn from women attending the antenatal clinic for the first time. Systematic sampling will be used to sample the antenatal Participants. The average number of women attending ANC in a month in Chilenje is 600, thus: **600/223 = 3**
Therefore, every **3rd** woman visiting antenatal care will be included in the study.

Inclusion Criteria
1. All women in the first and second trimester of pregnancy
2. Women who will consent to participate in the study

Exclusion Criteria
1. Women who would have had a blood transfusion in the previous three months before the study
2. Women with haematological conditions
3. Women on certain medications such as Calcium and Zinc supplements and Cisplastin, a chemotherapeutic agent which interferes with levels of selenium

Variables
The variables under study will be demographics, dietary recall, HIV status, levels of iron, zinc, selenium and calcium as predictors of low birth weight and preterm delivery.

Instruments

All women who will consent to be in the study will be subjected to respond to an interview schedule which will help to determine the dietary diversity scores. The initial part of the structured interview schedule will consist of the demographic characteristics of the participants; the next section will include information on dietary intake in the previous 24 hours and the last one on the vital signs and laboratory findings. Plasma Iron, Zinc, Selenium and Calcium measurements will be analysed using the OPTIMA DV 7000 Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES, Perkin Elmer, Waltham, USA). Analysis of the specimens will be conducted at the Tropical Gastroenterology and Nutrition Group (TROPGAN) Laboratory, Department of Internal Medicine, UTH.

Data collected on dietary diversity will be categorised into nine (9) foods for women (18). Each category consumed will be scored 1, if not eaten, 0. The food groups eaten will be totalled to give individual women dietary diversity scores. The mean and percentiles of the dietary diversity will be computed. The ratings will further be categorised as low, medium and high. A height measuring board and mid-upper arm circumference measuring tape will be used to collect anthropometric measurements of the study participants. A maternal height of <146 cm and 156 cm will be considered as statistically significant for low birth weight and mid-upper arm circumference of <22 and <23 cm will be regarded to be at risk of having low birth weight babies [19]. Maternal weight gain for gestational age will be obtained using a bathroom scale. The cut-off point of 43.5 kg to < 50 kg will be considered statistically significant for Low Birth Weight (19). The same range will be used for the participants each time that they will need their weight to be checked. They will be instructed to wear light clothing so that no extra weight will be recorded. Any participant who will deliver after 28 weeks but before 37 weeks gestation will be categorised as having had a pre-term birth.

Procedure methodology

The women will be given a chance to agree verbally before proceeding. The participants will then be subjected to the routine care they are obliged to have such as getting Information Education and Communication (IEC), height, weight and taking of vital signs, physical examination and obtaining blood for Human Immunodeficiency Virus (HIV), Rapid Plasma Reagin (RPR), including urine for urinalysis. Participants who will consent to take part in the study will fill in a consent form, and a serial number will be assigned to them. The medical technologist will collect 10 mls of blood in the first, second and third trimester on all participants using the antecubital vein. Test Information Sheets will specify the type of container to be used.

The blood will be placed in an Ethylenediaminetetraacetic acid (EDTA) tube of 10 ml containers. Biochemical measurements will be determined after that thoroughly mixed with the EDTA anticoagulant. Samples that are not filled correctly will not be tested. If haemolysis occurs or small clots are observed, the specimen will be discarded. Venous blood will be mixed about eight (8) times by hand inversion. The capped sample will be turned upside down and then back straight up. Plasma will be separated from the cells approximately two (2) hours after collection. The specimens will be transported in specimen containers provided by LabCorp. The sample will then be centrifuged for about 10 minutes. The tubes will remain closed during centrifugation process. Specimens will be refrigerated at a temperature of 4 ∘C after collection then at − 70 ∘C for later analysis. Controls will be run on a daily basis, after completion of calibration, after any major repairs and when patient results appear inaccurate. Controls will be analysed similarly to the patient specimen.

III. Analysis of Plasma Iron, Zinc, Selenium and Calcium

Iron Analysis of Plasma on OPTIMA DV 7000

The purpose of the test is to quantitatively measure the amount of Iron in plasma of the pregnant women. The samples will be collected in specimen bottles containing lithium anticoagulant. A sub-sample of each vial will be put in an acid-washed container using a pipette. Samples will be placed in a Class 100 wood to clean to prevent contamination. Digestion will after that be performed by using the Discovery SPD (CEM, Mathews,NC). In preparation for digestion, the samples will be vortexed and immediately pipetted to avoid settling from occurring before removing the sample. A volume of 250 µl each of serum, whole blood, pooled serum and pooled whole blood will be placed in an acid-washed glass microwave container. Samples will be prepared in triplicate for analysis each day. Thus nine similar samples will be for three analytical days to allow for interday (different day) and intraday (same day) precision.' Deionized water will be used for reagent blanks in place of the samples. Reagent blanks will be prepared by adding deionized water in place of the samples. For each analytical day, seven reagent blanks will be prepared to monitor concentrations of all analytes.

For serum samples, a volume of 300 µl concentrated nitric acid (HNO_3) (Ultrex purity, Fisher), 200 µl concentrated hydrochloric acid (HCL) (Ultrex, Fisher) will be added to each vial. Deionized water will be added to provide a final volume of 2 ml. An acid-washed stir-bar will be added to each vial for stirring purposes during digestion. Samples will then be sealed, placed in the microwave and digested. Trace mineral calibration

standards for ICP-MS analysis will be prepared using NIST-traceable 10 mg/L of Iron. Samples will then be analysed without dilution in the original storage containers to minimise the possibility of contamination. The analysis will then be conducted in kinetic energy discrimination (KED) mode with 10% H_2/He gas to minimise polyatomic interferences. Continuing calibration check samples will be analysed after ten samples and will consist a blank measurement to monitor carry over and mid-range calibration standards from the low-concentration calibration curve and the high-concentration calibration curve. For the calibration, measures will be accepted as passing if their back-calculated concentration will be found to be within ± 15% of the nominal frequency (± 25% of the nominal strength for the lowest concentration standard). Quality control checks will be accepted as passing if their back-calculated level will be found to be within ± 15% of the nominal concentration).

Zinc Analysis in Plasma on OPTIMA DV 7000

A diluent containing 100ml 2% Nitric acid and 100 ml 1% Triton will be prepared. Using the diluent, five (5) working standard solutions of 4ml each will be prepared, ranging from 0.7-2.0mg/L from Zinc stock solution. Quality control will be prepared using Seronorm L-1 1:9 v/v with diluent. Plasma samples will be diluted (1:9 v/v) with diluent. The instrument will then be initialised and stabilised. Working conditions will be on the following parameters; Resolution: normal; Wavelength: 206.2nm; Plasma gas flow: 1.5 L/min; Auxiliary gas flow: 0.2 L/min; Nebulizer gas flow: 0.6 L/min; RF Power: 1450 watts; Plasma view: Axial; Read delay: 15 sec;

Read parameters: 1.0 min; 5.0 max; Peristaltic pump flow rate: 1.5 ml/min; Replicates: 3. The OPTIMA DV 7000 will be standardised with the five working standard solutions (multi-point linear fitting). Samples will be measured in order, beginning with standardisation blanks (diluent), water, quality control samples and then samples. Quality Control rerun will be conducted after 25 samples. Data will be exported to an Excel spreadsheet. Correction for standardisation blanks, drift correction, and dilution factor application will be preset on the OPTIMA DV 7000 software (WinLab32).

Selenium Analysis of Plasma in OPTIMA DV 7000

Diluent containing 100ml 2% Nitric acid, 1ml 3M Hydrochloric Acid (HCl) and 99 ml 1% Triton will be prepared. Five standard working solutions of 4ml each, ranging from 0.5-2.5µmol/L with PerkinElmer Pure Instrument Calibration Standard 4(N930021) will be prepared using a diluent. Quality control will be prepared using Seronorm L-1 1:9 v/v with diluent. After that, Plasma samples will be diluted (1:9 v/v) with diluent. The OPTIMA DV 7000 will be Initialised and stabilised. Working conditions will be set at the following parameters: Resolution: normal; Wavelength: 196.026nm; Plasma gas flow: 1.5 L/min; Auxiliary gas flow: 0.2 L/min; Nebulizer gas flow: 0.6 L/min; RF Power: 1450 watts; Plasma view: Axial; Read delay: 90 sec; Read parameters: 2.0 min; 5.0 max; Peristaltic pump flow rate: 1.8 ml/min; Replicates: 3.

The instrument will be standardised with the five working standard solutions (multi-point linear fitting). Samples will be measured in order, beginning with standardisation blanks (diluent), water, quality control samples and then samples. Quality Control rerun will be conducted after 25 samples. Data will then be exported to an Excel spreadsheet. Correction for standardisation blanks, drift correction, and dilution factor application will be preset on the instrument software (WinLab32).

Calcium analysis in plasma on OPTIMA DV 7000

The purpose of the test is to quantitatively measure the amount of Calcium in plasma of the pregnant women. The samples will be collected in specimen bottles containing lithium anticoagulant. A sub-sample of each vial will be put in an acid-washed container using a pipette. Samples will be placed in a Class 100 wood to clean to prevent contamination. Digestion will after that be performed by using the Discovery SPD (CEM, Mathews,NC). In preparation for digestion, the samples will be vortexed and immediately pipetted to avoid settling from occurring before removing the sample. A volume of 250 µl each of serum, whole blood, pooled serum and pooled whole blood will be placed in an acid-washed glass microwave container. Samples will be prepared in triplicate for analysis each day. Thus nine similar specimens will be for three analytical days to allow for interday (different day) and intraday (same day). Precision' deionised water will be used for reagent blanks in place of the samples which will be prepared by adding deionised water in place of the samples. For each analytical day, seven reagent blanks will be prepared to monitor concentrations of all analytes.

For serum samples, a volume of 300 µl concentrated nitric acid (HNO_3) (Ultrex purity, Fisher), 200 µl concentrated hydrochloric acid (HCL) (Ultrex, Fisher) will be added to each vial. Deionized water will be added to provide a final volume of 2 ml. An acid-washed stir-bar will be added to each vial for stirring purposes during digestion. Samples will then be sealed, placed in the microwave and digested. Bulk mineral calibration standards for ICP-OES analysis will be prepared by dilution of NIST-traceable 1,000 mg/L of Calcium

standards. Samples will be diluted 5-fold and 25-fold using an acid matrix-matched blank solution and analysed at the wavelengths of 393.3. (396.8), and analysis of samples will be performed in axial mode. Continuing calibration check samples will be examined at most after every ten samples and will consist of a blank measurement to monitor carryover, a high-range calibration standard, and a low-range calibration standard to assess the performance of the instrument. The estimated limit of quantification (ELOQ) will be taken at the lowest calibration standard for each analyte, and continuing calibration blanks will be used to determine instrument detection limits (IDL) for Calcium.

Ethical considerations

Before initiation of the study, ethical approval will be obtained from the University of Zambia Biomedical Research Ethics Committee. Ethical guidelines will be adhered to throughout the study by the declaration of Helsinki on moral principles for medical research involving human subject [20]. The study will ensure that the rights, integrity and confidentiality of study participants are protected. Participants will be enrolled in the study protocol and consent forms approved by the relevant ethics committee. Privacy and confidentiality of all data and information collected will be strictly maintained for study participants. No names will be used to identify participants during data collection, report or publication of study findings. All data and information collected from the participants will be kept in a secure place, and accessibility will be restricted to authorised research teams. Resuscitative equipment and drugs will be made available in case of an emergency. Participants who will be discovered to be HIV positive will be provided with a full package of HIV management according to the National guidelines such as counselling and initiation of antiretroviral therapy. Those who will be found to be RPR positive will be treated accordingly. Whether the participants will decline or not participate in the study, standards will be adhered. The participants will be in the natural setting and so will not be exposed to any physical and emotional danger or harm. Immediate benefits may not be realised there and then but shortly. Informed consent will be obtained from antenatal women participating in the study by using the language conversant to them (preferably Nyanja and English will be used). A written consent form will be signed by both parties, and a copy will be given to the participant, and another copy will remain with the Researcher. Assent will be obtained from participants aged less than 18 years of age.

Statistical analysis

To compare the demographic characteristics, the Chi-squared test will be used. Means and standard deviations will be used to analyse continuous demographic variables if it will be normally distributed, whereas the median and interquartile range will be used if not normally distributed. Comparisons between the normal and the comparison group will be analysed using the unpaired t-test if data will be normally distributed and Mann-Whitney's U test for data which will not be normally distributed. To compare continuous variable groups at three-time points, repeated measure ANOVA will be used for normally distributed data and Friedman test for data that is not normally distributed. To determine the relationship between two continuous variables such as serum Iron with age, serum iron with mid-upper arm circumference or serum iron with women's dietary diversity score, Pearson's Correlation will be utilised if the data will be normally distributed and Spearman's Correlation if data will not be normally distributed. In the final analysis to rule out confounders, Multivariate Multiple Regression will be conducted after adjustment for baseline characteristics, Iron , Zinc, Selenium and Calcium as independent variables and birth weight and preterm labour as dependent variables with the probability of removal in the final model set at p = 0.2 (20%) and interpreted using regression coefficients and confidence intervals. All statistical analyses will be done using GraphPad Prism version 5.01 (GraphPad Software Inc., La Jolla, CA, USA) and STATA version 13 (Stata Corp, College Station, Texas, USA). A p value of <0.05 will be considered statistically significant for all statistical tests.

IV. Results

The results will be discussed according to the variables under study and will be used to determine whether the establishment of these Micronutrients are predictors of low birth weight and pre-term labour in women attending antenatal care in Chilenje. The results will further reveal the nutritional status and dietary intake of women attending antenatal care in Chilenje, Lusaka. Recommendations will be made according to the findings.

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