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Research Article

Evaluation of Serum Concentrations of Copper, Ferritin, Selenium, Zinc and Vitamin D at Birth in Women with Spontaneous Preterm Birth: A Case-Control Study in a Low Resource Country

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Abstract

Introduction: Micronutrients are elements and vitamins required in small amounts for the average growth and development of organisms. During pregnancy, micronutrients enable the fetus to develop and mature into a health neonate. Micronutrients provide immunity and anti-oxidative protection from the damaging effects of reactive oxygen species. Deficiency of micronutrients has various devastating health effects, including the lack of anti-oxidation and dysregulation of immunity. Considering Malawi's high preterm birth rate, which is currently at 18.1%, and the increased prevalence of micronutrient deficiency in women of reproductive age, we evaluated the maternal serum concentrations of copper, ferritin, selenium, zinc and vitamin D in spontaneous preterm birth. The primary objective was to assess the serum concentrations of these factors and how they relate to infections of histologic chorioamnionitis or placental malaria.

Materials and methods: We conducted this case-control study at Kamuzu Central and Bwaila Hospitals between June 2016 and March 2017. The target population was all women presenting for delivery at these hospitals. The study used gestation to define cases (28-<37 weeks) and controls (37-<41 weeks) and participants were randomization on admission.

Results: We sampled a total of 187 women, of which 95 were preterm, and 92 were term births. The mean serum concentrations for copper 2.5; SD0.5 (0.76-1.59mg/L), ferritin 60.3; SD92.9 (10-100µg/L), selenium 77.4; SD19.3 (47-142µg/L), zinc 0.8; SD 0.2 (0.59-1.11mg/L), vitamin D 90.7; SD 21.3(50-125nmol/L) were normal in the study population. Preterm birth group had significantly higher serum concentration of copper ($p=0.002$), ferritin ($p=0.001$), selenium ($p=0.033$), and zinc ($p=0.003$) compared to term birth group. The prevalence of histologic chorioamnionitis in the study participants was 10.2% (19/187) while for placental malaria was 20.3% (37/187)). Maternal serum zinc ($p=0.027$) and vitamin D ($p=0.031$) were significantly higher in women with histologic chorioamnionitis compared to those without histologic chorioamnionitis. And serum copper ($p=0.028$) and iron ($p=0.001$) were significantly higher in women with placental malaria compared to these without placental malaria.

Conclusion: We conclude that there is no deficiency of copper, ferritin, selenium, zinc and vitamin D in the study population. The preterm birth group has significantly higher serum micronutrients compared to term group. Serum zinc and vitamin D were increased in positive histologic chorioamnionitis while positive placenta malaria was associated with increased serum copper and iron concentrations.

Keywords: Chorioamnionitis; Copper; Iron; Placental malaria; Preterm birth; Selenium; Vitamin D; Zinc

Key Message: Increased maternal serum concentrations of copper, iron, selenium and zinc are associated with the prevalence of spontaneous preterm birth. Similarly increased maternal serum concentrations of zinc and vitamin D are associated with histologic chorioamnionitis while serum copper and iron are associated with placental malaria.

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Introduction

Micronutrients are elements and vitamins acquired from our diet in minute quantities that are necessary to support life and optimal physiological function [1]. During pregnancy, micronutrients such as copper, iron, selenium, zinc and vitamin D enable the fetus to develop and mature into a health neonate [2-5]. The micronutrients copper, iron, selenium, zinc and vitamin D also provide a potent innate and adaptive immunity regulating the infection/inflammatory response [6-10]. Additionally, these micronutrients provide anti-oxidative protection from the damaging effects of Reactive Oxygen Species (ROS) [11-13]. During pregnancy, the increased metabolic demands of the growing foetus up-regulate ROS [14]. Globally, widespread micronutrients exist affecting an estimated 2 billion people [15]. Micronutrient deficiency is reportedly high in women of reproductive age in Malawi [16]. Changes in the homeostasis of micronutrients during pregnancy affect the health of pregnant women and the growing fetus with increased mortality and morbidity [17].

Preterm birth remains a global health problem with an average preterm birth rate of 11.1% every year [18]. Malawi is one of the countries with a very high preterm birth rate of 18.1% [18]. Various pathological processes, including infections/inflammation and ROS, have been identified as precursors leading to a common pathway of increased myometrial contractility, cervical dilatation and chorioamnionic membrane weakening and rupture [19]. Spontaneous Preterm Birth (spPTB) has multiple risk factors with infections causally linked to it [20].

Considering the high preterm birth rate and increased micronutrient deficiency prevalence in women of reproductive age in Malawi, the critical role that copper, iron, selenium, zinc, and vitamin D play in anti-oxidation and immunity in humans, we evaluated the status of these micronutrients in spPTB. The null hypothesis was that there is no difference in maternal serum concentration of copper, iron, selenium, zinc and vitamin D between preterm and term births. The main objective of the study was to determine the maternal serum concentrations of copper, iron, selenium, zinc and vitamin D in pregnant women delivering spontaneously preterm in Malawi. The secondary objective was to find out how these micronutrients relate to infections of histologic chorioamnionitis or placental malaria. The knowledge gained will assist in determining the causal pathway in trying to reduce the rate of preterm birth.

Materials and Methods

We conducted this case-control study at Kamuzu Central Hospital (KCH) and Bwaila Hospitals (BH) between June 2016 and March 2017. KCH and BH are public and government-funded tertiary and general hospitals respectively, located within the city of Lilongwe in the central region of Malawi. Geographically, the two hospitals are within 4 kilometres of each other. The availability of neonatal services at KCH necessitates the management of early preterm births. The measured outcomes were maternal serum concentrations (copper, ferritin, selenium, zinc and vitamin D), histologic chorioamnionitis and placental malaria. Neonatal services are available at KCH only.

The target population was all pregnant women presenting with spPTB at the hospitals. The study used previous dating ultrasound to assess gestational age. We performed an ultrasound on admission in cases where the dating ultrasound result was not available. Upon delivery of the baby, we evaluated the baby maturity with the Ballard score [21]. The inclusion criteria were pregnant women presenting in spontaneous labour at gestation 28 - <37 weeks for preterm (cases) and 37 - ≤41 completed weeks for term (control) group and willing to participate in the study. The following were the exclusion criteria: maternal comorbidities (preeclampsia, gestational diabetes and heart disease), multiple pregnancies, Intrauterine Growth Restriction (IUGR), Fetal Anomaly and Preterm Premature Rupture of Membrane (PPROM). Participants were selected by systematic random sampling with numbers allocated to all eligible preterm cases and all with odd numbers recruited in the study to minimise confounding bias. Convenience sampling was done for controls as they presented in the labour ward and matched to the living area (within the boundaries of the urban city).

All data and samples were obtained soon after delivery. The study administered a structured questionnaire in a local language which study participants were conversant with to capture quantitative data on demographic characteristics of the participants and antenatal care package. We collected the self-reported information on herbal medicine ingestion. The study used the World Health Organization definition of herbal medicine ingestion [22]. The trained midwife proceeded to do bedside laboratory test of haemoglobin using HemoCue Hb 301 (HemoCue AB, Angelhalm, Sweden). Upon delivery of placenta and membranes, the midwife collected a full-thickness placental biopsy (approximately 2.5×2.5×1cm thick) from a third of the distance from the umbilical cord and the edge of the placental disc. At the same time, a membrane roll was also taken and together with the placenta biopsy, they were immediately placed in 10% neutral buffered formalin and sent to the histopathological laboratory. The trained midwife proceeded to withdraw 10 mL of venous blood using a standard vacutainer technique into a royal blue top vacutainer tube (lot No. BD368380). The blood samples were transported to the laboratory within 30 minutes of collection for centrifuging at 3000 g for 10 minutes and serum transferred into a screw-top vial and stored in the freezer at -80˚C. The study used metal-free vessels throughout the process. After obtaining the required number of specimens, we shipped the samples in the frozen state to the University Hospital of North Norway (UNN), Tromsø, Norway, for chemical analysis.

Placenta, membranes and cord specimens were processed and analysed within one week of submission. One specific pathologist did the histopathological analysis. The processing included paraffin embedding, cutting 5μm sections and staining with Hematoxylin and Eosin (H&E).The histopathologist used three key histologic of untreated malaria, including parasites, inflammation and pigment deposition (haemozoin) for the pathological diagnosis of placental malaria [23]. The presence of acute inflammatory infiltrates in the chorion and amnion of confirmed the diagnosis of acute histologic chorioamnionitis. Copper, ferritin, selenium, and zinc analysis were performed at the Norwegian laboratory in Tromsø, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Nexion 300D, Perkin Elmer, Waltham, Massachusetts, USA).Serum (100 μL) was diluted by an automated liquid handler Tecan Freedom Evo 200 (Männedorf, Switzerland) with an alkaline diluent solution. Concentrations were obtained by the standard internal method with a blank subtraction. Two sets of ClinCheck control material L-1 and L-2 from Recipe (Recipe, Munich, Germany), and Seronorm L-1 and L-2 (Sero, Billingstad, Norway), as well as 4 Milli-Q water blanks, were run together with each batch of samples for quality assurance and quality control. Background and instrumental carry over were monitored by injections of diluent blanks. The Laboratory for Analysis of Environmental Pollutants, UNN, Norway, participates successfully in the international quality control programme and/such as Quebec

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Multielement External Quality Assessment Scheme (QMEQAS) organised by the Centre de toxicologie du Quebec, Quebec and Canada. Measurement of 25 (O.H.) D was analysed by liquid chromatography-tandem mass spectrometry, as previously described by Sollid [24].

Ethical Approval

The ethics committee of the University of Malawi, College of Medicine Research Committee (COMREC) approved this study, P.05/15/1738. We also obtained permission from the Directors of KCH and BH to conduct the study at their respective hospitals. Participants in the study gave informed consent.

Data Management and Analysis

We reached a sample size of 95 through the power of test calculation. This sample size gives 80% power to detect a significant difference at 5% level of significance. Data were processed using IBM SPSS version 20.0 software. The SPSS dataset was imported into STATA 14.0 for analysis. A binary variable was generated for the primary outcome of spPTB, preterm birth (cases) coded as 1 and term birth (controls) coded 0. One-way frequencies were computed for categorical variables, while mean and standard deviation were calculated for continuous variables that were normally distributed. Log transformation was done where there was a presence of highly skewed data. Two-way associations between independent variables and the outcome variable of preterm birth were investigated using the t-test for continuous independent variables and the Chi-squared test for categorical variables. P-values from all tests of less than 0.05 were considered statistically significant.

Results

There were a total number of 187 participants in the study, divided into 95 preterm and 92 term groups (Table 1).

There was no significant difference in age group, gravidity, marital status and education between the two groups (p=>0.05). Increased number of women who took herbal medication were more likely to deliver preterm, p=0.032 compared to those who did not. In contrast, those who took intermittent preventive treatment in pregnancy with Sulfadoxine- pyrimethamine (IPTp-SP) were less likely to deliver preterm (p=0.033) compared to women who did not use it. Haemoglobin lower than 11g/dl was significantly present in women delivering preterm compared to women delivering at term (p=008). Similarly, women delivering preterm were more likely to have histologic chorioamnionitis (p=0.014) and placental malaria (p=0.003).

Two-way associations of micronutrients with preterm birth

The analyses used a two-way model to investigate several trace elements. There was no difference in maternal serum concentrations of copper, selenium and zinc between the two groups (Table 2).

The mean concentrations of ferritin, selenium, zinc, 25 (O.H.) D were within the normal reference range while that of copper was above the normal range in both groups. Women who delivered preterm were more likely to have significantly higher mean concentrations of copper (p=0.002), ferritin (p=0.001), selenium (p=0.033) and zinc (0.003) compared to women who delivered at term. There was no difference in the mean concentration of 25 (O.H.) D between women having preterm birth and those delivering at term, p=0.886. In the analysis of histologic chorioamnionitis, there were 19/187 (10.2%) women with positive histologic chorioamnionitis (Table 3A).

Characteristic	Preterm N=95	Term N=92	P-value
Maternal age (years), N (%)			
<20	28 (29.5)	15 (16.3)	0.063
20-29	48 (50.5)	49 (53.3)	
≥30	19 (20.0)	28 (30.4)	
Gestation, Mean(SD), weeks	33.4 (2.5)	38.0 (1.0)	<0.001*
28-<32, N (%)	22 (23.2)		
32-<37, N (%)	73 (76.8)		
Parity, N (%)			
0-1	64 (67.4)	48 (52.2)	0.083
2-4	25 (26.3)	38 (41.3)	
≥5	6 (6.3)	6 (6.5)	
Inter-pregnancy interval of >2years	39 (41.1)	59 (64.1)	0.015*
Marital status, N (%)			
Single	3 (3.1)	2 (2.2)	1.000
Married/cohabitating	90 (94.7)	89 (96.7)	
Divorced/separated	2 (2.1)	1 (1.2)	
Education Level, N (%)			
≤ Primary	59 (62.1)	44 (47.8)	0.076
Secondary	29 (30.5)	43 (46.7)	
College	7 (7.4)	5 (5.4)	
Herbal medication use, N (%)	22 (23.2)	10 (10.9)	0.032*
IPTp-SP Use, N (%)	85 (89.5)	90 (97.8)	0.033*
HIV positive, N (%)	13 (13.7)	15 (16.3)	0.379
Use of ART	9 (9.5)	15 (16.3)	0.193
Hemoglobin (g/dl), N (%)			
≥11	33 (34.7)	50 (54.4)	0.008*
<11	62 (65.3)	42 (45.6)	
Positive histologic chorioamnionitis, N (%)	15 (15.8)	4 (4.4)	0.014*
Positive placental Malaria, N (%)	27 (28.4)	10 (10.9)	0.003*

Table 1: Characteristics of study participants for preterm vs term births.

M: Mean, SD: Standard Deviation, N: Number, HIV: Human immunodeficiency Virus, ART: Anti-retroviral Treatment, IPTp-SP: Intermittent Preventive Treatment in Pregnancy with Sulfadoxine-Pyrimethamine, *: Significant.

Significantly higher maternal serum concentrations of zinc (p=0.027) and vitamin D (p=0.031) were present in women with histologic chorioamnionitis compared to women who were negative for histologic chorioamnionitis. In the analysis of placental malaria, there were 37/187 (19.8%) women with positive placental malaria (Table 3B).

Increased maternal serum concentrations of copper (p=0.028) and ferritin (p=0.001) were present in women with placental malaria compared with those who were negative for placental malaria.

Discussion

The concentrations of ferritin, selenium, zinc, and vitamin D were within normal range while serum copper was above average in both preterm and term groups. Thus, there was no deficiency of copper, ferritin, selenium, zinc and vitamin D in the study population.

Serum micronutrient	Lab Ref Values [25]	Overall mean values	Preterm births n=95	Term births n=92	p-value
Copper (mg/L), M (SD)	0.76-1.59	2.5 (SD 0.5)	2.6 (2.5)	2.4 (0.5)	0.002*
Ferritin (µg/L), M (SD)	10-100	60.3 (SD 92.9)	81.5 (123.2)	38.4 (31.7)	0.001*
Selenium (µg/L), M (SD)	47-142	77.4 (SD 19.3)	80.4 (21.3)	74.4 (16.4)	0.033*
Zinc (mg/L), M (SD)	0.59-1.11	0.8 (SD 0.2)	0.8 (0.2)	0.7 (0.2)	0.003*
Vitamin D (nmol/L), M (SD)	50-125	90.7 (SD 21.3)	90.5 (21.6)	91.0 (21.2)	0.886

Table 2: Maternal serum concentrations of copper, ferritin, selenium, zinc and vitamin D for term vs preterm births.

M: Mean, SD: Standard Deviation

Variable	Histologic Chorioamnionitis		p-value
	Positive N=19	Negative N=168	
Copper (mg/dl), M (SD)	2.6 (0.7)	2.5 (0.5)	0.421
Ferritin (µg/L), M (SD)	53.2 (59.1)	61.1 (96.0)	0.726
Selenium (µg/L), M (SD)	81.8 (12.7)	76.9 (19.8)	0.295
Zinc (mg/L), M (SD)	0.9 (0.4)	0.8 (0.2)	0.027*
Vitamin D (nmol/L), M (SD)	100.7 (20.0)	89.6 (21.2)	0.031*

Table 3A: Maternal serum micronutrients concentrations of copper, iron, selenium, zinc, and vitamin D for positive vs negative histologic chorioamnionitis/placental malaria.

M: Mean, SD: Standard Deviation

Variable	Placental Malaria		p-value
	Positive N=37	Negative N=150	
Copper (mg/dl), M (SD)	2.7 (0.6)	2.5 (0.5)	0.028*
Ferritin (µg/L), M (SD)	106.7 (126.9)	48.9 (78.8)	0.001*
Selenium (µg/L), M (SD)	79.7 (20.7)	76.9 (18.9)	0.432
Zinc (mg/L), M (SD)	0.8 (0.3)	0.8 (0.2)	0.676
Vitamin D (nmol/L), M (SD)	95.2 (23.6)	89.6 (20.7)	0.156

Table 3B: Maternal serum micronutrients concentrations of copper, iron, selenium, zinc, and vitamin D for positive vs negative histologic chorioamnionitis/placental malaria.

M: Mean, SD: Standard Deviation

Previous reports on these serum concentrations are different from these study findings. In Malawi, women of reproductive age group have prevalent micronutrient deficiencies [16]. This difference in the study population explains the discrepancies. The studies were done outside pregnancy and included participants from the rural community. Micronutrient deficiency is more prevalent in rural communities [16]. This study was hospital-based and within the urban setting. To the best of our knowledge, this is the first study to analyse copper, ferritin, selenium, zinc and vitamin D in Malawi pregnant women.

Mothers delivering preterm had significantly higher concentrations of copper, ferritin, selenium and zinc ($p < 0.05$). This finding is similar to results reported by other researchers [26,27]. Other studies have reported contrasting findings of increased maternal serum concentrations of copper, iron, selenium and zinc in women with term births [4,28]. The differences in study population, design and sample size contribute to the discrepancy. Both studies were done outside the sub-Saharan Africa region, and the study by Wang et al., [4] was prospective while that by Irwinda et al., [28] used small numbers of participants (25 term versus 26 preterm births). In this study, a significant number of women delivering preterm had placental malaria. The increased maternal serum concentrations of copper and ferritin in women with preterm birth is attributed from the presence of malaria parasites destroying red blood cells resulting in the efflux of copper andiron [29,30]. The inflammation from placental malaria also up-regulated the inflammatory marker ferritin, sequestering the serum irons from the malaria parasite providing nutritional immunity [31]. The haemolysis of red blood cells also caused the significant anaemia present in women with spPTB. Additionally, the elevated free copper ions caused the generation of damaging ROS further increasing the risk of spPTB [32].

The study findings do not fully explain the science behind an increased maternal concentration of selenium and zinc in women with spPTB. There is a possibility that hemolysis of red blood cells by the malaria parasites would cause this increase. Contrarily, women with placenta malaria (Table 3B) do not have increased selenium and zinc. Similarly, the maternal serum concentration of zinc is increased in the presence of histologic chorioamnionitis inflammation (Table 3A). However, the literature suggests that serum concentrations of zinc decrease during inflammation resulting from host defense in creating nutritional immunity [31]. The pro-inflammatory cytokine interleukin 6 up-regulate expression of ZIP14 [33]. The zinc ion transporter ZIP14 leads to accumulation of zinc bound to metallothionein in the liver resulting in hypozincemia [31].

Conversely, a significant number of women having preterm birth used herbal medication during this pregnancy. Most plants extract have unknown biological activity. Herbal medicines could have caused the efflux of selenium and zinc. The increased zinc microenvironment present in preterm birth group favoured the pathogen growth resulting in inflammation associated with spPTB. Both human and pathogens require zinc for survival [34]. Apart from causing the efflux of the elements, these herbs also produced uterotonic effects leading to spPTB [35].

Similarly, serum concentrations of vitamin D were significantly higher in the group with histologic chorioamnionitis. Published reports support association of low concentrations of vitamin D and infections and pro-inflammatory cytokines during pregnancy [36]. Vitamin D deficiency correlates with increased susceptibility to infections due to impaired localized innate immunity and defective antigen-specific cellular immune response [6]. This study finding suggests placental immune function is not dependent on serum vitamin D concentrations [37]. The role of maternal serum vitamin D concentrations on histological chorioamnionitis is yet to be determined. The limitation to be considered when interpreting these study findings is the absence of data on anthropometric measurements for nutritional status. As such, it was not possible to assess the nutrition status of the participants in the study. Despite the limitations, the study provides the basis for investigating further the micronutrient concentrations of the Malawi pregnant population.

Conclusion

There was a difference in maternal serum concentrations of copper, ferritin, selenium, and zinc between term and preterm births. Preterm birth is associated with increased concentrations of copper, ferritin, selenium and zinc. Malaria continues to be associated with significant morbidity in maternal health.

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Author Contributors

Grace Chiudzu, Alfred Maluwa, PhD and Jon Odland, MD, PhD, conceptualise the study, provided the methodology, including the design, data collection, analysis and the software. Grace Chiudzu did the formal analysis and results writing. Sandra Huber did the blood serum analysis of the trace elements. Ole-Martin Fuskevåg did the blood serum analysis of the vitamin D. Jon Odland provided the resources. Grace Chiudzu wrote the original manuscript draft. Alfred Maluwa and Augustine Choko guided statistics and data analysis. Alfred Maluwa, together with Jon Odland, edited the manuscript. All authors approved the manuscript to be submitted for publication consideration.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

1. Gernand AD, Schulze KJ, Stewart CP, West Jr KP, Christian P (2016) Micronutrient deficiencies in pregnancy worldwide: health effects and prevention. Nat Rev Endocrinol 12: 274-289.
2. Gambling L, Andersen HS, McArdle HJ (2008) Iron and copper, and their interactions during development. Biochem Soc Trans 36: 1258-1261.
3. Mariath AB, Bergamaschi DP, Rondó PHC, Tanaka ACD, Hinnig P de F, et al. (2011) The possible role of selenium status in adverse pregnancy outcomes. Br J Nutr 105: 1418-1428.
4. Wang H, Hu Y-F, Hao J-H, Chen Y-H, Wang Y, et al. (2016) Maternal Serum Zinc Concentration during Pregnancy Is Inversely Associated with Risk of Preterm Birth in a Chinese Population. J Nutr 146: 509-515.
5. van der Pligt P, Willcox J, Szymlek-Gay EA, Murray E, Worsley A, et al. (2018) Associations of Maternal Vitamin D Deficiency with Pregnancy and Neonatal Complications in Developing Countries: A Systematic Review. Nutrients 10: 640.
6. Adams JS, Hewison M (2008) Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab 4: 80-90.
7. Failla ML, Hopkins RG (1998) Is low copper status immunosuppressive? Nutr Rev 56: 59-64.
8. Berrak SG, Angaji M, Turkkan E, Canpolat C, Timur C, et al. (2007) The effects of iron deficiency on neutrophil/monocyte apoptosis in children. Cell Prolif 40: 741-754.
9. Kiremidjian-Schumacher L, Roy M, Glickman R, Schneider K, Rothstein S, et al. (2000) Selenium and immunocompetence in patients with head and neck cancer. Biol Trace Elem Res 73: 97-111.
10. Allen JI, Perri RT, McClain CJ, Kay NE (1983) Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. J Lab Clin Med 102: 577-589.
11. Wang Y, Walsh SW (1998) Placental mitochondria as a source of oxidative stress in pre-eclampsia. Placenta 19: 581-586.
12. Erdman JW, Macdonald IA, Zeisel SH (2012) Present Knowledge in Nutrition (10thedn). International Life Sciences Institute, Washington, D.C., USA.
13. Johnson MA, Fischer JG, Kays SE (1992) Is copper an antioxidant nutrient? Crit Rev Food Sci Nutr 32: 1-31.
14. Myatt L, Cui X (2004) Oxidative stress in the placenta. Histochem Cell Biol 122: 369-382.
15. Institute of Medicine (US) Committee on Micronutrient Deficiencies (1998) Prevention of Micronutrient Deficiencies: Tools for Policymakers and Public Health Workers. National Academies Press, Washington, D.C., USA.
16. National Statistical Office (2020) 2015-16 Malawi Micronutrient Survey (2015-16 MNS). National Statistical Office, Malawi.
17. Black R (2003) Micronutrient deficiency--an underlying cause of morbidity and mortality. Bull World Health Organ 81: 79.
18. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller A-B, et al. (2012) National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. Lancet 379: 2162-2172.
19. Romero R, Espinoza J, Kusanovic J, Gotsch F, Hassan S, et al. (2006) The preterm parturition syndrome. BJOG 113: 17-42.
20. Gonçalves LF, Chaiworapongsa T, Romero R (2002) Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev 8: 3-13.
21. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, et al. (1991) New Ballard Score, expanded to include extremely premature infants. J Pediatr 119: 417-423.
22. WHO (2000) General Guidelines for methodologies on research and Evaluation of traditional medicine. WHO, Geneva, Switzerland.
23. Garnham PCC (1938) The placenta in malaria with special reference to reticulo-endothelial immunity. Trans R Soc Trop Med Hyg 32: 13-34.
24. Sollid ST, Hutchinson MYS, Fuskevåg OM, Figenschau Y, Joakimsen RM, et al. No effect of high-dose vitamin D supplementation on glycemic status or cardiovascular risk factors in subjects with prediabetes. Diabetes Care 37: 2123-2131.
25. Rahil-Khazen R, Bolann BJ, Ulvik RJ (2000) Trace element reference values in serum determined by inductively coupled plasma atomic emission spectrometry. Clin Chem Lab Med 38: 765-772.

26. Hao Y, Pang Y, Yan H, Zhang Y, Liu J, et al. (2019) Association of maternal serum copper during early pregnancy with the risk of spontaneous preterm birth: A nested case-control study in China. Environ Int 122: 237-243.

27. Saha CK, Jain V, Gupta I, Varma N (2000) Serum ferritin level as a marker of preterm labor. Int J Gynecol Obstet 71: 107-111.

28. Irwinda R, Wibowo N, Putri AS (2019) The Concentration of Micronutrients and Heavy Metals in Maternal Serum, Placenta, and Cord Blood: A Cross-Sectional Study in Preterm Birth. J Pregnancy 5062365: 1-7.

29. Attri S, Sharma N, Jahagirdar S, Thapa BR, Prasad R (2006) Erythrocyte metabolism and antioxidant status of patients with Wilson disease with hemolytic anemia. Pediatr Res 59: 593-597.

30. White NJ (2018) Anaemia and malaria. Malar J 17: 1-17.

31. Hennigar SR, McClung JP (2016) Nutritional Immunity: Starving Pathogens of Trace Minerals. Am J Lifestyle Med 10: 170-173.

32. Angelé-Martínez C, Goodman C, Brumaghim J (2014) Metal-mediated DNA damage and cell death: Mechanisms, detection methods, and cellular consequences. Metallomics 6: 1358-1381.

33. Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, et al. (2005) Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proc Natl Acad Sci USA 102: 6843-6848.

34. Gammoh NZ, Rink L (2017) Zinc in Infection and Inflammation. Nutrients 9: 624.

35. Veale DJ, Oliver DW, Havlik I (2000) The effects of herbal oxytocics on the isolated “stripped” myometrium model. Life Sci 67: 1381-1388.

36. Akoh CC, Pressman EK, Cooper E, Queenan RA, Pillittere J, et al. (2018) Low Vitamin D is Associated With Infections and Proinflammatory Cytokines During Pregnancy. Reprod Sci 25: 414-423.

37. Liu NQ, Hewison M (2012) Vitamin D, the placenta and pregnancy. Arch Biochem Biophys 523: 37-47.