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Effets de l'anémie maternelle et ses causes sur le développement cognitif des enfants agés de 1 an au Bénin

Michael Osei Mireku

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THESE DE DOCTORAT DE L'UNIVERSITE PIERRE ET MARIE CURIE

Spécialité **EPIDEMIOLOGIE**

Ecole doctorale Pierre Louis de santé publique

ED 393 Epidémiologie et Sciences de l'Information Biomédicale

Présentée par

M. Michael Osei MIREKU

Pour obtenir le grade de

DOCTEUR de l'UNIVERSITÉ PIERRE ET MARIE CURIE

**THE EFFECTS OF ANEMIA DURING PREGNANCY AND ITS RISK FACTORS ON THE
COGNITIVE DEVELOPMENT OF ONE-YEAR-OLD CHILDREN IN BENIN**

Soutenue le 7 Juillet 2016

Devant le jury composé de :

M. le Pr. Patrick Truffert	Rapporteur
Mme. le Dr. Valériane Leroy	Rapporteur
M. le Pr. Pascal Astagneau	Examineur
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This thesis is dedicated:

To my mum, M. Biamah and my dad, D. Mireku,
for pushing me beyond the limits they themselves reached

and

To the Owusu and Marfo families, for making France feel like home.

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With the exception of work that formed part of jointly authored publications that has been included, the candidate confirms that the thesis submitted is his own work. Any contributions of the candidate and the authors to this work have been explicitly indicated. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Acronyms and Abbreviations

ANC	Antenatal Care
AGS	American Guidance Service
AMD	Adjusted Mean Difference
AVI	Années Vécues avec de l'Incapacité
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CRP	C-reactive Protein
DAGs	Direct Acyclic Graphs
EL	Expressive Language
ELC	Early Learning Composite
EPDS	Edinburgh Postnatal Depression Scale
FM	Fine Motor
G6PD	Glucose-6-phosphate dehydrogenase
GM	Gross Motor
Hb	Hemoglobin
HbF	Fetal Hemoglobin
HIV	Human Immunodeficiency Virus
HOME	Home Observatory Measurement of the Environment
IC	Intervalle de Confiance
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
IMC	Indice de Masse Corporelle
IPTp	Intermittent Preventive Treatment in Pregnancy
IQ	Intelligence Quotient
IRD	Institut de Recherche pour le Développement
LBW	Low Birth Weight
LLITNs	Long-Lasting Insecticide Treated Nets
MiPPAD	Malaria in Pregnancy Preventive Alternative Drugs
MQ	Mefloquine
MSEL	Mullen Scales of Early Learning
NIH	National Institutes of Health

OMS	Organisation Mondiale de la Santé
PI	Principal Investigator
RBCs	Red blood cells
RL	Receptive Language
RPM	Raven's Progressive Matrices
SD	Standard Deviation
SGA	Small-for-Gestational Age
SP	Sulfadoxine-Pyrimethamine
STHs	Soil-Transmitted Helminths
VP	Visual Perception
WHO	World Health Organization
YLD	Years Lived with Disability

Abstract

The objective of this thesis was to investigate the impact of anemia during pregnancy and its risk factors on the cognitive development of one-year-old children.

Our prospective cohort study included 636 mother-singleton child pairs from 828 eligible pregnant women who were enrolled during their first antenatal care (ANC) visit in Allada, Benin, into a clinical trial comparing the efficacy of mefloquine and sulphadoxine-pyrimethamine. Venous blood samples of women were assessed for ferritin and hemoglobin (Hb) concentrations at the first and second ANC visit of at least one-month interval and at delivery. Stool samples of pregnant women were also collected during these follow-up periods to test for helminths using the Kato-Katz technique. All pregnant women were administered a total of 600 mg of mebendazole (100 mg two times daily for 3 days) to be taken after the first ANC visit. Women were also given daily iron and folic acid supplements throughout pregnancy. The intake was not directly observed. At age one year, cognitive and motor functions of children were assessed using the Mullen Scales of Early Learning (MSEL).

The prevalence of iron deficiency (ID) among pregnant women at first and second ANC visits, and at delivery was 30.5%, 34.0% and 28.4%, respectively. Prevalence of helminth infection was 11.5%, 7.5% and 3.0% at first, second ANC visits and at delivery, respectively. Prevalence of anemia decreased from 67.1% at first ANC visit [mean gestational age (Standard deviation, SD), 22.1(4.0) weeks] to 40.1% at delivery. Children of mothers who were infected with hookworms at the first ANC visit had 4.9 (95% confidence interval, CI: 1.3 - 8.6) lower mean gross motor scores compared to those whose mothers were not infected with hookworms at the first ANC visit, in the adjusted model. Helminth infection at least once during pregnancy was associated with infant cognitive and gross motor functions. We observed a significant negative quadratic relationship between infant gross motor function and Hb concentration at first and second ANC visits.

Prenatal helminth infection is associated with poor with infant cognitive and motor development. However, in the presence of iron supplementation, ID is not associated with infant neurocognitive development. Further, there appears to be an Hb concentration range (90-110 g/L) that may be optimal for better gross motor function of one-year-old children.

Keywords: maternal anemia, hemoglobin, pregnancy, iron deficiency, helminths, child cognitive development, Mullen Scales of Early Learning

Résumé

L'objectif de cette thèse était d'évaluer l'impact de l'anémie pendant la grossesse et ses facteurs de risque sur le développement cognitif d'enfants à un an.

Notre étude de cohorte a inclus 636 couples de mères et d'enfants singletons nés de femmes enceintes incluses dans un essai clinique comparant l'efficacité de la méfloquine et de la sulfadoxine-pyriméthamine dans le district d'Allada au Bénin. Les prélèvements sanguins ont été réalisés lors des première et deuxième visites prénatales (VP) espacées d'au moins un mois et à l'accouchement afin d'évaluer la concentration en hémoglobine (Hb) et la ferritine sérique. Les selles des femmes enceintes ont été aussi collectées pour tester la présence d'œufs d'helminthes par la technique de Kato-Katz. Toutes les femmes enceintes ont reçu 600 mg de mébendazole (100 mg deux fois par jour pendant 3 jours) lors de la première VP. Ces femmes qui ne recevaient pas d'héminiques avant l'étude, ont reçu des suppléments de fer et d'acide folique à partir de leur inclusion. À l'âge d'un an, le développement psycho-moteur des enfants a été évalué par le Mullen Scales of Early Learning (MSEL).

La prévalence de la carence en fer chez les femmes enceintes aux première et seconde VP et à l'accouchement était de 30,5%, 34,0% et 28,4%, respectivement. La prévalence des infections helminthiques était de 11,5%, 7,5% et 3,0 % à la première, la seconde VP et à l'accouchement, respectivement. La prévalence de l'anémie a diminué de 67,1% à la première VP [moyenne l'âge de gestationnel (écart-type), 22,1 (4,0) semaines d'aménorrhée] à 40,1% à l'accouchement. Les enfants nés des mères infectées par ankylostomes à la première VP avaient en moyenne un score de motricité globale plus bas que les enfants nés de mères non-infectées [différence de 4,9 (Intervalle de Confiance à 95% (IC95%) : 1,3 ; 8,6)]. L'infection par helminthes au moins une fois pendant la grossesse était associée chez les enfants à des scores cognitifs et moteurs plus bas que l'absence d'infection. Nous avons observé une relation quadratique négative significative entre la motricité globale de l'enfant et la concentration d'hémoglobine à la première et la seconde VP.

Dans ce contexte de supplémentation en fer des femmes enceintes, la carence en fer maternelle n'était pas associée au développement neurocognitif de l'enfant. De plus, il semble que des concentrations en hémoglobine légèrement en-dessous de la normale (comprises entre 90 et 110 g/L) soient optimales pour la motricité des enfants à un an.

Mots-clés: Anémie maternelle, hémoglobine, grossesse, carence en fer, helminthes, développement cognitif, Mullen Scales of Early Learning

Résumé Long

Introduction

Le développement cognitif des enfants est formé par les processus psychologiques qui jouent un rôle significatif dans la pensée, les caractéristiques émotionnelles et comportementales des enfants. Bien qu'il soit surtout objectivable pendant la période postnatale par le développement moteur, socio-émotionnel, et l'apprentissage de la langue, ces composantes fonctionnelles dépendent directement de l'organisation structurelle du cerveau qui se forme principalement pendant la période prénatale. Les travaux de Bystron *et al*¹ ont montré que le début de la neurogenèse dans le diencéphale primaire du lobe frontal et du télencéphale basal pourrait commencer dès le 31^{ème} jour après conception¹.

Pendant les étapes prénatales, le fœtus subit une série d'événements liés au développement cérébral incluant la migration neuronale, la synaptogenèse, le développement hippocampique et la myélination². Le cerveau fœtal est vulnérable aux atteintes prénatales comme les infections, le stress, les toxines et les carences en nutriments.³ Les déficiences dans le transport du fer et d'oxygène suite à l'anémie prénatale pourraient être préjudiciable au neurodéveloppement du fœtus.

L'anémie pendant la grossesse est définie comme une concentration en hémoglobine (Hb) de moins de 110 g/l au niveau de la mer pendant toute la grossesse par l'Organisation Mondiale de la Santé (OMS).⁴ Les *Centers for Disease Control and Prevention* (CDC) retiennent le même seuil pour les femmes enceintes pour les premiers et troisièmes trimestres de grossesse et un seuil de 105 g/l pour le deuxième trimestre.⁵

Au cours du siècle écoulé, d'énormes efforts ont été faits pour l'amélioration de la santé maternelle et infantile dans le monde et particulièrement en Afrique sub-Saharienne où le fardeau de la mortalité et de la morbidité maternelle et infantile reste le plus élevé. L'anémie est considérée comme un des principaux problèmes de santé affectant les femmes enceintes en Afrique. Des évaluations mondiales récentes révèlent que l'anémie représente plus de 68 millions d'années vécues avec dincapacité (AVI)⁶ Dans 83% des pays dans le monde, l'anémie maternelle est considérée comme une préoccupation modéré ou majeure de santé publique.⁴ Bien que

l'anémie pendant la grossesse soit un problème de santé publique globale, les pays les moins avancés sont les plus affectés.

Selon Benoist *et al.*, plus de 40 % de femmes enceintes sont anémiées dans le monde.⁷ Cependant, l'Asie du Sud-Est et l'Afrique supportent la plus grande part du fardeau de l'anémie pendant la grossesse avec une prévalence de 48,7 % et 46,3 %, respectivement comparé à 24,9 % et 25,8 % en Amérique et en Europe, respectivement.⁸

L'étiologie de l'anémie pendant la grossesse est multifactorielle. L'infection par le paludisme, la carence en fer (ID) et l'infection par helminthes en sont les principaux facteurs de risque. Les infections par helminthes intestinaux sont une des maladies tropicales négligées les plus fréquentes, puisqu'elles touchent plus de deux milliards de personnes dans le monde avec la fréquence la plus haute en Asie et en Afrique subsaharienne. Le fardeau des infections par helminthes intestinaux est évalué à cinq millions d'années de vie perdues corrigées pour l'incapacité (AVCI).⁹ Les infections par helminthes sont rarement associées à une augmentation de la mortalité, mais sont en revanche associées à une augmentation de la morbidité résultant de la chronicité et des conséquences de l'infection.¹⁰ Bien que l'OMS recommande fortement la prophylaxie antihelminthique pour des femmes enceintes pendant le deuxième trimestre,¹¹ les bénéfices par rapport à l'anémie, les anomalies congénitales et la mortalité périnatale demeurent controversés.¹² En Afrique subsaharienne, il est estimé qu'un tiers de femmes enceintes sont infectées par des helminthes transmis par le sol¹³ bien que plusieurs études aient montré d'importantes différences entre pays, de 11,1 % au Bénin,¹⁴ à 25,7 % au Ghana¹⁵ et 49 % au Gabon.¹⁶ Au Bénin, les antihelminthiques font partie de la prise en charge anténatale après le premier trimestre.¹⁷

Malgré les besoins en fer pour les processus cellulaires impliquant l'ADN, l'ARN et de la synthèse de protéines,^{18,19} la carence en fer reste la carence en micronutriments la plus répandue au monde affectant environ 2 milliards de personnes.⁷ Bien que la carence en fer soit un problème de santé publique dans le monde entier, ce sont les enfants avant l'âge de cinq ans et les femmes enceintes qui sont le plus en danger dans les pays les moins avancés. Pendant la grossesse, la demande de fer est augmentée pour satisfaire les besoins de l'unité fœto-placentaire. Au deuxième trimestre de grossesse, les exigences quotidiennes augmentent à 6.8 mg, soit trois fois celles d'une femme non enceinte.²⁰ Cette augmentation de la demande physiologique en fer rend

les femmes enceintes fortement vulnérables à la carence en fer en l'absence de supplémentation ou d'une alimentation appropriée. La carence en fer est supposée être à l'origine de plus de la moitié des anémies pendant la grossesse.²¹

La carence en fer et l'infection par helminthes aussi bien que d'autres facteurs de risque d'anémie pourraient avoir des conséquences délétères sur la santé maternelle et infantile via la moindre disponibilité en fer pour le neurodéveloppement fœtal.^{22,23} Ces facteurs de risque d'anémie ont aussi été associés à des issues de grossesse défavorables elles-mêmes associées à des déficits neurocognitifs chez l'enfant.²⁴

Considérant la demande accrue en fer pendant la grossesse, son rôle dans le transport d'oxygène et l'oxygénation cérébrale, l'hypothèse dans ma thèse était que l'anémie maternelle pendant la grossesse et ses facteurs de risque pourraient être associés aux déficits cognitifs de l'enfant. La littérature existante sur ce sujet était limitée au début de la thèse en octobre 2012 et reste peu concluante.

Le diagramme ci-dessous (*direct acyclic graphs* (DAGs)) représente les liens hypothétiques entre l'anémie maternelle et le développement de l'enfant, ainsi que les facteurs de confusion potentiels.

Les causes de l'anémie et la fonction cognitive :

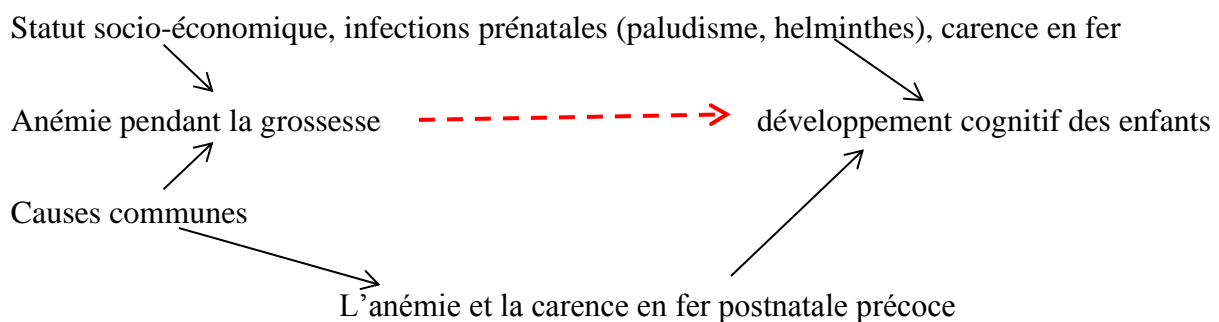


Fig I. DAG montrant l'impact causal de l'anémie pendant la grossesse sur la fonction cognitive des enfants

L'objectif principal de ma thèse était donc d'examiner l'association entre l'anémie maternelle pendant la grossesse et ses facteurs de risque et le développement psychomoteur de l'enfant à un an au Bénin. Les objectifs spécifiques étaient :

- D'étudier l'impact de l'anémie et de la concentration en Hb pendant la grossesse sur le développement psychomoteur de l'enfant à un an,
- De déterminer l'effet des infections par helminthes pendant la grossesse sur le développement psychomoteur de l'enfant à un an
- D'examiner l'impact de la carence en fer prénatale sur les fonctions cognitives et motrices de l'enfant à un an.

Méthodes

Population

Notre cohorte prospective a inclus des singletons nés de femmes enceintes incluses avant 28 semaines d'aménorrhée dans l'essai clinique *Malaria in Pregnancy Preventive Alternative Drugs* (MiPPAD, NCT00811421) comparant deux traitements préventifs intermittents contre le paludisme pendant la grossesse, la sulfadoxine-pyriméthamine (SP) et la méfloquine (MQ). L'étude a été conduite dans le district d'Allada au Bénin. De janvier 2010 à mai 2011, 1005 femmes enceintes séronégatives ont été recrutées lors de leur première visite prénatale (VP) dans les maternités de Sékou, Allada et Attogon. L'essai MiPPAD a été décrit par ailleurs dans le détail.²⁵

Tous les enfants survivants nés de ces femmes enceintes ont été invités à participer à l'étude TOVI, 12 mois après leur naissance. Les enfants mort-nés, ou décédés entre la naissance et un an ainsi que les naissances multiples ont été exclus.

Variables

(a) Femmes Enceintes

Des données socio-démographiques et cliniques ont été collectées lors de trois visites prénatales (VP), lors du recrutement (première VP, première administration de l'IPTp), à l'occasion de la 2ème dose d'IPTp (la seconde VP, au moins un mois après le recrutement) et à l'accouchement. Lors de la première VP, la gravidité, l'âge gestationnel, le poids et la taille de la mère et des caractéristiques sociodémographiques comme l'âge, l'éducation, l'indice de masse corporelle (IMC), la situation de famille et l'emploi ont été collectés.

Les prélèvements de selles ont été immédiatement préparés dans les six heures en utilisant la technique de Kato-Katz comme décrit par l'OMS. Pour obtenir les comptes d'œufs fécaux standardisés, le nombre d'œufs de chaque espèce d'helminthes a été multiplié par vingt-quatre.

Huit millilitres de sang veineux ont été obtenus pour analyses lors des VP et à l'accouchement. Pour chaque femme participante, 10 µL de sang a été prélevé et analysé par un dispositif de terrain Hemocontrol (EFK Diagnostics, Barleben/Magdeburg, Allemagne) pour mesurer la concentration d'hémoglobine. Le contrôle de qualité a été assuré par le calibrage quotidien de l'Hemocontrol par des techniciens de laboratoire et la vérification par recoupement de la concentration d'hémoglobine de 10% des échantillons au laboratoire Hospitalier Central d'Allada en utilisant un automate d'hématologie (Laboratoire Erma, Tokyo, Japon). L'électrophorèse en milieu alcalin (Laboratoires Helena, Beaumont, TX, USA) a été utilisée pour déterminer le génotype de l'hémoglobine.

La ferritine sérique a été mesurée à partir de 500 µL de sérum avec un Analyseur AxSym (Laboratoires Abbot, Abbot Park, IL, USA). La concentration en protéine C-reactive (CRP) dans le sang a aussi été mesurée (CRP Latex; Cypress Diagnostics Inc., Campbellsville, Ontario, Canada). L'inflammation a été définie comme une CRP supérieure à 5 mg/L. La carence en fer a été définie par une concentration en ferritine sérique inférieure à 12 µg/L ou une concentration en ferritine sérique entre 12 µg/L et 70 µg/L en cas de CRP élevée.

L'anémie chez la femme enceinte a été définie comme une concentration en hémoglobine inférieure à 110 g/L. Après chaque évaluation, les femmes enceintes avec une concentration en hémoglobine entre 70 g/L et 110 g/l ont été traitées par 400 mg de sulfate ferreux oral (200 mg deux fois par jour) conformément aux directives nationales. Celles dont la concentration en hémoglobine était inférieure à 70 g/L ont été adressées à un hôpital régional (de zone) pour transfusion sanguine. Conformément aux directives du Ministère de la Santé Béninoise, des suppléments en fer (200 mg quotidiennement) et en acide folique (5 mg quotidiennement) ont été prescrits pour toutes les femmes enceintes à partir de la première VP jusqu'à 3 mois après l'accouchement. De plus, 600 mg de mébendazole (100 mg deux fois par jour pendant 3 jours) ont été remis à chaque femme enceinte pour prise à domicile sans vérification de l'observance. Pendant la période d'étude, les médicaments et les suppléments jugés nécessaires ont été prescrits aux femmes gratuitement.

(b) Enfants

L'hauteur utérine a été utilisée pour déterminer l'âge gestationnel des enfants à la naissance. Les selles ont été collectées pour un sous-échantillon d'enfants seulement (N=186) à 6, 9 et 12 mois pour la recherche d'helminthes.

Dans les centres de santé, des infirmiers formés spécifiquement pour l'étude ont évalué le développement psychomoteur de 636 enfants d'un an avec le Mullen Scales of Early Learning (MSEL).²⁶ Le MSEL comprend cinq échelles : la motricité globale, la motricité fine, la perception visuelle, la compréhension et l'expression verbale.

Les scores bruts ont été convertis en scores standardisés en fonction de l'âge de l'enfant. Les scores standardisés des 4 échelles perception visuelle, motricité fine, compréhension et expression verbale ont été combinés pour former le score *Early Learning Composite* (ELC).²⁶

Trois jours plus tard après les évaluations neurocognitives, un examinateur différent a effectué des visites à domicile. Les mères ont répondu aux questionnaires concernant les possessions familiales, l'échelle du *Home Observatory Measurement of the Environment* (HOME),²⁷ l'échelle sur la dépression du *Edinburgh Postnatal Depression Scale* (EPDS)²⁸ et les matrices de Raven (*Raven's Progressive Matrices* RPM)²⁹. Un score a été calculé concernant les possessions de la

famille, incluant des informations sur la présence d'électricité dans la maison, la possession d'un poste de radio, de télévision, d'une bicyclette, d'une moto, ou d'une voiture. Ce score correspondait à la somme des scores individuels donnés à l'électricité et la voiture (trois points pour chaque), une moto et la télévision (deux points) et une radio, bicyclette, moto, et les bovins (un point). Le HOME, adapté pour l'étude, a été utilisé pour évaluer la qualité de l'environnement domestique incluant les interactions parent-enfant et les opportunités d'apprentissage pour l'enfant à la maison. L'EPDS et les matrices de Raven ont été utilisés pour évaluer la dépression postnatale maternelle et le quotient intellectuel maternel, respectivement.

Une double saisie des données a été réalisée avec EpiData 3.1 (Danemark) et vérifiée pour la cohérence. En cas d'incohérence, le questionnaire sous format papier a été consulté. Les analyses statistiques ont été réalisées avec Stata IC/11.2 pour Windows (StataCorp Lp, College station, TX) au début de la thèse et Stata IC/14.1 pour Mac (StataCorp Lp, College station, TX) par la suite. La signification statistique a été définie comme une valeur de p inférieure à 0,05.

Considérations éthiques

Le Comité d'Éthique de l'Hospital Clinic de Barcelone en Espagne, le Comité d'Éthique Consultatif de l'Institut de Recherche pour le Développement (IRD) en France et les organismes de réglementation et le Comité National pour l'Éthique de la Recherche en Santé du Bénin ont approuvé le protocole d'étude de MiPPAD et les formulaires de recueil du consentement éclairé. Avant l'inclusion dans l'étude MiPPAD, le consentement volontaire a été obtenu auprès de chaque femme après explication de l'étude dans la langue locale. Pour les mineurs, le consentement éclairé a été obtenu auprès d'un parent ou représentant légal.

L'approbation éthique pour l'étude TOVI a été obtenue auprès des comités d'éthique de l'Université d'Abomey-Calavi (Bénin), de New York University (USA), Michigan State University (USA) et du Comité d'Éthique Consultatif de l'IRD (France). Nous avons obtenu le consentement éclairé de toutes les femmes qui ont participé à cette étude, pour elles et leurs enfants.

Dans les deux études, les femmes étaient libres de cesser leur participation à n'importe quelle étape.

Résultats

Un total de 963 nouveau-nés a été enregistré à l'accouchement dont 39 mort-nés. Les naissances multiples (n=61) n'ont pas été incluses dans l'étude TOVI. Par conséquent 863 singletons ont été inscrits dans la cohorte de naissance. L'âge médian au moment des évaluations MSEL était de 12,11 mois (de 11,3 à 15,3 mois). Les caractéristiques maternelles étaient comparables entre les femmes dont les enfants ont été évalués pour la fonction cognitive et ceux dont les enfants ne l'ont pas été.

À la première VP, la fréquence des infections par helminthes était de 11,5 % parmi lesquelles les infections par ankylostomes étaient les plus répandues (9,5 %). Il y avait 1 (0,1 %) et 4 cas (0,5 %) d'infections par plusieurs espèces d'helminthes lors de la première et seconde VP, respectivement. Parmi les 52 cas d'infections par ankylostomes lors de la deuxième VP, 12 étaient déjà infectés par la même espèce lors de la première VP.

Les enfants des mères infectées par ankylostomes lors de la deuxième VP avaient un score composite de 4,7 points plus bas (Intervalle de Confiance à 95% (IC95%) : 0,5 ; 8,9) comparé à ceux dont les mères qui n'étaient pas infectées à la deuxième VP après ajustement. Les enfants des femmes enceintes infectées par des helminthes au moins une fois pendant la grossesse avaient des scores composites plus bas de 4,1 points (IC95% : 7,0 ; 1,3) comparés à ceux de mères qui n'étaient pas infectées pendant la grossesse après ajustement.

Après ajustement sur la gravidité, l'éducation maternelle, les possessions familiales et le score du HOME, l'infection par helminthes à la première VP était négativement associée à la motricité de l'enfant ($p=0,017$). Les enfants nés de mères infectées par des helminthes au moins une fois pendant la grossesse avaient des scores de motricité plus bas que les enfants nés de mères jamais infectées pendant la grossesse [-3,3 (IC95% :-6,1 ; -0,5)].

La carence en fer pendant la grossesse n'était pas associée à la concentration de la ferritine sérique du sang de cordon. Les scores d'ELC et de motricité globale étaient similaires entre les enfants dont les mères avaient carence en fer ou anémie par carence en fer prénatals et ceux dont la mère n'a pas eu carence en fer ou anémie par carence en fer pendant la grossesse. De même, l'anémie et la concentration de ferritine à la naissance n'étaient pas associées aux scores d'ELC et

de motricité globale à l'âge d'un an. Dans le modèle ajusté, il n'y avait pas de différence de concentration en ferritine sérique au sang du cordon entre les enfants nés de mères avec une carence en fer et ceux nés de mères non carencés. Cependant, la concentration en ferritine sérique au sang du cordon était plus basse chez les enfants nés de mères avec une anémie par carence martiale comparé aux enfants nés de mères sans anémie par carence martiale [différence moyenne ajustée (IC95%): -0,2 (-4,0 ; -0,0)].

La concentration moyenne en hémoglobine (ET) à la première VP était de 103,7 (12,3) g/L, de 105,4 (10,6) g/L à la seconde et de 112,4 (14,1) g/L à l'accouchement. Une concentration en hémoglobine basse ou haute était associée à des scores de motricité plus bas qu'une concentration en hémoglobine comprise entre 90 et 110 g/L (coefficient quadratique significatif). Les scores de motricité les plus élevés étaient observés pour des valeurs d'Hb de 98 g/L et 97 g/L à la première et la deuxième VP, respectivement.

Discussion

Nous présentons une brève discussion de chaque résultat. La première porte sur l'impact des helminthiases prénatales, la seconde sur l'impact de la carence en fer prénatale sur le développement neurocognitif des enfants et enfin, sur la relation entre la concentration d'hémoglobine prénatale et le développement psychomoteur du nourrisson.

L'infection helminthique prénatale et le développement cognitif des enfants

Notre étude a montré qu'après ajustement sur des facteurs de risque connus de déficits neurocognitifs, l'infection par helminthes intestinaux à la première VP était associée à un moins bon développement psychomoteur des enfants à l'âge d'un an par rapport aux enfants nés de mères non infectées. Dans notre population d'étude, l'infection prénatale par ankylostomes était associée à une baisse des performances motrices des enfants. Nos résultats montrent aussi qu'au moins une infection par helminthes pendant la grossesse peut avoir des conséquences négatives sur le développement cognitif et moteur des enfants.

Malgré le faible nombre de coinfections, nos résultats ne suggèrent pas d'impact sur le développement des infections conjointes de paludisme et d'helminthes. Étant donné la prescription de traitements antihelminthiques aux femmes lors de leur première VP, le nombre d'infections persistantes était bas dans notre étude, ce qui ne permettait pas d'évaluer l'effet des infections helminthiques chroniques. Les femmes qui présentaient des infections par helminthes peuvent avoir été chroniquement infectées avant leur première VP.

Mis à part une étude de cohorte nichée dans la *Entebbe Mother and Baby Study in Uganda*,²³ nous n'avons pas identifié d'étude sur l'impact des infections prénatales par helminthes sur le développement des enfants. La relation négative entre l'infection maternelle par helminthes et le développement de l'enfant dans notre étude est en accord avec la conclusion de l'étude en Ouganda mentionnée ci-dessus.

Le mécanisme par lequel l'infection prénatale par helminthes influence le développement de l'enfant est inconnu. Cependant, l'infection helminthique, et particulièrement les ankylostomes, est un facteur de risque connu de la carence en fer chez les mêmes individus. Quand les ankylostomes pénètrent dans la muqueuse intestinale d'un hôte, ils ingèrent le sang de l'hôte en causant une perte intestinale de sang et une lyse des érythrocytes.³⁰ Ceci pourrait aboutir à une carence en fer³¹ potentiellement désavantageuse pendant la grossesse à cause de la demande physiologique accrue de fer. Des études ont montré que chez les mères déficientes en fer, la ferritine sérique maternelle était corrélée avec celle du nouveau-né³² tandis que la ferritine sérique fœtale était associée à une diminution du fer au niveau cérébral³³ qui pourrait à son tour altérer le développement hippocampique du nouveau-né.³⁴

Les infections par helminthes peuvent être associées à différentes issues de grossesse défavorables, elles-mêmes situées sur le chemin causal entre les infections prénatales par helminthes et les altérations du développement cognitif de l'enfant.

Les études portant sur l'impact des infections prénatales par helminthes sur les issues de grossesse montrent des effets contradictoires.^{35,36} Dans une étude randomisée et contrôlée en Ouganda, les auteurs n'ont constaté aucun effet bénéfique d'antihelminthiques pendant la grossesse sur le poids à la naissance ou la mortalité périnatale ou des anomalies congénitales³⁵ tandis qu'une autre étude a rapporté une amélioration du poids de naissance quand les mères ont

reçu deux doses de antihelminthiques par rapport à qui n'en ont pas reçu³⁶. Les issues de grossesse défavorables ont des effets négatifs sur le développement cognitif de l'enfant.³⁷⁻³⁹ Nos résultats, après des analyses de sensibilité, suggèrent cependant que d'autres facteurs non mesurés sont probablement impliqués dans l'association observée entre les infections prénatales par helminthes et le développement de l'enfant.

Il est peu probable que l'association soit expliquée par une augmentation des infections par helminthes chez l'enfant (donc pendant la période post-natale), étant donné que nous n'avons trouvé aucune association entre les infections prénatales par helminthes et les infections par helminthes chez les enfants à l'âge de un an. Par ailleurs, les espèces étaient différentes chez les mères et les enfants. *T. trichiura* était l'espèce la plus répandue chez les enfants (20,9 %) tandis que les ankylostomes étaient plus fréquents chez les femmes enceintes, ce qui peut s'expliquer par des modalités d'infestation distinctes (par voie digestive pour les trichocéphales et par voie transcutanée pour les ankylostomes).

La relation entre la carence en fer prénatale et le développement cognitif de l'enfant

Dans notre population d'étude, la fréquence de l'anémie et de la carence en fer est restée élevée pendant la grossesse même après prescription de suppléments quotidiens en fer et acide folique. Nous n'avons trouvé aucune relation entre la carence en fer ou l'anémie par carence en fer prénatale et le développement psychomoteur des enfants.

Considérant la vulnérabilité du fœtus aux atteintes prénatales et le rôle essentiel du fer dans le neurodéveloppement de l'enfant, il est surprenant que nous n'ayons trouvé aucune association entre la carence en fer prénatale et le développement cognitif de l'enfant en bas âge. Cependant, pendant la grossesse, le fœtus a la priorité sur les besoins de la femme enceinte. La mère peut être déficiente en fer même avec prise de suppléments sans répercussion notable sur le fœtus.^{32,40} Ceci pourrait expliquer l'absence d'association entre la ferritine dans le sang du cordon et la ferritine chez la mère bien que l'anémie pendant la grossesse ait été associée à une diminution de la concentration en hémoglobine du sang du cordon, dans notre étude.

Dans notre population d'étude, après la première VP, des suppléments en fer et en acide folique ont été prescrits quotidiennement à toutes les femmes enceintes en plus du traitement préventif

intermittent contre le paludisme. L'absence d'association entre la carence en fer prénatale et le développement cognitif de l'enfant peut probablement être expliquée par des apports en fer adéquats chez le fœtus, même lorsque les femmes enceintes étaient carencées.

Association entre la concentration prénatale en Hb et le développement cognitif et moteur de l'enfant

Cette étude montre qu'il y a une relation en U inversée entre l'Hb prénatale lors de la première et la deuxième VP et la motricité de l'enfant indiquant que des concentrations basses et hautes en hémoglobine pendant la grossesse peuvent être nuisibles. Les enfants avec les meilleurs scores de motricité ont été observés pour une concentration en Hb prénatale de 98 g/L et 97 g/L à la première et la deuxième VP, respectivement.

Pendant la grossesse, il y a une augmentation physiologique d'environ 50 % du volume du plasma, atteignant un niveau maximal entre 28 et 34 semaines de gestation et une augmentation de 20 à 30 % de la masse de globules rouges, conduisant à une concentration en hémoglobine diminuée.⁴¹ Cette hémodilution pendant les deuxièmes et troisièmes trimestres de grossesse peut être essentielle pour la croissance fœtale car la réduction de la viscosité du sang augmente le flux efficace d'oxygène et d'autres substances nutritives pour le fœtus.⁴¹ Ceci pourrait expliquer les deux observations dans nos résultats : premièrement, les scores de motricité élevés parmi les enfants de femmes anémiées en deuxième VP quand plus de 50 % de femmes étaient entre 28 et 34 semaines de gestation ; deuxièmement, les scores de motricité optimaux observés parmi les enfants de femmes enceintes avec une hémoglobine entre 90 et 110 g/L. Cet intervalle d'Hb (90-100 g/L) pourtant considéré comme pathologique, soulève des questions à propos de la valeur seuil pour la définition de l'anémie.

Des scores de motricité plus bas ont été observés pour une concentration en hémoglobine basse. Les niveaux bas observés d'hémoglobine parmi les femmes enceintes à la première VP pourraient être liés à des pathologies telles que le paludisme, les infections par helminthes et les carences en micronutriments. Les niveaux d'hémoglobine bas lors de la deuxième VP pourraient être expliqués par une combinaison des étiologies mentionnées ci-dessus et de l'augmentation physiologique du volume plasmatique diminuant la concentration en hémoglobine.⁴² Une concentration en hémoglobine extrêmement basse implique un transport d'oxygène déficient au

foetus. Bien que le fœtus dispose de mécanismes d'adaptation à un apport d'oxygène diminué, les coûts d'une telle adaptation pourraient être élevés, menant probablement à une croissance fœtale détériorée.

Des niveaux d'hémoglobine bas pendant la grossesse pourraient diminuer la quantité de fer disponible pour le fœtus. La disponibilité du fer est essentielle pour la croissance fœtale et la fonction de plusieurs systèmes d'organes incluant le cerveau.⁴³ La carence en fer prénatale est associée à une diminution de la longueur des dendrites et pourrait modifier le développement hippocampique même en début de grossesse.^{43,44} Les dendrites de l'hippocampe jouent un rôle substantiel dans la mémoire et les fonctions sensorielles.

A l'inverse, des concentrations élevées d'Hb pourraient être dues à la non-expansion du plasma et en conséquence, une viscosité du sang accrue. La viscosité accrue implique un flux sanguin réduit dans l'espace intervilleux.⁴¹ Ceci pourrait mener au stress fœtal en raison de la baisse de perfusion placentaire fœtale et détériorer par conséquent le développement d'organes fœtaux essentiels.⁴¹ Cette hémococoncentration pourrait expliquer l'extrémité droite des courbes quadratiques où des concentrations élevées d'hémoglobine pendant la grossesse ont été associées à une diminution des scores de motricité et pourrait refléter les inconvénients liés à l'hémococoncentration pendant la grossesse.

La nature curviligne de la relation observée dans notre étude est analogue à celle entre Hb prénatale et le faible poids de naissance.^{45,46} La concentration en hémoglobine maternelle est source de complications de grossesse et d'issues de grossesse défavorables comme le faible poids de naissance, le décès à la naissance et la naissance prématurée qui pourraient être des facteurs intermédiaires dans le chemin causal entre l'Hb prénatale et la fonction motrice des enfants.⁴⁷

Conclusion et perspectives

Des améliorations considérables ont été faites au cours du dernier siècle dans la prévention, la gestion et le traitement de l'anémie, en particulier chez les femmes enceintes, ayant pour conséquence la baisse de l'anémie globale et l'augmentation de la concentration moyenne de

l'hémoglobine au monde. Malgré cela, les controverses entourent encore la classification de l'anémie pendant la grossesse. Par exemple, nos résultats, ainsi que les résultats d'autres chercheurs ont identifié un risque plus faible d'issues de grossesse défavorables et de déficits neurocognitifs chez les enfants nés de femmes avec une anémie considérée légère ou modérée (90 -110 g / L). Cela soulève des questions sur le seuil pour définir l'anémie pendant la grossesse. De même, des études devraient être menées chez des femmes vivant en altitude et ayant une concentration en hémoglobine plus élevée. Celle-ci pourrait avoir un effet délétère en raison d'une plus grande viscosité du sang.

Notre étude a montré que, dans une région où le paludisme est endémique, une carence en fer pendant la grossesse n'est pas associée avec la concentration en ferritine au sang du cordon ni avec le développement cognitif et moteur de l'enfant. Ces résultats sont observés dans une population avec suivi des recommandations actuelles de supplémentation en fer et nous ne pouvons rapporter ce qui aurait pu se produire en l'absence de supplémentation en fer. Par conséquent, nos résultats ne remettent pas en question les recommandations internationales actuelles concernant la supplémentation en fer chez les femmes enceintes.

Les résultats de cette recherche révèlent l'importance des conséquences de l'anémie pendant la grossesse et de ses facteurs de risque sur le développement cognitif précoce d'enfants à l'âge d'un an. Une conclusion importante de nos études est l'impact négatif des helminthes intestinaux chez les femmes enceintes sur le développement neurocognitif de l'enfant qui est compatible avec la conclusion de la seule étude publiée sur le sujet.²³ Nous avons constaté que les enfants nés de mères infectées par ankylostomes avant traitement antihelminthique avaient des scores plus bas que ceux nés de mères non infectées. Ces résultats renforcent les recommandations de l'OMS pour administrer des vermifuges aux femmes enceintes vivant dans les régions endémiques après leur premier trimestre.

Étant donné que la littérature est limitée sur les sujets abordés dans cette thèse, de nouvelles études seront nécessaires afin de corroborer nos découvertes. Le suivi des enfants pour confirmer cette association à un âge ultérieur est aussi nécessaire. Les études de suivi pour vérifier si cette association persiste même dans la vie ultérieure des enfants est également nécessaire et sera réalisée par le biais du projet EXPLORE avec un suivi des enfants à l'âge de 6 ans. En outre, les

mécanismes sous-jacents les associations observées dans notre étude ne sont pas bien compris bien qu'un certain nombre d'hypothèses ont été formulées et pourraient être explorées en utilisant des modèles animaux ou des études in vitro.

List of Publications and Conference Presentations

Published Articles on Thesis (and inserted in the manuscript)

1. **Mireku MO**, Davidson LL, Koura GK, Ouédraogo S, Boivin MJ, Xiong X, Acrombessi MMK, Massougbodji A, Cot M, Bodeau-Livinec F. (2015) Prenatal Hemoglobin Levels and Early Cognitive and Motor Functions of One-Year-Old Children. *Pediatrics*; peds.2015–0491. doi: 10.1542/peds.2015-0491
2. **Mireku MO**, Boivin MJ, Davidson LL, Ouédraogo S, Koura GK, Alao MJ, Massougbodji A, Cot M, Bodeau-Livinec F. (2015) Impact of Helminth Infection during Pregnancy on Cognitive and Motor Functions of One-Year-Old Children. *PLoS Negl Trop Dis* 9(3): e0003463. doi:10.1371/journal.pntd.0003463

Published Articles outside Thesis

3. Quansah E, Ohene LA, Norman L, **Mireku MO**, Karikari TK (2016) Social Factors Influencing Child Health in Ghana. *PLoS ONE* 11(1): e0145401. doi:10.1371/journal.pone.0145401
4. Moya-Alvarez V, **Mireku MO**, Ayotte P, Cot M, Bodeau-Livinec F (2016) Elevated Blood Lead Levels Are Associated with Reduced Risk of Malaria in Beninese Infants. *PLoS ONE* 11(2): e0149049. doi:10.1371/journal.pone.0149049

Articles Submitted

5. **Mireku MO**, Davidson LL, Boivin MJ, Zoumenou R, Massougbodji A, Cot M, Bodeau-Livinec F. (2016) Impact of Prenatal and Early Postnatal Iron Deficiency on Infant Cognitive and Motor Functions –*Under Review- Pediatrics*
6. **Mireku MO**, Davidson LL, Zoumenou R, Massougbodji A, Cot M, Bodeau-Livinec F. (2016) Consequences of prenatal geophagy on maternal health, birth outcomes and child development- *Under Review- American Journal of Clinical Nutrition*

Presentations at Scientific Conferences

1. **Mireku MO**, Davidson LL, Boivin MJ, Zoumenou R, Massougbdji A, Cot M, Bodeau-Livinec F. The impact of iron deficiency during pregnancy on the cognitive and motor functions of one-year-old children. *64th ASTMH Annual Meeting*. October 2015, Philadelphia, USA (Poster)
2. **Mireku MO**, Cot M, Bodeau-Livinec F. Conséquences des infections helminthiques pendant la grossesse sur le développement cognitif du nourrisson. *Journée de l'ED 393*. October 2015, St. Malo, France (Invited speaker)
3. **Mireku MO**, Cot M, Bodeau-Livinec F. Out of sight but not out of mind: The impact of hookworm infection during pregnancy on infant cognition. *6^{ème} Rencontres Scientifiques du Réseau Doctoral en Santé Publique*. April 2015, Paris, France (Oral)
4. **Mireku MO**, Davidson LL, Koura GK, Ouédraogo S, Boivin MJ, Xiong X, Accrombessi MMK, Massougbdji A, Cot M, Bodeau-Livinec F. Relationship between hemoglobin levels during pregnancy and cognitive and motor development in early childhood. *63rd ASTMH Annual Meeting*. November 2014, New Orleans, USA (Oral)
5. **Mireku MO**, Cot M, Bodeau-Livinec F. Hemoglobin concentration during pregnancy and child psychomotor development: A cohort study in Benin. *Journée de Jeunes Chercheurs en Périnatalité : DHU Risque Et Grossesse*. July 2014, Paris, France (Poster)
6. **Mireku MO**, Boivin MJ, Davidson LL, Ouédraogo S, Koura GK, Alao MJ, Massougbdji A, Cot M, Bodeau-Livinec F. Helminth infection during pregnancy and cognitive function of one-year-old children in Benin. *6th MiM conference*. October 2013, Durban, South Africa. (Poster)

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Introduction

For most parts of the century, enormous efforts have been made into improving maternal and child health worldwide and especially in sub Saharan Africa where the burden and prevalence of maternal and infant mortality and morbidity remains highest. Of the many health problems affecting pregnant women in Africa, anemia is considered a major public health problem. Globally, anemia is estimated to account for 8.8% (thus 68.4 million years lived with disability, YLD) of the total burden of disease for all conditions.⁶ In fact, of all the population at-risk of anemia, it is only among pregnancy women that anemia is a public health problem globally (thus prevalence of more than 5% in every country).⁷ The prevalence of anemia is estimated to be 38.2% among pregnant women worldwide with an even higher prevalence among pregnant women in Africa, 46.3%.⁸

Anemia in pregnancy is defined as hemoglobin (Hb) concentration less than 110 g/L throughout pregnancy according to the World Health Organization (WHO)²¹, thus 10 g/L less than the cut-off for non-pregnant women. In normal pregnancy, beginning at the end of the first trimester, there is a physiologic expansion of plasma volume of about 50% and an increase of red blood cells (RBCs) by about 30%. This disproportional increase in plasma and RBCs reduces the Hb concentration of the pregnant woman and depending on the Hb concentration, prior to this expansion, the pregnant woman could be rendered anemic. This physiologic hemodilution accounts for the even lower cut-off for defining anemia in the second trimester by the Centers for Disease Control and Prevention (CDC)⁵ and some other institutions. However, by the end of the third trimester, there is a slight increase in RBC production and thus an increase in Hb concentration⁴⁸.

The prevalence of anemia is particularly highest in developing countries because they harbor the highest prevalence of the various causes of anemia including nutritional deficiencies (particularly, ID but also folic acid and vitamin B₁₂ deficiencies), malaria, helminth infections and human immunodeficiency virus (HIV) infections.⁶ In some situations, these etiologies coexist in an anemic individual making it difficult to attribute the cause of anemia to one particular factor. Due to the vulnerability of the fetus and the increased demand for nutrients by the

pregnant woman and the fetus, the burden and impact of these causes of anemia may be most severe during pregnancy.

Prenatal phase is the most delicate developmental period of life. Since the prenatal phase marks the beginning of organ development, the sensitivity of the growing fetus makes it particularly vulnerable to insults that could be injurious to its development. During this phase, the fetus undergoes extensive series of events involving neural migration and hippocampal development that become the base for proper structural brain development. Further, knowing that neurogenesis begins in the early stages of pregnancy, questions are raised if exposure to prenatal insults could impair fetal brain development and subsequently, cognitive function after birth.

Maternal anemia and its causes have been associated with several consequences in birth outcomes such as low birth weight (LBW), preterm birth and small-for-gestation age (SGA). These adverse birth outcomes are risk factors for poor cognitive development of children after birth. Further, considering the increased demand for iron during pregnancy and the role that iron plays not only in oxygen transport and brain oxygenation but also more importantly, in energy metabolism of the hippocampus, anemia and its risk factors during pregnancy were hypothesized to be associated with infant cognitive development. Existing literature on this subject was also limited at the start of the thesis in October 2012 and still remains inadequate.

This hypothesis was the basis of a study that was conducted in Allada, Benin called the TOVI study which assessed the cognitive function of one-year-old children whose mothers had been enrolled in a clinical trial called *Malaria in Pregnancy Preventive Alternative Drugs* (MiPPAD). My thesis is based on the TOVI study therefore addresses particular objectives that are meant to respond to the questions posed by the investigators of the TOVI study.

The main objective of thesis was therefore to investigate the association between maternal anemia and its risk factors and infant cognitive and motor functions. Specific objectives set for the thesis were:

- to study the impact of anemia in pregnancy on the cognitive development of one-year-old children as well as investigating the relationship between maternal Hb levels and infant cognitive and motor scores,

- to determine the effect of helminth infection during pregnancy on the cognitive development of one-year-old children, and
- to investigate the impact of prenatal ID on the cognitive and motor functions of children.

I hope that the findings of this thesis will help inform policy makers on interventions to prevent anemia during pregnancy and also contribute to the growing knowledge of prenatal risk factors for poor cognitive development of children.

This thesis is divided into seven chapters. Chapter 1 presents the background elements of this thesis. It elaborates on the definitions of anemia in different contexts; the epidemiology, risk factors and consequences of anemia in pregnancy; and the methods of assessment of infant cognitive function. These provide adequate knowledge and vocabulary to understand the subsequent chapters.

Chapter 2, the materials and methods section, provides information on the sources of data that were used in this thesis. It details the study design and population, data collection and the laboratory techniques used in the biological assessments of blood and stool samples. This chapter also explains data collected in the TOVI study including how the cognitive assessment tool was adapted to the study setting.

The results of our assessment of the impact of helminth infection during pregnancy on the cognitive and motor functions of children contribute to the main theme of Chapter 3. The article published in *PLoS Neglected Tropical Diseases* from our analysis is also reproduced in this chapter.

The focus of Chapter 4 is the second article published in *Pediatrics* that investigated the relationship between maternal Hb concentration during pregnancy and infant cognitive and motor development. This chapter also addressed the main aim of the TOVI study, thus determining the impact of anemia in pregnancy on infant cognition.

Chapter 5 addresses the effect of prenatal ID on the cognitive abilities of one-year-old children. This chapter is therefore the third part of the findings of this thesis. The manuscript from this part is yet to be submitted for publication.

An overall discussion of our findings in light of the existing knowledge on the effect of prenatal anemia, its risk factors and infant cognitive development is presented in Chapter 6. The chapter draws on the strengths and limitations of the TOVI study and the articles published from that study as well as explaining how the results in the various articles and manuscript interrelate in the study context.

A concluding chapter, Chapter 7 explores the knowledge drawn from the three distinct findings and addresses the implications of these findings for future research and existing policies on maternal and child health. The conclusion also includes recommendations that are based on the findings of this thesis.

Where needed, an extra introduction is given to some chapters to prepare the reader for the detailed contents.

Chapter 1. Background: Anemia, Pregnancy and Infant Cognition

1.1 Definition of Anemia

Anemia is a pathological condition that is depicted by a decrease in red cell mass resulting from either insufficient production of RBCs or excessive removal of RBCs via blood loss or increased cell death. Since RBCs contain Hb, the protein responsible for the transport of oxygen, a decrease in RBCs implies inadequate oxygen transport to tissues and organs.

Clinically, anemia is diagnosed by measuring the concentration of Hb in blood (in g/dl or g/L) or by measuring hematocrit levels (i.e. the proportion of the volume of RBCs in whole blood in %/L).⁴⁹ In order to assess population prevalence of anemia using Hb levels, the cyanomethemoglobin method or the hemocue method is used.²¹ The WHO approves both methods for surveys in low or moderate resource-limited areas.²¹ Determination of hematocrit levels is performed by centrifugation.

To diagnose anemia, cut-offs are set for both Hb levels and hematocrit levels below which an individual can be said to be anemic. The cut-offs for hematocrit and Hb are different for different groups of individuals as a number of physiological factors influence the levels of Hb. The WHO thresholds for anemia diagnosis using Hb and hematocrit levels are shown in Table 1.1.

Table 1.1 Hemoglobin and hematocrit thresholds for anemia diagnosis at sea level

Population	Hemoglobin threshold (g/L)	Hematocrit threshold (% 1/L)
Children (6 months to 59 months)	110	33
Children (5-11 years)	115	34
Children (12-14 years)	120	36
Non-pregnant women (≥ 15 years)	120	36
Pregnant women	110	33
Men (≥ 15 years)	130	39

Source: Adapted from Iron deficiency anaemia: assessment, prevention and control. WHO, 2001.²¹

As shown in Table 1.1, correctly interpreting Hb and hematocrit levels are dependent on factors such as age, gender, pregnancy status as well as altitude and smoking habits.

1.1.1 Definition of Anemia by Age

Children between 6 months and 14 years generally have lower Hb levels compared to adults as shown in Table 1.1 mainly because children have lower red blood cell counts. After birth, the concentration of Hb measured in cord blood is dependent on factors such as the gestational age at birth and maternal hematologic factors.⁵⁰

Neonates have very high Hb levels, mainly fetal hemoglobin (HbF) which has a very high affinity for oxygen.⁵¹ By the 6th-12th months after birth, adult Hb almost completely replaces HbF. The switch from the high oxygen-affinitive HbF into low oxygen-affinitive Hb does not come at an oxygen-cost to the infant. Instead, the reduced oxygen affinity allows for increased oxygen unloading to tissues.⁵²

Due to the increased demand for oxygen and other nutrients for developing tissues, children are among the group that is highly at risk of anemia. Table 1.2 shows the mean evolution in Hb and hematocrit levels in healthy infants younger than 6 months.

Table 1.2 Hemoglobin and hematocrit levels in healthy infants

Age	Hemoglobin (g/L)		Hematocrit (%)	
	Mean	Mean - 2 SD	Mean	Mean - 2 SD
1-3 days	185	145	56	45
1 week	175	135	54	42
2 weeks	165	125	51	39
1 month	140	100	43	31
2 months	115	90	35	28
3-6 months	115	95	35	29

Source: Adapted from Hematology: Diagnosis and Treatment (p 418 to 425)⁵³

1.1.2 Definition of Anemia by Gender

Prior to the age of menarche, healthy females and males do not show any significant difference in Hb concentration.⁵⁴ For this reason, the loss of blood during menstruation in adult women has been explained to be a reason for the observed differences in hemoglobin concentration.⁵⁵ A study in Jos, Nigeria following nulliparous women showed a significant decline in mean Hb concentration during the menstruation phase (120.5 g/L) compared to the ovulatory (132.7 g/L) and follicular phases (122.3 g/L) of the menstrual cycle.⁵⁶

1.1.3 Definition of Anemia by Pregnancy Status

During pregnancy, although menstruation ceases, other physiological changes occur to alter Hb concentration. Beginning from the second month of pregnancy, plasma volume increases by about 50%, peaking between 28 to 34 weeks of gestation, with a disproportionate 20 to 30% increase in red cell mass, which results in a decrease in Hb concentration.⁴² It is for this reason that Hb cut-off for pregnant women is 10 g/L lower than that of adult childbearing females. The CDC sets the cut-off even lower to 105 g/L for pregnant women at the second trimester.⁵⁷ Detailed physiopathology of anemia pregnancy will be explained in Chapter 1.2.2

1.1.4 Definition of Anemia by Altitude

The WHO cut-offs for diagnosing anemia as shown in Table 1.1 are only applicable at sea level. Above sea level, there is an inverse relationship between altitude and ambient barometric pressure. The low pressure at high altitudes results in a decrease in the partial pressure of oxygen.⁵⁸ To compensate for this plummet in oxygen levels, at high altitudes, there is an increase in erythropoiesis which results in an increase in circulating Hb levels and the oxygen carrying capacity of the blood.⁵⁹

In order to prevent under-diagnoses of anemia due to the influence of altitude on Hb concentration, it is essential to account for altitude in the cut-off for diagnosing anemia among residents of high altitudes.

Cohen and Haas⁶⁰ also propose a different formula for altitude adjusted Hb concentration among non-pregnant women as shown below:

$$\text{Hemoglobin (g/L)} = 120 + 16.3 [\exp^{(0.00038 \times (\text{altitude}-1000))}]$$

Altitude-specific Hb and hematocrit adjustments to complement the cut-offs in Table 1.1 have been proposed by the CDC (Table 1.3)

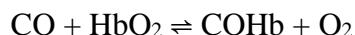
Table 1.3 Hemoglobin and hematocrit adjustments for diagnosing anemia at different altitudes

Altitude (feet)	Adjustment to Hemoglobin concentration threshold (g/L)	Adjustment to Hematocrit level threshold (%)
Less than 3000	No adjustment	No adjustment
3000-3999	+2	+0.5
4000-4999	+3	+1.0
5000-5999	+5	+1.5
6000-6999	+7	+2.0
7000-7999	+10	+3.0
8000-8999	+13	+4.0
9000-9999	+16	+5.0
10000-11000	+20	+6.0

Source: Adapted from Recommendations to prevent and control iron deficiency in the United States. Centers for Disease Control and Prevention, 1998.⁵⁷

1.1.5 Definition of Anemia by Cigarette Smoking Habits

Dissolved carbon monoxide (CO) in the blood, resulting from smoking, binds to available Hb to form a fairly stable carboxyhemoglobin as shown in the reversible reaction equation below:



Because the affinity of CO for Hb is about 200 times that of oxygen the forward reaction is more favored. The formation of carboxyhemoglobin restricts the binding of oxygen to Hb resulting in reduced oxygen transport to tissues.

To compensate for the reduced oxygen transport, there is increased production of RBCs. Due to the high Hb levels in smokers, diagnosing anemia using the thresholds as shown in Table 1.1 may not reveal anemic smokers. Adjustments to Hb thresholds for smokers have been proposed.⁶¹ Table 1.4 shows adjustments to Hb and hematocrit cut-offs for smokers.

Table 1.4 Hemoglobin and hematocrit adjustments for diagnosing anemia among cigarette smokers

Cigarettes smoked (per day)	Adjustment to Hemoglobin concentration threshold (g/L)	Adjustment to Hematocrit level threshold (%)
Less than 10	No adjustment	No adjustment
10-19	+2	+1.0
20-39	+3	+1.5
More than 40	+5	+2.0
Smoker*, amount unknown	+7	+1.0

*More than 100 cigarettes ever smoked

Source: Adapted from Sullivan *et al.*, 2008. ⁶²

In pregnancy, smoking has been shown to have adverse consequences on maternal health and fetal development. Adverse effects of smoking on micronutrient deficiencies such as vitamin C, vitamin B6, vitamin B12 and folic acid have been reported.⁶³ Others have also reported significantly lower levels of serum pro-hepcidin and low cord blood ferritin levels, transferrin and total iron in pregnant smokers compared to non-pregnant smokers.⁶⁴

Adverse birth consequences including sudden infant death syndrome have also been linked with smoking during pregnancy.⁶⁵ Further, in smoking pregnant women, the high levels of Hb concentration masks anemia and as a result, in regions where hematologic indices such as Hb or hematocrit concentrations are the sole indicators of health status, smoking pregnant women may not receive the adequate care required thus compounding to the negative consequences of cigarette smoking.

In our study setting however, smoking among pregnant women is rare and only 3 per cent of pregnant women smoked cigarette, pipe or cigars.

1.2 Anemia in Pregnancy

1.2.1 Epidemiology of Anemia in Pregnancy

Anemia in pregnancy is defined as Hb concentration less than 110 g/L at sea-level throughout pregnancy by the WHO.⁷ The CDC considers the same cut-off of 110g/L for pregnant women in the first and third trimesters of pregnancy and a cut-off of 105 g/L for pregnant women in their second trimester.⁵ The global definition of anemia during pregnancy remains subjective as some studies, especially in Asia, use a Hb cut-off of 100 g/L to diagnose anemia in pregnancy.^{66,67}

Anemia in pregnancy is a serious public health problem worldwide. Recent global estimates reveals that anemia accounts for more than 68 million YLD.⁶ Considering the WHO criteria for classifying anemia as a public health problem, anemia in pregnancy is an ubiquitous public health problem worldwide.⁷ Even though anemia in pregnancy is a public health problem in almost every country, less developed countries are the most affected bearing most of the burden of anemia (Figure 1.1).

According to deBenoist *et al.*, over 40% of pregnant women worldwide are estimated to be anemic⁷ while a recent publication of the global prevalence of anemia in 2011 by the WHO⁸ reports a global anemia prevalence of 38.2 among pregnant women. However, South-East Asia and Africa bear most of the burden of anemia in pregnancy with prevalence of 48.7% and 46.3%, respectively in comparison with the 24.9% and 25.8% in the Americas and Europe, respectively (Table 1.5).⁷ Although a recent systematic analysis of Hb data revealed a slight decline in global prevalence of anemia, the disparity in the prevalence of anemia in pregnancy between developed and less developed countries still persisted.⁶⁸ Narrowing down on the anemia prevalence among pregnant women in some rural regions in less developed countries reveals even higher proportions of affected pregnant women.^{14,69,70}

At the individual and population level, anemia is associated with socioeconomic determinants such as education, wealth and cultural practices. Characterization of anemia by socioeconomic status is also evident in pregnant women among whom other factors such as gravidity, duration between successive pregnancies, and early pregnancy onset further increases the risk of anemia.^{71,72} Balarajan *et al.* presents a concise conceptual framework of the determinants of

anemia (Figure 1.2) which encompasses the socio-political context, structural and intermediary determinants and the biological factors.⁷³

b. Prevalence of anaemia, pregnant women aged 15-49 years, 2011

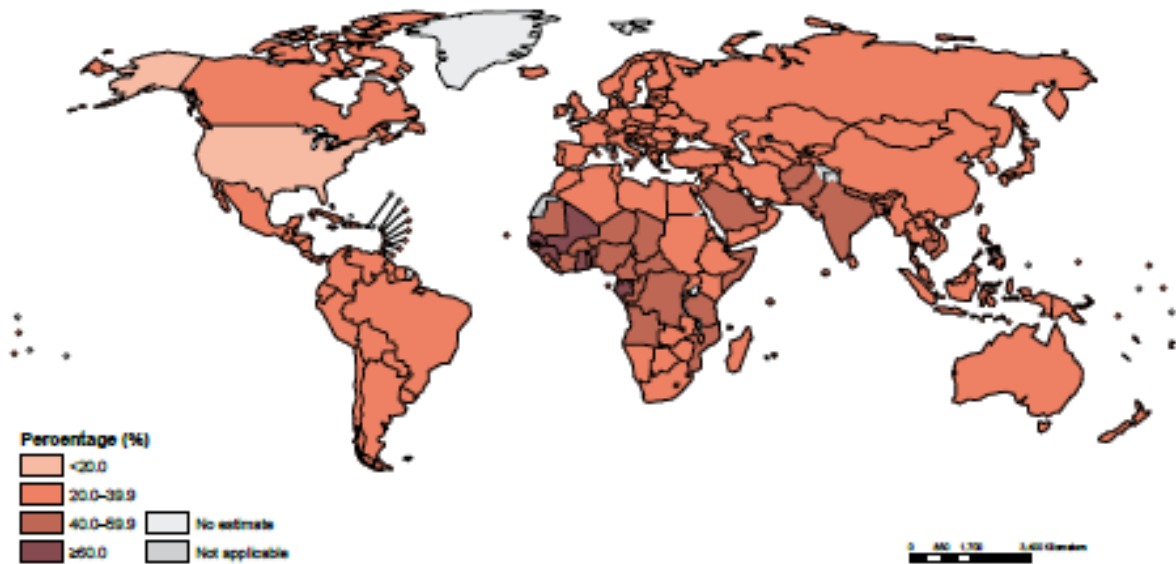


Figure 1.1 Prevalence of anemia among pregnant women (15-49 years) in 2011

Source: WHO. The Global Prevalence of Anaemia in 2011. Geneva. World Health Organization, 2015.⁸

In order to prioritize management or treatment of anemia in pregnancy, it is necessary to ascertain the severity of anemia in pregnancy using Hb cut-offs set by the WHO. Anemia in pregnancy can be said to be mild (Hb 100-109 g/L), moderate (70-99 g/L) or severe (lower than 70 g/L).⁷⁴ In most epidemiologic studies, the highest proportion of anemic pregnant women are mildly or moderately anemic. The prevalence of mild anemia among anemic pregnant women could range from 64.0% in Gondar, Ethiopia⁷⁵, 75.0% in Hyderabad, Pakistan⁷⁶ to 90.7% in

Enugu, Nigeria⁷⁷. Mild anemia usually goes unrecognized as it hardly presents evident clinical manifestations.

Table 1.5 Prevalence of anemia and number of affected pregnant women

WHO region	Prevalence % (95% CI)	Number affected in millions (95% CI)
Africa	46.3 (40.6-51.0)	9.2 (8.1-10.1)
South-East Asia	48.7 (36.1-58.9)	11.5 (8.5-13.9)
Eastern Mediterranean	38.9 (32.7-46.3)	3.9 (3.3-4.6)
Western Pacific	24.3 (15.1-37.7)	3.6 (2.2-5.5)
Europe	25.8 (19.8-33.6)	1.8 (1.4-2.3)
Americas	24.9 (19.0-32.5)	2.4 (1.8-3.1)
Global	38.2 (33.5-42.6)	32.4 (28.4-36.2)

Source: Adapted from WHO. The Global Prevalence of Anaemia in 2011. Geneva. World Health Organization, 2015.⁸

1.2.2 Pathophysiology of Anemia in Pregnancy

During pregnancy, there is an increase in the demand for iron to accommodate the needs of the fetal-placental unit, mainly. To cope with these increased needs, some physiological changes occur in the pregnant woman. Hematological changes that occur during normal pregnancy include the increase in red blood cell production and disproportionately higher increase in plasma volume which results in increased blood volume.⁷⁸ The exact mechanism for plasma volume expansion during pregnancy however remains unknown.

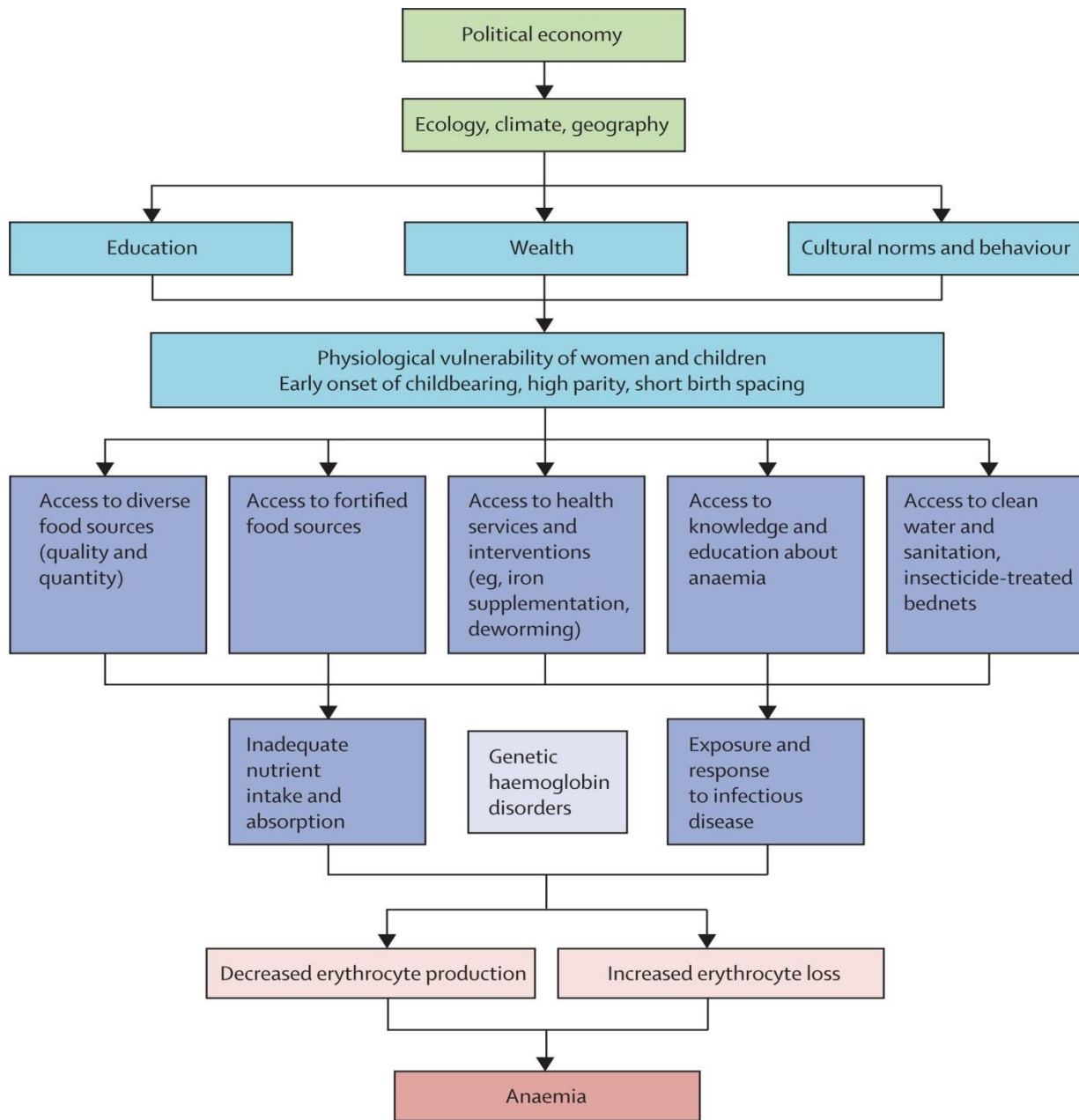


Figure 1.2 Conceptual model of the determinants of anemia

Source: Balarajan *et al.* Anaemia in low-income and middle-income countries. *The Lancet*, 2011.⁷³

In normal pregnancy, plasma volume expansion begins in the 6th week of gestation⁷⁹ and peaks between the 28th and 34th week by about 40-50% in greater volume than in non-pregnant women^{80,81}. The relatively higher increase in plasma volume to RBCs by late first trimester and

in the second trimester of pregnancy results in a decline in Hb concentration, a condition referred to others as ‘**physiologic anemia**’ of pregnancy.

The hypervolemic condition created by the relatively higher plasma volume causes the viscosity of the blood to reduce. The reduced blood viscosity from the physiologic hemodilution renders pregnant women hypercoagulable over the course of pregnancy and thus optimizes the circulation of oxygen-carrying blood from the pregnant women to the fetus and protects the pregnant women from bleeding at delivery.⁸² Hemodilution during pregnancy is accompanied by an increasing demand for iron of up to about 1g of total iron⁸¹. Hb concentration however increases in the third trimester towards the later stages of pregnancy because of increased RBC production. This U-shaped relationship between hemoglobin concentration and gestational age is highlighted in the works of Bodeau-Livinec *et al.* (Figure 1.3)¹⁷ and Pacheco *et al.* (Table 1.6)⁸³.

Table 1.6 Mean hemoglobin values during pregnancy

Gestational age	Mean Hb value (g/L)
12 weeks	12.2
28 weeks	11.8
40 weeks	12.9

Source: Adapted from Pacheco *et al.* Physiologic changes during Pregnancy. 2013.⁸³

In the absence of plasma expansion, as it is in the case for pre-eclamptic pregnant women, hemoconcentration is likely to occur. The increasing blood viscosity as a result of hemoconcentration leads to impaired oxygen transport to the fetus. Further, pregnant women regarded as non-anemic and presenting high concentrations of serum iron is not necessarily a reflection adequate nutritional status as this may reflect pregnancy induced hypertension and eclampsia⁶⁰. Apart from physiologic-induced anemia, several other factors including nutrient deficiencies and infectious and parasitic diseases could explain for anemia in pregnancy.

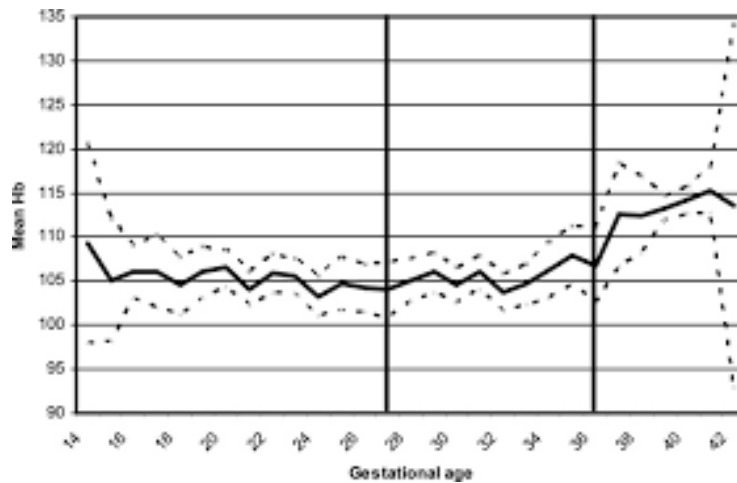


Figure 1.3 Mean hemoglobin concentration, in g/L (95% CI) by gestational age (in weeks) of pregnant women in Benin

Source: Bodeau-Livinec *et al.* Maternal Anemia in Benin: Prevalence, Risk Factors, and Association with Low Birth Weight. AJTMH, 2011¹⁷

1.3 Risk Factors of Anemia in Pregnancy

The etiology of anemia in pregnancy is multifactorial and the factors responsible rarely act in isolation. In most situations, the risk factors of anemia co-exist. The causes of anemia in pregnancy are detailed in Table 1.7. Apart from physiologic anemia during pregnancy, reduced Hb concentration is an indication of impaired erythropoiesis or increased hemolysis or both. In either of these scenarios, genetics, infectious or parasitic diseases and nutrient deficiencies could be responsible. The presentation of the causes of anemia in this thesis will fall under these three categories. Since the latter two are of more interest to this thesis, more emphasis will be given to them in comparison to the genetic causes of anemia, which will be briefly touched on. Also, brief information on some of the consequences of these particular causes of anemia will be sighted although elaborate consequences of anemia are further discussed in Chapter 1.4.

1.3.1 Genetic-related Anemia

Alteration of a gene(s) responsible for the structure of one of the globin chains of the Hb molecule or the inheritance of such gene could lead to anemia. This structural variation often arises from a substituted amino acid in the chain which could also alter the structure and function of the protein. Although over 700 of Hb variants exist only the HbS, HbC and HbE are common and of public health significance.⁸⁵

Sickle cell anemia and thalassemias are the major types of blood disorders that are associated with impaired Hb and as a result, impaired oxygen transport. It is estimated that about 5% of the world's population are carriers of a sickle cell or thalassemia gene⁸⁶ although HbS allele frequency of 15% have been reported in some studies.^{87,88} This is due to well-described spatial heterogeneities in the distribution of Hb anomalies (especially Hb C and Hb S in Africa).

Other genetic Hb disorders that could account for anemia in pregnancy are Glucose-6-phosphate dehydrogenase (G6PD) deficiency disease and ovalocytosis. G6PD is an enzyme involved in the pentose phosphate pathway which produces the only source of the reduced form nicotinamide adenine dinucleotide phosphate for the reduction of glutathione in RBCs. A deficiency in G6PD therefore means a reduction in the protective effect of RBCs from oxidation by free radicals⁸⁹. The resulting oxidative stress of the RBCs results in hemolytic anemia. Hereditary ovalocytosis or elliptocytosis, the condition where over 25% of the RBCs of an individual have an oval or elliptical rod-like shapes arising from gene mutations that influence the cytoskeleton and membrane integrity of the RBCs.⁹⁰

1.3.2 Nutritional Causes of Anemia

Nutritional anemias are preventable and reversible types of anemia that occur due to deficiencies of nutrients particularly, micronutrients, vitamins and proteins, arising either from low dietary intake or malabsorption or abnormal intestinal loss. Nutritional anemias are common in low and middle income countries where nutrient deficiencies are highest and usually co-exist with other causes of anemia.⁹² The major causes of nutritional anemia that are of major concern to pregnant women include ID, folic acid deficiency, vitamin B12 deficiency and vitamin A deficiency.

Table 1.7 Causes of anemia in pregnancy

Nutritional deficiencies	Iron Folic acid Vitamin B12 Vitamin C, Vitamin A Protein
Hemolysis and abnormal hemoglobin synthesis	Malaria Glucose-6-phosphate dehydrogenase deficiency Thalassemias Sickle cell disease
Blood loss and defective iron absorption and metabolism	Helminthiasis, especially hookworm infestation Amoebiasis and Giardiasis Schistosomiasis Abnormal iron metabolism Bleeding hemorrhoids Antepartum hemorrhage Trauma
Chronic conditions	High parity Malignancies Tuberculosis Chronic renal disease including urinary tract infection Sexually transmitted infections including bacterial vaginosis Human Immune deficiency Virus (HIV) infection Chronic rheumatic and rheumatoid disease

Source: Adapted from Goonewardene *et al.* Anemia in Pregnancy. Best Practice & Research Clinical Obstetrics and Gynaecology. 2012.⁹¹

Iron Deficiency

Iron is an essential micronutrient that plays significant roles in several human biochemical processes including enzymatic functions, DNA synthesis, functioning of the immune system and oxygen transport. In normal adults, most of the iron body (approximately 70%) is responsible for the transport of oxygen in the form of Hb in erythrocytes.⁹³

In spite of the abundance of iron and in various foods^{94,95}, ID remains the world's commonest nutrient deficiency affecting an estimated 2 billion (over 30%) people worldwide according to the WHO⁹⁶. In addition, ID is estimated to contribute to about 50 per cent of anemia globally with

Africa and Asia bearing most of the global burden of ID and anemia.⁹⁷ For this reason and the ease of measuring Hb concentration in comparison to ferritin in serum, some studies use Hb levels below 110 g/L during pregnancy (anemia) as a proxy for ID instead of measuring ferritin or transferrin concentrations.²⁴ This use of Hb concentration as an indicator for ID in population studies, especially in places with limited resources, is supported by the WHO.⁹⁸ However the proportion of anemia that is attributable to iron deficiency in some studies is usually lower than estimated (19% in a Germany and 12% in Benin).^{99,100} It is however undisputable that globally, iron deficiency anemia (IDA) accounts for the highest burden of anemia.⁶ In West Africa, ID accounts for about 30 per cent of the burden of anemia according to recent studies on the global burden of diseases in 2010.⁶

Maternal and fetal iron demands are least during the first trimester of pregnancy following the absence of menstruation¹⁰¹ and as a result, a well-nourished healthy pregnant woman is likely to have adequate iron stores. Reduction of iron stores begin in the late first trimester or second trimester of pregnancy when the maternal and fetal iron requirements increase following the development and upkeep of the fetal-placental unit.¹⁰² Iron status is therefore expressed as a continuum from iron overload (excessive iron stores) to normal iron status to ID without anemia and then to IDA.²¹

The role of iron in the body can be divided into two: functional iron (for metabolic processes) and storage iron (in the form of ferritin). Ferritin is an ubiquitous spherical-shaped protein responsible for the intercellular storage of iron in the ferric state (Fe^{3+}). Ferritin sequesters reactive ferrous irons (Fe^{2+}) and converts them to Fe^{3+} for storage thereby preventing the formation of reactive oxygen species.¹⁰³ Transferrin on the other hand is a glycoprotein responsible for the transport of iron via the transferrin receptor.¹⁰⁴

In the context of IDA, ferritin concentrations decrease whereas transferrin levels increase. The regulation of iron in the body includes the inter-organ transport of iron which involves transferrin/transferrin-receptor system transporting non-heme irons to cells which increases in the context of depleted iron stores.^{105,106} Also, the size of the bound iron complex influences intracellular gene expression of ferritin and transferrin after transcription.¹⁰⁷ For these reasons, ferritin and transferrin concentrations are indicators used in determining ID in an individual.

However, in the presence of an inflammation there is a decline in the levels of transferrin (between 20% - 50%) and an increase in ferritin levels (30% - 100% or more) with a paralleled increase in C-reactive protein (CRP).¹⁰⁸ Inflammations affect several iron biomarkers and as a result in regions where inflammation is expected to be high due to infections, it is essential to account for the changes in concentration of iron biomarkers due to inflammation. Table 1.8 displays the influence of inflammation on iron biomarkers.

Evidence from observational studies reveals how ID and anemia could be detrimental to the health of the pregnant woman and her baby. Several studies following groups of pregnant women show an increased risk of adverse birth outcomes such as LBW, preterm birth and SGA babies, among anemic pregnant women compared with non-anemic pregnant women.^{109–111} A systematic review published in 2013 concluded that pregnant women who were anemic at first trimester had 1.10 (95% CI: 1.02–1.19), 1.17 (95% CI: 1.03–1.32), and 1.14 (95% CI: 1.05–1.24) times higher risk of preterm birth, LBW and SGA birth, respectively, compared to non-anemic women.¹¹²

Evidence of possible effect of gestational ID, anemia and severe anemia on the cognitive and motor development of offspring after birth have also been reported in animal and humans studies.^{113–115} In order to prevent IDA during pregnancy and its consequences several interventions have been rolled out including food fortification, dietary control and iron supplementation of which iron supplementation is the most implemented.¹¹⁶ Daily supplementation of 30–60 mg of elemental iron throughout pregnancy with the higher end of the dose recommended for pregnant women in areas where the prevalence of anemia exceeds 40% irrespective of anemia or iron status have been recommended by the WHO.¹¹⁷

In malaria endemic regions however, iron supplementation during pregnancy have been debated over recent years as inconsistent benefits have been seen in the risk of malaria.¹¹⁸ The debate surrounds evidence of increased risk of malaria among iron supplemented individuals^{119,120}. In addition this debatable disadvantage of iron supplementation, mass administration of daily iron supplementation in at-risk populations, especially pregnant women, have been criticized by some researchers who have shown that iron supplementation to already iron replete pregnant women is not beneficial to maternal health and childbirth.^{121,122} In depth implications of iron

supplementation for iron replete and non-anemic pregnant women especially in the light of the findings of this thesis are deliberated upon in Chapter 6.

Table 1.8 Effects of inflammation on iron biomarkers

Blood biomarkers	Impact of inflammation		Mechanism of response
	Speed	Response	
Iron	Within 8 hours	Fall by ~50%	Inhibition of iron release from macrophages and uptake by newly released apoferritin
Hemoglobin	Slow or none	Fall by 20-30%	Insufficient iron in serum to allow normal replacement of old red cells and inhibition of erythropoiesis by TNF and IFN- γ
Hematocrit	Slow or none	Fall by 20-30%	
Zinc protoporphyrin (ZPP)	Slow	4–5-fold increase in ACI	Where there is a decreased production of haemoglobin, ZPP will increase
Transferrin	Rapid in 24 hours	Fall by 20-30%	Increased vasodilation and capillary permeability
Transferrin receptors	24 hours	Fall by 30-50%	Impaired erythroid growth or inhibition of TfR gene expression mediated by TNF and IFN- γ
Ferritin	Rapid in 8 hours	Rise paralleled CRP increase 30–100% or more	TNF, IL-6 and IFN- γ exert a stimulatory role on ferritin synthesis

ACI, anaemia of chronic inflammation; CRP, C-reactive protein; TNF, tumour necrosis factor; IFN, interferon, IL, interleukin

Source: Adapted from Thurnham and McCabe. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. World Health Organization. 2012.¹⁰⁸

Folic Acid Deficiency

Folic acid (also called pteroglutamic acid or vitamin B₉) is a naturally occurring water soluble B-vitamin that acts as a precursor to the active coenzyme, tetrahydrofolate. The active form of tetrahydrofolic acid plays a significant role in DNA, RNA and protein synthesis. Folic acid plays a significant role in the synthesis of heme and as a result the maturation of RBCs.

During pregnancy, there are increased demands for folic acid as it is essential for the growth and development of the fetus. The increased demand increases the tendency of the pregnant woman to be deficient in the absence of consumption of adequate folate-rich foods or folic acid supplements. Folic acid deficiency can also be due to malabsorption of folic acid arising from severe alcohol consumption¹²³, relatively rare in sub-Saharan Africa. Folic acid deficiency could result in megaloblastic anemia, the condition of abnormally large RBCs arising from slowed nuclear maturation of the cells.

The global prevalence of folic acid deficiency is unknown as a result of limited data, prevalence of folic acid deficiency among the pregnant women in Benin, who were part of the study used in this thesis, was 31%¹⁴. Further, in this group of women, folic acid deficiency has been shown to be a risk factor of anemia.¹⁴ Folic acid deficiency has been associated with neural tube defects and consequently, spontaneous abortions among other adverse birth outcomes.¹²⁴ The supplemental daily dose of folic acid recommended by the WHO was increased from 300µg in 1968 to 400µg in 1998.¹¹⁷ The present recommendations of Beninese authorities are much too high (5 mg daily), with a risk of interaction with the antifolate activity of SP, thus a possible decrease in IPTp efficacy.

Vitamin B₁₂ Deficiency

Vitamin B₁₂ or cobalamin is also a naturally occurring water soluble B-vitamin that is essential for healthy nervous system functioning, DNA synthesis and red cell formation. Vitamin B₁₂ also plays a significant role in the synthesis of the active form of folate from its inactive, methylated form (5-methyl tetrahydrofolate). As a result a deficiency in vitamin B₁₂ could induce folic acid deficiency. Vitamin B₁₂ is produced by bacteria and can be obtained from animal diet. The

vitamin is available in animals that ingest cobalamine producing bacteria, which are the sole source of bisynthesized vitamin B₁₂.

Due to the significant role of vitamin B₁₂ in the appropriate functioning of nervous system, prenatal requirements are usually elevated to accommodate for the development and functioning of the nervous system of the growing fetus. In the case of low intake of animal diets (such as in vegans), micronutrient malabsorption or gastro-intestinal disorders during pregnancy, vitamin B₁₂ deficiency could arise. Pernicious anemia, an autoimmune destruction of gastric parietal cells which are responsible for secreting intrinsic factor protein, is another potential cause of vitamin B₁₂ deficiency.¹²⁵

Like folic acid deficiency, vitamin B₁₂ deficiency could also lead to megaloblastic anemia. Although the global prevalence of vitamin B₁₂ deficiency is not well documented, the limited evidence shows that the prevalence is highest among adults (12 to 60-year-olds) and in sub-Saharan Africa.¹²⁶ Further little is known of what proportion of anemia is due to folic acid deficiency. In Benin, 5% of anemia in pregnancy was attributable to folic acid deficiency.¹⁴ The global prevalence of vitamin B₁₂ deficiency is unknown due to inadequate data.¹²⁷

Vitamin A deficiency

Vitamin A or retinol is a liposoluble vitamin that is mainly responsible for the formation of rhodopsin (photoreceptor molecule) by binding to opsin in the retina. Vitamin A also plays a significant role in erythropoiesis and has been shown to modulate the iron metabolism through erythropoietic mechanisms.¹²⁸ Vitamin A is abundant in varying amounts in cod-liver oil, liver, carrots, palm oil, pumpkins, eggs, etc.

For pregnant women, a daily intake of 800µg of vitamin A is recommended by the WHO.¹²⁹ Following the low intake of vitamin A-rich animal diets, beta carotene-rich plant diets or vitamin A supplements, an individual could be at risk of vitamin A deficiency. Vitamin A deficiency is common in low and middle-income countries. Globally, approximately 19 million pregnant women are estimated to be vitamin A deficient.¹³⁰ In Southeast Asia, vitamin A deficiency is estimated to affect about 5% of pregnant women.¹³¹ Vitamin A deficiency results in nyctalopia (night blindness), xerophthalmia, and complete blindness.^{132,133} Studies in the Republic of Congo

among pregnant women show 33% prevalence of clinical signs of xerophthalmia in rural communities.¹³⁴ Vitamin A deficiency during pregnancy is also associated with increased mother-to-child HIV-1 transmission.¹³⁵

Although the role of vitamin A in the erythropoiesis is not well understood, it is suggested that vitamin A supplementation drives iron from existing stores to promote erythropoiesis¹³⁶ thereby reducing the risk of anemia. Although vitamin A supplementation during pregnancy has been shown to reduce anemia risk¹³⁷, the current WHO guidelines do not recommend vitamin A supplementation in pregnancy except in regions where the prevalence of night blindness exceeds 5%¹³⁰.

1.3.3 Infectious and Parasitic Causes of Anemia

Globally, evidence points to nutritional deficiencies, particularly ID, as the leading bearer of the burden and prevalence of anemia⁶. However, in tropical and sub-tropical countries, preventable infectious diseases exacerbate the prevalence and burden of anemia. In fact, the combined burden of anemia attributable to a collection of these infectious diseases surpasses that of nutritional deficiencies as shown in figure 1.4.⁶

Helminth infections

The term helminths refer to worm parasites that infect humans. There are different types of helminths but of interest to this thesis are *the nematodes* particularly, soil-transmitted helminth infections. Soil-transmitted helminths (STHs) are parasitic worms that infect humans through a contaminated soil. The major species of STHs are hookworms (*Ancylostoma duodenale* and *Necator americanus*), roundworms (*Ascaris lumbricoides*) and whipworms (*Trichuris trichiura*).

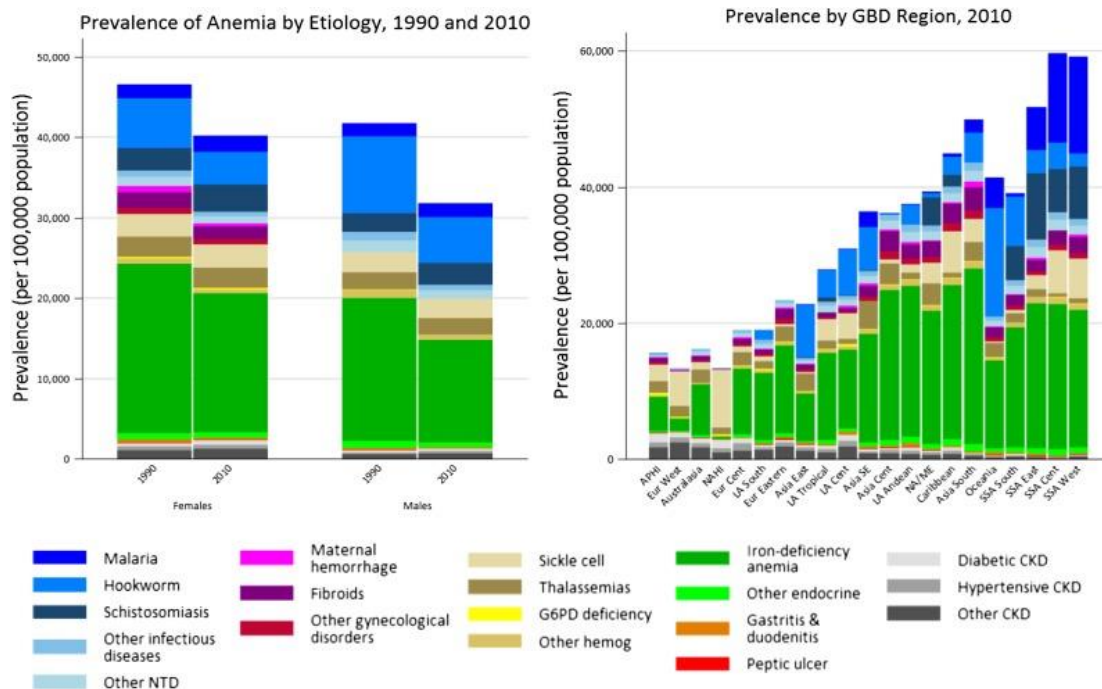


Figure 1.4 Global and regional cause-specific anemia prevalence for 1990 and 2010

Source: Kassebaum *et al.* A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. 2014.

There are two main modes of transmission for these species of helminths; cutaneous penetration and ingestion of eggs or larvae. *Ascaris lumbricoides*, *Trichuris trichiura* and *Ancylostoma duodenale* are mainly transmitted through ingestion of a contaminated soil or a contaminated food (Figure 1.5)¹³⁸.

Hookworms are transmitted mainly by cutaneous penetration of the eggs from a contaminated soil. Although *Ancylostoma duodenale* anecdotally been reported to be transmitted by ingestion, *Necator americanus* is strictly transmitted by skin penetration. The feces of an infected person, which contains the eggs of the helminths, contaminate soils. Helminth infection during pregnancy particularly, hookworm infection, has been associated with increased risk of anemia in an earlier systematic review.¹⁰ Figure 1.6 explains the lifecycle of hookworms.

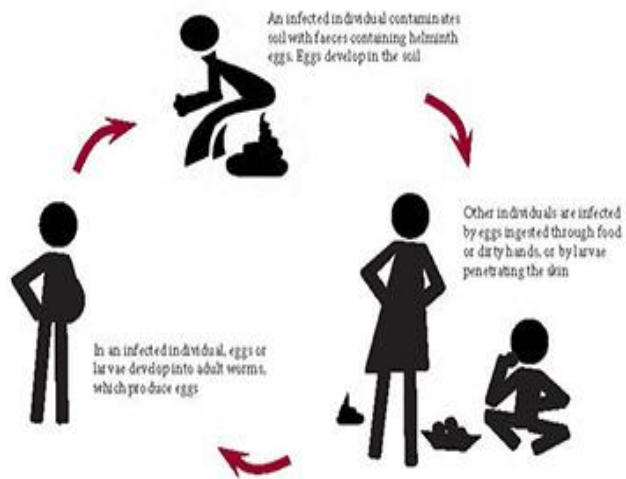


Figure 1.5 Transmission cycle of soil-transmitted helminths by ingestion

Source: WHO. Epidemiology of intestinal worms. World Health Organization. 2015

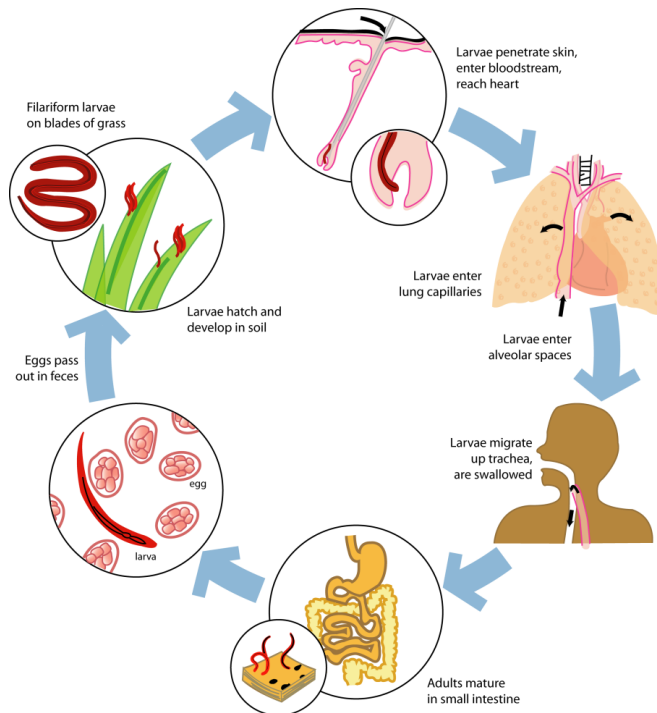


Figure 1.6 Lifecyle of human hookworm, *Necator Americanus*

Source: Hotez *et al.* Hookworm: “The Great Infection of Mankind”. 2005.¹³⁹

The evidence of the impact of helminth infection on increased anemia among pregnant women has been reported in recent studies as well.^{14,140} Hookworms cause anemia by causing intestinal blood loss that arises when they cut through, suck and ingest blood from the intestinal mucosa of their host.¹⁴¹ The degree of intestinal blood loss is however dependent on the type of hookworm that an individual is infected with. *N. americanus* infestation in general is associated with lesser blood loss compared to *A. duodenale*.³⁰ However, differentiating these species in population studies, especially in resource-limited settings is complicated. Among pregnant women, studies have shown that moderate and high intensities of hookworm and whipworm infestation [>2000 eggs per gram (epg) of stool] is associated with increased risk of anemia¹⁴² whereas other studies also show that even light infections have been shown to be associated with decreased Hb concentration.¹⁰ However, the mechanism by which *T. trichiura* causes anemia is less understood.¹⁴³

The global prevalence of helminth infection is unknown but it is estimated that over 1.5 billion people are infected with one or more of the species of STHs worldwide.¹⁴⁴ In sub-Saharan Africa, it is estimated that one-third of pregnant women are infected with soil-transmitted helminths¹³ although several studies have shown wide variation in prevalence across different countries, 11.1% in Benin¹⁴, 25.7% in Ghana¹⁵ and 49% in Gabon¹⁶. There are, too, important differences between sites within the same country. In tropical and sub-tropical countries, helminth infection often coexists with malaria leading to a combined burden.

To control helminth infection, WHO recommendations suggest that pregnant women living in endemic regions should be administered 400 mg of albendazole or to 500 mg of mebendazole beginning in the second trimester.¹⁴⁵ However, evidence from a recent systematic review and meta-analysis shows no beneficial effect of anthelmintic administration on anemia risk, preterm birth, LBW and perinatal mortality.¹⁴⁶ Some authors have also been raised concerns about the teratogenicity and adverse birth effects due to mebendazole and albendazole administration during pregnancy.¹⁴⁷

Malaria

Malaria is common in tropical and sub-tropical regions of the world and most prevalent in sub-Saharan Africa. The female anopheles mosquito transmits malaria. Of the five species of plasmodium (*P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*) known to cause malaria, *P. falciparum* is the most dangerous to malaria transmission and it is more prevalent in sub-Saharan Africa. This explains why approximately 90% of the death due to malaria is estimated to occur in sub-Saharan Africa.¹⁴⁸

During the reproductive life cycle of the Plasmodium parasite in humans, the merozoite forms of the parasite attack the RBCs upon entering the blood stream and subsequently the spleen engulfs the infected RBCs. The hemolysis caused by the infection explains how malaria leads to anemia. Malaria contributes to about one-third of the burden of anemia in sub-Saharan Africa.⁶

During pregnancy, the vulnerability of the woman and the developing fetus makes them particularly susceptible to the consequences of malaria compared to non-pregnant women.¹⁴⁹ Malaria in pregnancy is thus considered a serious public health issue with over 54 million pregnancies occurring in malaria-endemic regions with stable *P. falciparum* malaria in 2007.¹⁵⁰ Generally, the regions of Africa with stable transmission bears the highest burden of *P. falciparum* malaria.¹⁵¹

Malaria in pregnancy has been associated with increased risk of anemia in pregnancy¹⁴, increased risk of intra-uterine growth restriction,¹⁵² increased risk of LBW¹⁵³ and preterm birth¹⁵⁴ although some studies have published contradictory findings.¹⁵⁵ A recent study using verbal autopsy in Mozambique shows that more than 20% of maternal deaths that occurred during pregnancy were attributable to malaria.¹⁵⁶

In its recent guidelines, the WHO recommends that pregnant women living in malaria-endemic regions to use long-lasting insecticidal nets (LLINs) and in regions of sub-Saharan Africa with stable malaria transmission, in combination with intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine.¹⁵⁷

Human Immunodeficiency Virus Infection

HIV attacks and destroys the immune system of the infected person thereby rendering them vulnerable to pathogens and cancers which otherwise would have been fought off by the immune system. HIV has been shown in many studies to be associated with increased risk of anemia^{158,159}, which further increases with the progression of HIV infection¹⁵⁹. Aside the apparent blood loss, such as in the case of neoplastic disease, impaired red blood cell production and increased hemolysis could also account for the increased risk of anemia in HIV infected persons.^{160,161}

HIV prevalence has declined over the years with increasing antiretroviral treatment (ART).¹⁶² However, the disease remains common in some countries in sub-Saharan Africa where an estimated 66% of the 37 million people infected worldwide live.¹⁶³ Likewise, the rate of mother-to-child transmission of HIV has significantly declined. According to the Joint United Nations Program on HIV/AIDS (UNAIDS), the prevalence of newly infected HIV children has fallen from 26.9% in 2009 to 15.3% in 2013 when the mother is infected.¹⁶⁴

HIV infection during pregnancy has been showed to be associated with increased risk of helminth infection and malaria^{165,166}, all of which are strong predictors of anemia in pregnancy in endemic regions. Severity of HIV in pregnancy has also been associated with other adverse outcomes such as LBW and preterm births.¹⁶⁷

Although HIV is curable, about half of the people living with HIV are unaware of their infection¹⁶⁸ and increased screening and information dissemination on testing are required. Even in developing countries, routine HIV screening has been proposed to be effective in averting deaths.¹⁶⁹

1.4 Consequences of Anemia in Pregnancy

Although anemia is common during pregnancy, depending on its severity, complications may develop that could negatively impact on the health of the pregnant woman and the growing fetus. Anemia or low Hb concentrations during pregnancy could have an impact on the weight of neonates and the gestational age at delivery. These consequences are more pronounced in women

with severe anemia (Hb < 70 g/L), in which case it could lead to maternal or neonatal mortality. The consequences of anemia in pregnant women on maternal health, pregnancy outcomes and child health are detailed in the subheadings that follow.

1.4.1 Consequences of Anemia on Maternal Health

Due to the impaired oxygen supply to tissues, arising from reduced Hb concentration in anemic pregnant women, fatigue, dizziness, increased rate of heartbeat, and loss of concentration are some of the mild symptoms expressed. Apart from these morbid situations, anemia in pregnancy has also been associated with maternal mortality. Using national data from 44 countries, a strong correlation was found between the prevalence of anemia in women and maternal mortality ratios.¹⁷⁰ Five years after the publication of the aforementioned study, another study in Ghana also reported increased risk of maternal deaths among anemic women compared with non-anemic women.¹⁷¹ A recent iron report from the Child Health Epidemiology Reference Group revealed a 29% reduction in maternal mortality for a 10g/L increase in Hb concentration late in pregnancy.¹⁷² It is however worthy of note that except for severe anemia, anemia in pregnancy may not be directly responsible for the increased maternal mortality. Other causes of anemia that could have such detrimental consequences on maternal health, such as HIV, could contribute to maternal death.

1.4.2 Consequences of prenatal Anemia on Fetal Development and Birth Outcomes

Researchers have investigated the impact of anemia in pregnancy on birth outcomes for over half a century. In 1962, Klein¹⁷³ published a paper in the *American Journal of Obstetric Gynecology* to report the findings of his study concerning preterm birth and prenatal anemia (Hb < 10 g/L). In this paper, Klein observed that the incidence of preterm deliveries was higher in anemic pregnant women compared to non-anemic pregnant women thus, 12.6% versus 7.2%, respectively.¹⁷³ Since his publication, numerous studies have been conducted to investigate the impact of anemia on other birth outcomes including intrauterine growth restrictions (IUGR), small-for gestational-age (SGA), birth weight, still births and perinatal deaths.

IUGR also called fetal growth restriction refers to the failure of the fetus to achieve proper growth. Accurately, IUGR is diagnosed using fetal characteristics such as fetal abdominal

circumference, head circumference, biparietal diameter, femur length provided by ultrasound scan to compute fetal weight.¹⁷⁴ When the estimated weight of the fetus is below the 10th percentile for its gestational age, it is diagnosed to be growth-restricted.¹⁷⁴ SGA is defined as the birth weight below the 10th percentile for the gestational age of the neonate. LBW is also defined as birth weight less than 2500 g while preterm birth describes neonates born before the 37th week of gestation.

Considering the increased demand for nutrients and oxygen during pregnancy, it is not unlikely to anticipate poor fetal growth for children of anemic women. Anemia in pregnancy has a negative impact on birth outcomes.¹⁷⁵ Specifically, anemia in pregnancy has been shown to be associated with increased risk of intrauterine growth restriction, LBW, SGA, preterm deliveries, and perinatal mortality in some studies.

Although the absence of adequate maternal nutrients for the growing fetus could directly be accounting for the poor growth, other researchers have hypothesized that anemia impacts on fetal endocrine regulation as they identified higher levels of growth hormone, placental lactogen and insulin-like growth factor in the cord blood of anemic women compared to non-anemic mothers.¹⁷⁶ The authors explain that the higher levels of the growth hormones in the cord blood are reflective of in-utero adaptive measures of the fetus to overcome growth-inhibiting insults.¹⁷⁶

Intrauterine growth restriction is a complex combination of factors that inhibit the normal growth of a fetus. Over the years, several studies have shown that prenatal anemia is directly associated with IUGR. A recent case-control study in India showed that the mean maternal Hb levels were significantly higher for normal fetuses compared to IUGR fetuses although the association was crude.¹⁷⁷ Fetuses with such growth restrictions are highly at risk of preterm birth, LBW, perinatal morbidity and mortality.

Anemia during pregnancy has often been linked with LBW. LBW is defined as birth weight less than 2500 g and is an indication of poor fetal growth. Most of the published literature on maternal anemia and LBW show an irrefutable evidence of the increased risk of LBW and SGA deliveries, among severely anemic pregnant women even after adjusting for potential confounders.^{17,69} The existing evidence therefore points to increasing risk of adverse birth outcomes as the severity of the anemia increases. However, controversies still surround the impact of prenatal anemia on the

birth weight of neonates. Using the cut-off of 110 g/L to compare the risk of LBW in anemic and non-anemic women, Finkelstein *et al.*¹⁷⁸ and Ahmad *et al.*¹⁷⁹ reported increased risk of LBW among anemic women whereas Koura *et al.*¹⁸⁰, Demmouche *et al.*¹⁸¹, and Buzyan¹⁸² found no relationship between prenatal anemia and LBW.

Ambivalent findings have also been published on the relationship between prenatal anemia and preterm birth (gestational age < 37 weeks).^{180,183} A recent study in India reported a 2.4 fold increase in the risk of preterm births among anemic pregnant women compared to non-anemic pregnant women.¹⁷⁸

Although many researchers have used the clinical threshold of 110 g/L to define prenatal anemia, others who have used the Hb or hematocrit levels or categories of severity of anemia (mild to severe or quantiles of Hb) have reported a non-linear relationship between prenatal anemia and the risk of some adverse birth outcomes. Beginning in the late 20th century, reports of increased risk of adverse birth outcomes among non-anemic, iron supplemented pregnant women were published.¹⁸⁴ In this study by Koller *et al.*,¹⁸⁴ the authors observed an inverse relationship between birth weight and Hb concentration in the late trimester among 113 non-anemic pregnant women with uncomplicated pregnancies. Following this study, in 1983, Murphy *et al.*¹⁸⁵ published in the *Lancet*, a larger study of over 54 000 singleton pregnancies, in which they observed an increased risk of preterm birth, LBW and perinatal mortality among pregnant women with Hb less than 104 g/L and those with Hb greater than 132 g/L in the first and second trimesters.¹⁸⁵ This U or V- shaped relationship was further reported in a publication by Steer *et al.*¹⁸⁶, in a retrospective study among 153,602 pregnancies, in 1995. (Figures 1.7 and 1.8) In that study, the authors concluded that the Hb range 95-105 g/L was associated with the least incidence of LBW and preterm deliveries.¹⁸⁶

Several recent studies have also reported certain prenatal Hb ranges for optimum birth weight and gestation, and have shown increased risk of adverse birth outcomes for abnormally low and high Hb levels.^{187,188} In fact, a recently published systematic review of observational epidemiological studies on prenatal Hb or anemia and birth weight revealed that, high Hb levels as well as anemia during pregnancy doubled the risk of LBW deliveries.¹⁸⁹ Another recently published systematic review on the benefits of iron supplementation in pregnancy however concludes that, although

iron supplementation in pregnancy improves maternal hematologic indicators, the risk of LBW, preterm birth, neonatal death and congenital anomalies were not significantly lower among women who received daily iron supplements during pregnancy.¹⁹⁰

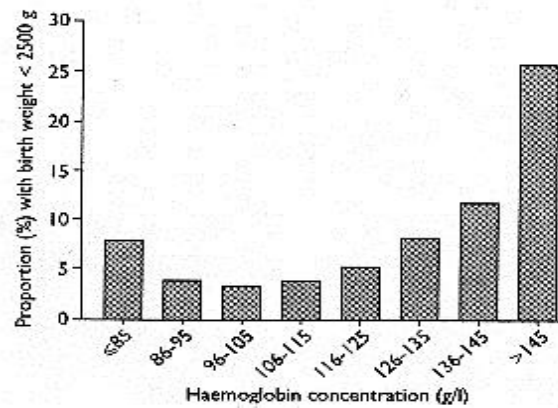


Figure 1.7 Incidence of LBW (<2500 g) by hemoglobin concentration (g/L)

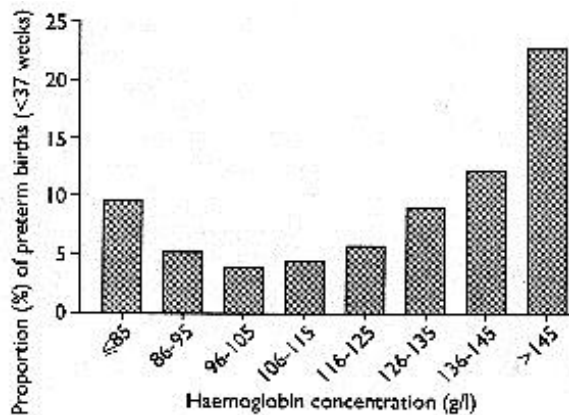


Figure 1.8 Incidence of preterm births (<37 weeks) by hemoglobin concentration (g/L)

Source (Figures 1.7 and 1.8): Steer Philip *et al.* Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. *BMJ*. 1995.¹⁸⁶

Anemia before pregnancy has also been shown to have a significant impact on adverse birth outcomes with the risk of fetal growth restriction, LBW, SGA, and preterm birth increasing with increasing severity of prepregnancy anemia.^{191,192}

1.4.3 Prenatal Anemia on Infant Anemia and Child Development

Since the fetus is solely dependent on maternal nutrient supply for its development, inadequate maternal nutrient availability will impair supply to the fetus and as a result hinder its development. The few studies that have investigated the relationship between maternal anemia and fetal anemia or cord blood hematologic indices have however produced conflicting results. Concretely, a few studies have shown that newborns of anemic mothers have significantly lower cord blood ferritin and Hb levels compared to non-anemic mothers^{193–196}, while other researchers have published contradictory findings indicating there is no relationship between maternal anemia and anemia in infants until 18 months¹⁸⁰.

The fetus however has adaptive mechanisms that permit the uptake of adequate nutrients even at the expense of the mother. First, HbF permits adequate uptake of oxygen by the fetus as it has a greater affinity for oxygenated blood than the maternal Hb. This implies that, even at low maternal Hb levels, the fetus could still scavenge as much oxygen from maternal blood as possible.¹⁹⁷ A similar mechanism exists to ensure adequate iron uptake at the expense of maternal iron stores. Generally, the uptake of iron by the fetus increases as gestational age progresses.¹⁹⁸ Evidence from animal studies indicate that the availability of transferrin receptor sites on the placenta regulates the uptake of iron by the fetus.¹⁹⁹ Thus, as the ferritin levels of the mother decreases, the number of receptor binding sites on the placenta increases and the fetus' supply of iron is prioritized.²⁰⁰ This may explain why there is no difference between mean cord blood ferritin and Hb levels between anemic and non-anemic pregnant women, except when women are severely iron deficient, as shown in some studies.^{32,201}

Although the important role iron plays in the cognitive development of children has been highlighted in a number of studies^{202,203}, not many studies have investigated the impact of anemia in pregnancy on the cognitive development of offspring. Prior to the start of this thesis, only one observational study²⁰⁴ existed that objectively aimed to assess the impact of prenatal anemia on child behavior. The aforementioned study found no relationship between maternal anemia and

infant behavior and was severely flawed by inadequate sample size. In 2013, Chang *et al.*²⁴ published a paper in *Pediatrics* that reported an observed negative impact of anemia on child cognitive and executive functions. The impact of prenatal anemia on the psychological processes of infants has therefore, in recent years, become a topic of interest among researchers and pediatricians.

As the Barker hypothesis states, exposure to prenatal insults during the sensitive periods of fetal development could lead to adaptations by the fetus, thus altering the structure and physiology of the body which could potentially lead to diseases later in life.²⁰⁵ Strong evidence points to the significant roles of stress and unbalanced nutrition on neurodevelopment and poor cognition later on in life.^{206,207} It has been explained that epigenetic regulation of inflammatory pathways presents a plausible mechanisms for changes in brain function resulting from prenatal stress including nutrition.^{206,208}

1.5 Infant Cognitive Development

The psychological processes that play a significant role in the thought, emotional and behavioral characteristics of an infant constitute the infant's cognitive development. Although mostly evident during the postnatal stages of life through the developmental domains of socio-emotional, fine-gross motor and language-cognition, these functional aspects of brain development relies heavily on the structural organization of an infant's brain, which begins in the prenatal phase of development. In fact, the works of Bystron *et al*¹ have shown that the onset of neurogenesis in the forebrain's primordial diencephalon and basal telencephalon could begin as early as the 31st day following conception¹.

During the prenatal stages of life, the fetus undergoes a series of brain developmental events that provide the structural support for brain functioning after birth. Some of these events include neural migration, synaptogenesis, hippocampal development and myelination². The **brain growth spurts** within the first two years after birth and as has been explained in the works of early psychologists and neuroscientists, **about 25% of the adult brain weight is achieved by birth** in normal individuals.²⁰⁹

Although many psychologists admit to the stepwise growth of the human brain²¹⁰, many find the stages more closely to what has been reported by Jean Piaget in the *Piaget Stages of Reasoning Development* involving sensorimotor (birth through 18-24 months), preoperational toddlerhood (18-24 months through 7 years), concrete operational childhood (7 to 12 years) and formal operational adolescence through adulthood (12 years and older).²¹¹ Although extensively explaining the stages of reasoning development, the stages of development as explained by Piaget pay little or no attention to the most vulnerable stages of human brain development, the fetal phase. Contrary however, the works of Dobbing and Sands^{209,212} mostly highlighted the importance of the prenatal phase in the development of the human brain. (Figures 1.9 and 1.10)

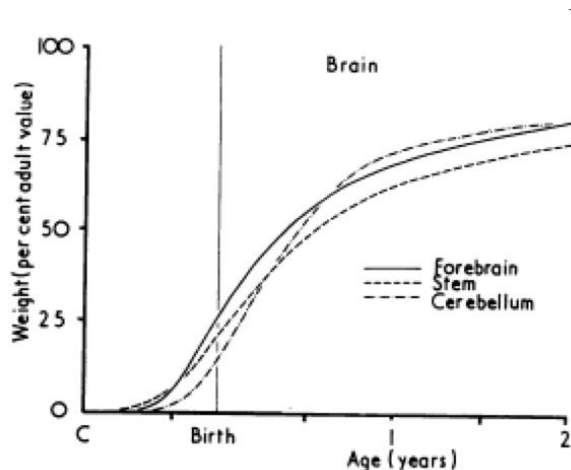


FIG. 10.—Comparative fresh weights of 3 brain regions during growth. Weights shown in Fig. 3 for forebrain, cerebellum, and stem have been calculated as a percentage of adult value, and smooth lines drawn by eye through the points.

Figure 1.9 Comparative fresh weights of three human brain regions

Dobbing and Sands. Quantitative growth and development of human brain. *Arch Dis Child.* 1973²⁰⁹

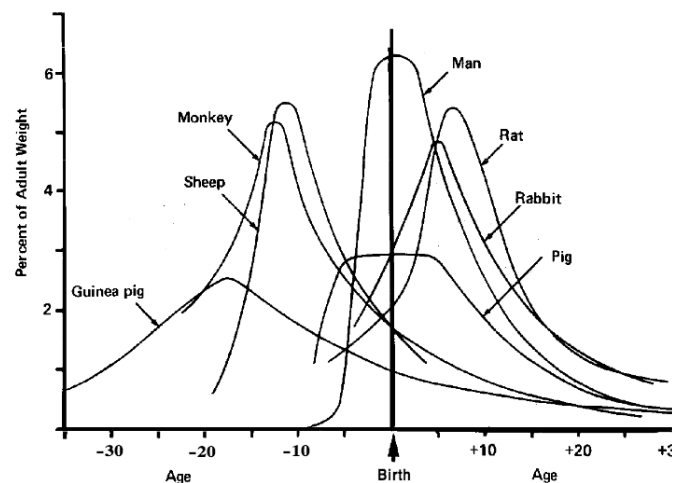


Figure 1.10 Comparative fresh weights of the brains of seven mammals

Source: Dobbing and Sands. Comparative aspects of the brain growth spurt. *Early Hum Dev.* 1979²¹²

Thus, in quantitative terms, the critical stages of human brain development are the prenatal phases of development to the about two years postnatal, by which time brain weight is about 80%

of the adult brain²¹³. The rapid increase in the brain development within the first two years after birth is partly attributable to increasing grey matter (149% increase by age one year)²¹⁴. The rapid development of the human brain thus allows the assessment of brain function through a number of cognitive, attentions, emotional or behavioral assessment tools.

Several assessment tools have been designed to capture different aspects of brain function of infants to be able to determine if some children need special attention or not. Tests including the Bayley Scales of Infant and Toddler Development (Bayley III, 1 - 42 months)²¹⁵, the Fegan Test of Infant Intelligence (FT III, 27 weeks - 12 months)²¹⁶, Griffiths Mental Development (1 - 96 months)²¹⁷ and the MSEL (1 - 68 months)²⁶ have been used in various studies to assess several domains of brain function. During the early stages of child development, the motor functions of an infant is essential in differentiating between poor and normal brain function and as a result, in most of these assessment tools, the motor function scale is separated from the other scales (even if the other scales are combined to form a composite) for infants less than 18 months. With the exception of the MSEL, most of the other cognitive assessment batteries separate the scores of the different scales and thus do not provide a single scale that encompasses the various scales to reflect the cognitive function of the child.

Since infant brain develops actively after birth, the environment he or she grows in, including the socio-economic environment, also influences the development of a child's cognition^{218,219}. Infants who score low on cognitive assessment test are thus likely to have had impaired or delayed brain development, which may have arisen during the prenatal phases or the early postnatal phases.

Factors that could account for low infant cognition include:

- Genetic disorders and adverse birth outcomes (such as Williams Syndrome, Fragile X syndrome X, preterm birth, etc.)
- Environmental toxicity (lead, mercury, organic pollutants, electromagnetic frequencies)
- Infectious diseases (HIV infection, malaria)
- Poor nutrition (nutrient deficiencies, including ID)

Although of interest to this thesis are the latter two, which are also associated with poor socioeconomic status, some mention will be given the first two factors. In depth impact on the role of birth outcomes, anemia and its risk factors on infant cognitive development have been mentioned in the previous sub-sections of this chapter.

The rare inheritance of altered genes could have significant impact on the cognition of infants born with such syndromes. The Williams syndrome, for example, is a rare genetic aberration that is caused by a micro deletion of approximately 26 genes on chromosome 7q11.23.²²⁰ Children with Williams syndrome have impaired expressive language, poor cognition and behavior.²²¹

Also, exposure to heavy metals and chemical products such as pesticides has been reported to have adverse consequences on brain development and child intelligence and behavior. In a study of 1 to 3-year-old children, children who had blood lead levels of more than 10 µg/dl had 6.2 lower scores in Mental Development Index of the Bayley's Scale of Infant Development (second edition), compared to children with blood lead levels between 0 and 9.9 µg/dl.²²² Although the CDC's acceptable threshold for blood lead levels remains at 10 µg/dl, it is recommended that public health actions be initiated for children between 0 and 5 years who have more than 5 µg/dl blood lead level.²²³ Childhood cognitive delay has also been reported for children who are exposed to high levels of mercury even after adjusting for consumption of fish.²²⁴ Evidence of prenatal exposure to levels of mercury also show poor negative consequences on cognitive function three years after birth among children of highly exposed pregnant women (top decile) compared to those of mothers below the top decile of mercury.²²⁵ Exposure to different organic pesticides, mostly the organophosphates, organochlorines, pyrethroids, and carbamates families have also been associated poor neurocognitive development in children.²²⁶ Another exposure that has raised attention on its possible link with poor infant neurocognitive development is the exposure to electromagnetic frequencies, particularly from mobile phone use.²²⁷

Evidence of the impact ID in infancy on the cognitive development has been extensively studied in the independent and sometimes collective works of Lozoff²²⁸⁻²³⁰, Georgieff^{22,231,232} and Beard^{233,234} although no causal inference of ID on cognitive development has been established. Notwithstanding the extensive work by these researchers, the literature on the role of ID or anemia on the cognitive development of infants remains very scanty. Further, with the exception

of HIV infection, prenatal infectious diseases have been rarely studied in relation to the cognitive functions of children. Findings of this thesis in relation to the impact of prenatal ID on infant cognitive development are mentioned in Chapter 5.

Chapter 2. Materials and Methods

My thesis was set to address the questions posed by a study (called TOVI) conducted in Benin to determine the impact of anemia during pregnancy on the cognitive development of children. Considering that the thesis draws part of its data from other previous studies, which followed a group of pregnant women and their offspring from the first ANC visit till their offspring were one year old, I will use the early part of this chapter to present these other studies before explaining the implementation of the TOVI study.

The Materials and Methods chapter therefore presents the sources of data required for the thesis, as well as the study designs and methods of each individual study. Specific methods pertaining to addressing specific objectives of the thesis (particularly, statistical analysis) are detailed in chapters 4, 5 and 6 and included in the various published articles included in this thesis.

The objectives of this section are therefore to:

- a. explain the research problem and implementation of each of the studies involved, including the study designs, procedure of enrolment, and methods of assessments adopted in data collection,
- b. present the study setting, population and data sources for pregnant women and children,
- c. outline the follow-up of pregnant women and children until age one year,
- d. describe the method of assessment of neurocognitive function of one-year children,
- e. present other postnatal assessments performed for the mothers and children.

2.1 Data Sources

Biological, sociodemographic and anthropometric data on pregnant women from their first ANC visit until delivery were obtained from the *Malaria in Pregnancy Preventive Alternative Drugs* (MiPPAD) clinical trial and a parallel cohort study, called *Anaemia in Pregnancy: Aetiologies and Consequences* (APEC), that followed the first 1005 women enrolled in the MiPPAD clinical trial. These two data sources on pregnant women also provided some biological and anthropometric data on children as the MiPPAD study further followed children born to the women in the clinical trial at 1, 9 and 12 months.

The TOVI study (Fon language of Benin: *Tovi* means child from the country), from which my thesis is based, was a cross sectional study that assessed the cognitive functions of one-year-old singleton children born to the pregnant women who were enrolled in MiPPAD/APEC. The TOVI study also assessed several postnatal factors that were essential to understanding the impact of prenatal anemia and its risk factors on infant cognitive development.

2.2 The MiPPAD Clinical Trial

Research Problem and Implementation

Malaria, although preventable, accounts for over half a million deaths annually.²³⁵ Most of the deaths occur among children and pregnant women, the groups most at risk of malaria infection. The deaths attributable to malaria occur in sub-Saharan Africa where an estimated 50 million pregnancies occur every year.¹⁴⁹ In addition to maternal mortality, malaria in pregnancy (MiP) is also a risk factor for maternal morbidity and several adverse birth outcomes including LBW deliveries.²³⁶ These problems caused by malaria makes their prevention and control during pregnancy a very crucial topic.

Recent efforts to prevent MiP have been successful mainly due to the availability of intermittent preventive treatment of malaria in pregnancy (IPTp), specifically sulfadoxine-pyrimethamine (SP) and the availability of LLITNs.¹⁴⁹ At the onset of the MiPPAD trial, the WHO recommended the administration of a minimum of two doses of IPTp-SP to pregnant women in their second trimester with at least one month interval between the two doses, to prevent

malaria.²³⁷ Now, the minimum two doses are no longer recommended. The WHO recommends IPTp at each ANC visit in endemic regions, with a schedule of four visits.²³⁸ IPTp-SP is contraindicated during the 1st trimester of pregnancy with potential teratogenic effects. Further, growing concerns about the possibility of resistance to IPTp-SP has resulted in the search of an alternative candidate IPTp. Mefloquine (MQ) was chosen as the potential candidate since it met the attributes required for an IPTp.^{239,240} Furthermore, the only two existing clinical trials of MQ in Africa also showed promising results.^{241,242} The MiPPAD clinical trial was therefore set up to assess the efficacy and safety of MQ as a candidate IPTp in comparison to the existing IPTp-SP.

MiPPAD was a multi-country clinical trial that was enrolled in five different countries in sub-Saharan Africa with the aim of contributing to the improvement of clinical interventions to the fight against malaria in pregnancy. Actually, there were 2 distinct MiPPAD trials: one in HIV-negative women (Benin, Gabon, Mozambique, Tanzania) and one in HIV-infected women (Mozambique, Kenya, Tanzania), with the same ClinicalTrials.gov ID number. The trial that enrolled non-HIV pregnant women was only in 4 countries (Kenya was not included) (PACTR 2010020001429343). Countries where MiPPAD was implemented are shown in Figure 2.1.

This clinical trial was funded by the European Developing Countries Clinical Trials Partnership (EDCTP; IP.2007.31080.002), the Malaria in Pregnancy Consortium and the following national agencies: Instituto de Salud Carlos III (PI08/0564), Spain; Federal Ministry of Education and Research (BMBF FKZ: da01KA0803), Germany; Institut de Recherche pour le Développement (IRD), France. CANTAM provided infrastructure help in the study.²⁴³ The MiPPAD clinical trials in all five countries are registered with ClinicalTrials.gov under number NCT 00811421 and also with the Pan African Clinical Trials Registry under numbers PACTR 2010020001813440 (HIV-positive pregnant women) and PACTR 2010020001429343.²⁴⁴

The MiPPAD study was an open-labeled randomized clinical trial with three arms of treatment. The three arms of IPTp were IPTp-SP, IPTp with full dose mefloquine (15 mg/kg) and IPTp with split dose of mefloquine (total of 15mg/kg) administered over 2 days. The random allocation of pregnant women to treatment arms was performed in 1:1:1 ratio throughout all the five countries.

For the purpose of this thesis, only data from the MiPPAD clinical trial at the Benin study site were used. Detailed information on study site and data collection is explained in the subsequent pages.



Figure 2.1 The four countries where the MiPPAD clinical trial was initiated (Study site of interest to this thesis in yellow highlight)

2.3 The APEC Study

Research problem and implementation

Although extensive work has been done on anemia during pregnancy, very little is still known on the contribution on the etiologies of anemia in pregnancy to the development of the disease, especially in malaria endemic regions.

Following the same pregnant women enrolled in the MiPPAD clinical trial, the APEC study was set up to investigate the etiological factors of anemia in pregnancy and the consequences of anemia in pregnant women and children in the context of a malaria endemic community.

The APEC study was a prospective cohort study that ran parallel to the MiPPAD clinical trial in Benin that enrolled pregnant women between January 2010 and May 2011. APEC was in fact an ancillary study of the MiPPAD study that performed several other assessments that were not planned for in the MiPPAD clinical trial. The assessment of the physical and clinical characteristics of the pregnant women in both the MiPPAD and APEC studies are the same except for the assessment of vitamin B12 and folic acid from venous blood, and helminths from the examination of stool samples which were strictly performed in the APEC study. Further explanation of the study procedures will follow that of the MiPPAD study clinical trial, as it was the principal study.

2.4 MiPPAD/APEC Study Procedures

2.4.1 Study Site and Population

The study was conducted in Allada, a semirural district that is located about 50 km north of Cotonou, Benin. The Allada district is made up of 84 villages with a total of 91,788 inhabitants as of 2002.²⁴⁵ Malaria in Allada is perennial with two transmission peaks in April to July and October to November.²⁴⁶ Figure 2.2 shows the location of the study site, Allada, on the map of Benin.

All pregnant women who were attending their first ANC visit in three maternity clinics in the three sub districts of Allada (Allada, Attogon and Sékou) were requested to participate in the clinical trial. Of these, only the first 1005 pregnant women were included in the APEC study and in the analysis in this thesis. When the women had consented to be part of the study, they were recruited into the study if they passed the eligibility criteria. Inclusion criteria for recruitment into the clinical trial included being less than 29 weeks pregnant at first ANC visit, HIV negative, having signed informed consent, being a permanent resident in Allada and agreeing to deliver in

one of the three maternity clinics. Women were excluded from the studies if their gestational age was 29 weeks or more, if they were HIV positive or had taken IPTp, anthelmintic therapy or iron or folic acid or vitamin B12 supplements prior to their first ANC visit. Pregnant women who were participating in a different study or had known history of allergy to sulfa drugs or mefloquine were also excluded from the study.



Figure 2.2 Study site in Benin

Source : <http://www.ias-ch.org/siteias/static/Carre-Benin.GIF>

2.4.2 Anthropometric and Sociodemographic Data during Pregnancy

Women who were enrolled in the clinical trial were examined at two ANC visits of at least one month interval and at delivery. Anthropometric and sociodemographic data of pregnant women were collected at the first ANC visit when they were included in the study. Sociodemographic data collected at the first ANC visit includes age, gravidity, marital status, occupation, area of residence and level of education.

Maternal height, weight, mid upper arm circumference and gestational age were also clinically determined. Maternal weight and height were measured using an electronic scale (to the nearest 0.1 kg; Seca Corp., Hanover, MD) and a body meter device (to the nearest 0.1 cm; Seca 206 Bodymeter; Seca Corp).

Maternal prepregnancy body mass index (BMI) was calculated using the weight (kilograms), height (meters) and gestational age at recruitment. During pregnancy, it is estimated that pregnant women gain 1kg per month from the end of the first trimester until delivery.²⁴⁷ Hence, using the gestational age of the pregnant women at recruitment, their weight prior to pregnancy was roughly estimated by subtracting the expected weight gain during pregnancy. Table 2.1 shows how prepregnancy BMI was estimated depending on the gestational age at recruitment.

Gestational age was determined by measuring the fundal height.

Table 2.1 Estimation of prepregnancy body mass index (BMI)*

Gestational age (weeks)	Prepregnancy weight (kg)
8-12	W_i
13-16	W_{i-1}
17-20	W_{i-2}
21-24	W_{i-3}
24-28	W_{i-4}

*Prepregnancy BMI (kg/m²) = prepregnancy weight (kg)/height (m) X height (m); W_i is the weight at recruitment

Source: Adapted from Ouédraogo *et al.* Maternal anemia at first antenatal visit: prevalence and risk factors in a malaria-endemic area in Benin. AJTMH, 2012.¹⁴

2.4.3 Biological Assessments

Apart from information on previous pregnancies and history of known chronic illness that were recorded at first ANC visit, blood and stool samples were also collected for several biological examinations. The biological examinations were repeated at the second ANC visit and at delivery. Of the 4 ml of venous blood taken from each pregnant woman at each of the three visits, 2 ml were introduced into an iron-free dry tube and the other 2 ml was put into a tube containing potassium EDTA. Serum was separated by centrifugation and analyzed.

Each pregnant woman was given a container to collect stool samples from the next morning after the ANC visits. Since it was difficult to retrieve stool samples when women were in labor, where possible, stool samples were collected 15 days prior to the expected date of delivery. In the event that the pregnant woman gave birth before 15 days of the expected delivery date, stool samples were taken within a week after delivery. The containers were then retrieved from pregnant women within the first six hours after defecation for examination for the presence of the eggs of intestinal helminths in stools.

The section that follows presents the specific biological assessments and interventions that followed after blood and stool samples were taken at each ANC visit and at delivery.

Helminth assessment

After stool samples were retrieved soon after defecation, smears of retrieved stool samples were immediately prepared and assessed using the Kato-Katz concentration technique²⁴⁸ (Vestergaard Frandsen, Lausanne, Switzerland). The Kato-Katz technique was chosen because it provides a quantitative estimate of the intensity of helminth infection. The Kato-Katz technique was used to identify the eggs of *A. lumbricoides*, *T. trichiura*, *N. americanus* and *A. duodenale*. Since it is hard to distinguish between the eggs of *N. americanus* and *A. duodenale*, they were generally defined as hookworms.

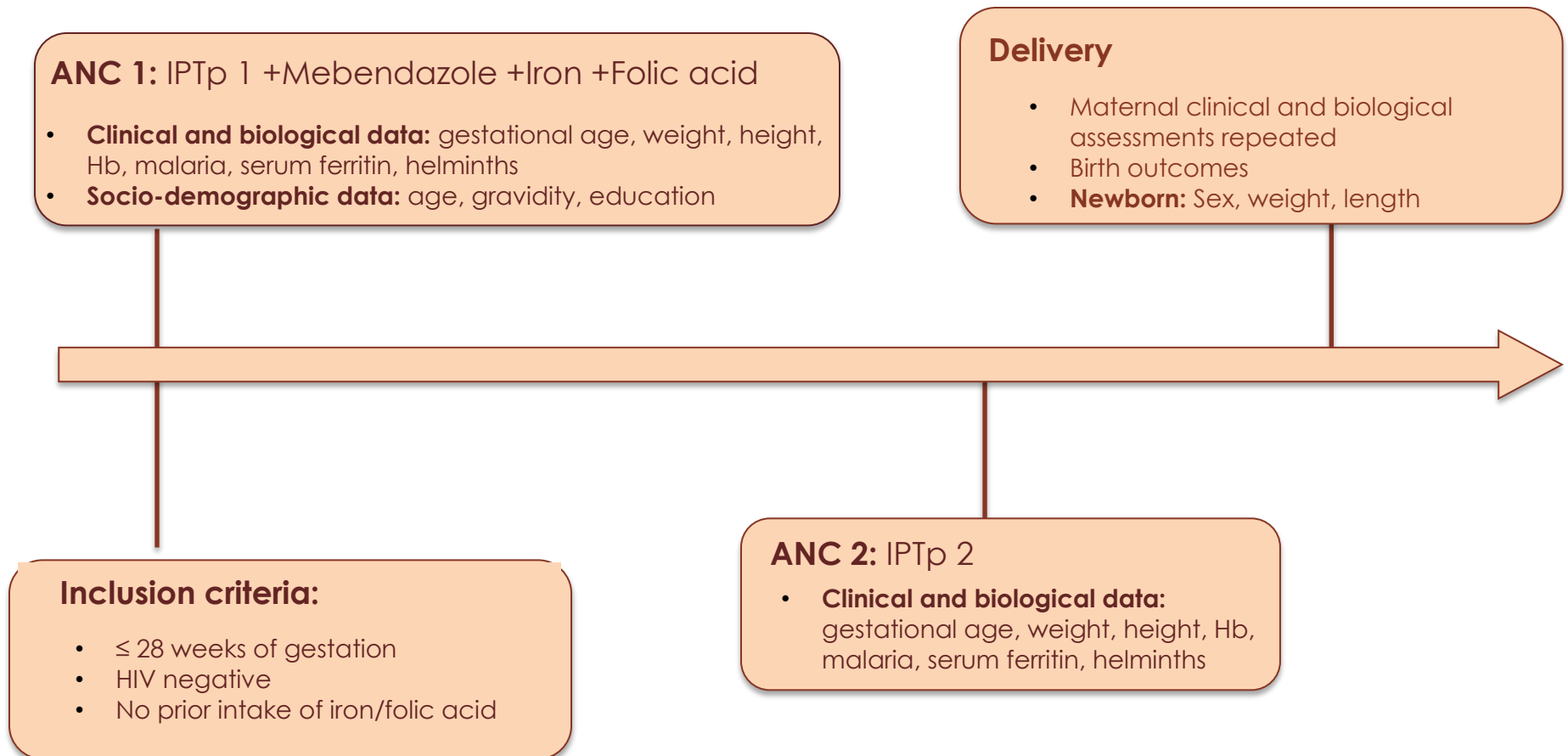


Figure 2.3 Flowchart of data collected during pregnant women in the MiPPAD trial (Credit: Ouédraogo S., 2013)

Because fecal samples had to be processed soon after obtaining them, no external controls were set. Instead, two laboratory technicians systematically and independently examined the slides of fecal smears under a microscope. The number of eggs in each individual stool sample were counted and recorded. The number of eggs counted for each species of helminth was then multiplied by twenty-four to obtain the standardized fecal egg counts (FEC), in eggs per gram of stool (epg).

An individual was said to be infected with intestinal helminths if the FEC was at least 24 epg of any species.

Malaria assessment

Malaria infection was determined by using the Lambaréné technique (detection threshold of 5 parasites/ μ L). Briefly, 10 μ L of blood was smeared on a 1.8 cm X 1 cm rectangular area of a slide then stained with Giemsa. The stained parasites were read with an oil immersion lens at a magnification of 1000 X. To obtain the number of parasites per microliter of blood, a multiplication factor was applied to the average parasitemia/field.

Hemoglobin assessment

The Hemo-Control photometer (EKF Diagnostics, Barleben/Magdeburg, Germany) device was used to determine the concentration of Hb in 10 μ L of venous blood taken from each pregnant woman. Quality control was ensured by daily calibration of the Hemo-Control photometer device by laboratory technicians and by crosschecking the Hb of one in ten consecutive samples at the Allada Central Hospital laboratory using a hematology analyzer (Erma Laboratory, Japan).

Hb concentration was recorded in g/L. Hb concentration less than 110 g/L was considered anemia in pregnant women.

Alkaline electrophoresis on cellulose acetate (Helena Laboratories, Beaumont, TX) was used to determine Hb genotypes. Fifty microliters of each participant's blood was diluted in a hemolysate reagent at a ratio of 1:3. A portion of the blood-hemolysate mixture and a control hemolysate were placed in sample wells and electrophoresis was run.

Iron, folic acid, vitamin B12 and C-reactive protein assessment

Body iron stores were measured by determining serum ferritin concentration. From each participant's centrifuged blood, 500µL of serum was obtained and used to measure folic acid and vitamin B12 by an AxSym Immuno-Assay Analyzer (Abbot Laboratories, Abbot Park, IL).

Participants were identified to be folic acid deficient when the serum ferritin concentrations were less than 6ng/mL. Vitamin B12 deficiency was defined as having vitamin B12 concentrations less than 150 pg/mL.

A rapid test (CRP Latex; Cypres Diagnostics Inc., Campbellville, Ontario, Canada) was used to measure the concentrations of CRP. Participants were said to have inflammations if the CRP concentration was less than 5mg/L. ID was defined as serum ferritin concentrations less than 12µg/L or serum ferritin concentrations less than 70µg/L if the participant had an inflammation. IDA was defined as being iron deficient and anemic.

2.4.4 Interventions during Pregnancy

In accordance with the national guidelines for women attending their first ANC visit, all pregnant women in their second trimester were systematically administered a total of 600 mg of mebendazole (100 mg twice daily for three days) irrespective of helminth infection status. The administration of mebendazole was repeated only for pregnant women found to be infected with helminths at the second ANC visit or at delivery.

As part of the ANC package in Benin for pregnant women, all pregnant women were given daily oral ferrous sulfate (200 mg per day) and folic acid (5 mg per day) supplements to be taken at home. Further, those with Hb concentration between 70 g/L and 110 g/L were treated with the administration of 400 mg of oral ferrous sulfate (200 mg twice daily). When Hb concentrations fell below 70 g/L, pregnant women were treated locally or referred to the tertiary hospital of the district. In addition, women were encouraged to eat more iron rich meals such as green vegetables, beef and eggs.

Since enrolled women belong to either IPTp treatment arm [SP or MQ (full or split dose)], all women received two doses (three for IPTp mefloquine-split dose) of antimalarial preventive treatment within a minimum one-month interval. Women who belonged to the sulfadoxine-

2.4.5 Follow-up of Pregnant Women

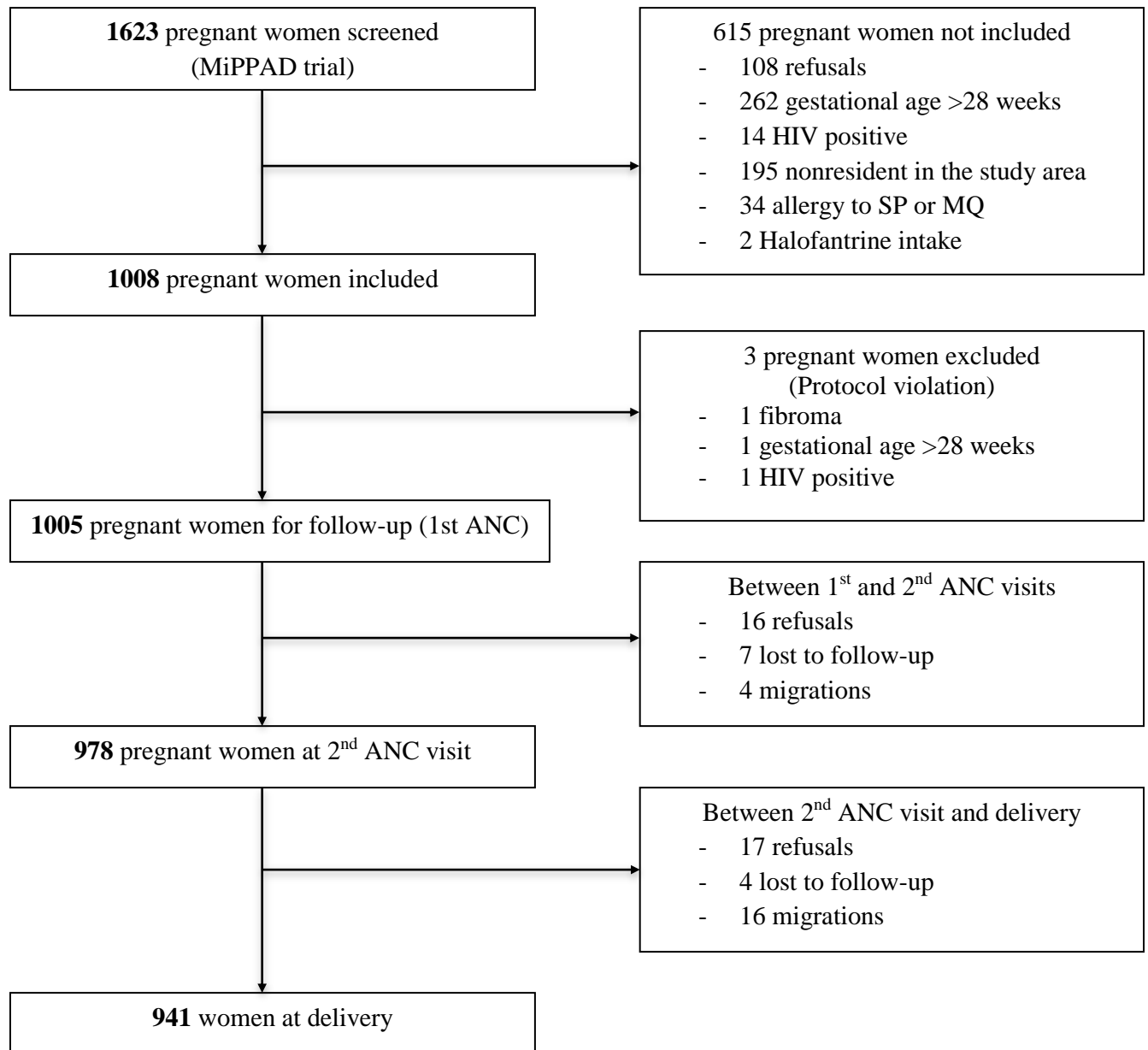


Figure 2.4 Follow-up of pregnant women during the MiPPAD study (Credit: Moya-Alvarez V., 2015)

pyrimethamine arm received standard IPTp (tablets of fixed combination therapy of 500 mg sulfadoxine and 25 mg pyrimethamine, Malastop, Sterop). Women who did not belong to the sulfadoxine-pyrimethamine arm were given 15 mg/kg of mefloquine (tablets of 250 mg of mefloquine, Lariam, Roche). In addition, all women in the clinical trial were administered long-lasting insecticide treated nets (LLITNs - PermaNet, Vestergaard Fransen) at recruitment.

2.4.6 Follow-up of Children

Of the 1005 women enrolled, 941 were followed until delivery. The last delivery among the enrolled women occurred in June 2012. In all, 863 live born singleton children born to pregnant women were followed from birth until age one year. Specifically, children were followed at 1, 9 and 12 months where physical and biological assessments were taken. The follow-up of children until age one year was completed in June 2013.

2.4.7 Physical Examination of Children

At birth, neonate's anthropometric measures were taken. Newborns weight and recumbent length were measured using digital calibrated scales and a calibrated length board, respectively within the first week of birth. Head circumference and infant sex were also recorded at birth. Gestational age at birth was evaluated using the Ballard score.²⁴⁹

At 1, 9 and 12 months children's weight (in grams), length at incumbent position (in centimeters), middle upper arm circumference (MUAC, in centimeters) and head circumference (in centimeters) were measured and recorded.

2.4.8 Biological Assessments of Children

At birth cord blood was assessed for plasmodium parasitemia. Hemoglobin concentration in cord blood was also determined. Plasmodium parasitemia and hemoglobin concentration in venous blood were also assessed at 1, 9 and 12 months during scheduled maternity visits to the clinic. Stool samples of a section of children (N=186) were assessed for helminth infection at any of the three follow-up periods. Techniques used in biological assessments did not differ from that used in maternal biological assessments during pregnancy which have been highlighted in the early part of this chapter.

2.5 The TOVI study

2.5.1 Research Problem and Implementation

The high prevalence of anemia among pregnant women, its multiple etiologies and consequences on maternal and child health makes anemia in pregnancy a severe public health problem especially in sub-Saharan Africa. Knowledge on the consequences of prenatal anemia infant cognitive development is however limited.

Following the results of the APEC study that showed a substantially high prevalence of anemia in pregnant women in the Allada region and the justification of the objectives of this thesis explained in the earlier chapters, the TOVI study was designed to assess the impact of anemia in pregnancy on the cognitive function of these children at age one year. My thesis was set up to respond to research questions of the TOVI study.

The TOVI study was a cross sectional study that assessed the cognitive function of one-year-old children whose mothers were enrolled in the APEC/MIPPAD studies. TOVI project began in June 2010 and ended in June 2013. The TOVI study was funded by the NIH (National Institutes of Health; NICHD: R21-HD060524 Project). Dr. Florence Bodeau-Livinec was the principal investigator (PI) for this project. Dr. Ghislain Koura coordinated the earlier part of the study including the recruitment of field personnel.

The TOVI team consisted of 10 key personnel (1 psychologist and field coordinator, 4 research nurses, 2 technicians of laboratories, 1 administrative assistant and 2 support staff) who assisted in training, administration of cognitive tests, laboratory assessments and data entry. I joined the TOVI team after the departure of Dr. Koura following the completion of his PhD. When I commenced my PhD, I continued the coordination of the TOVI project mainly through emails with field personnel and weekly meetings with the PI. During this period I checked the validity of the double data entered.

Four months following the start of my PhD, I went to the field to coordinate the completion of child cognitive assessments among other assessments that were carried out within the TOVI project. My mission between February and April 2013, funded by the NIH grant, was also to

ensure that accurate data entry were being conducted and to speed the data collection process so that the June 2013 deadline of the TOVI study could be met. Furthermore, while on the field, I prepared database questionnaires to capture some data in the MiPPAD study that had been omitted in previous data entries.

During this period of intense field activity, I observed the assessment of cognitive function of some of the children in the study and followed the research nurses during the home visits of study participants. I participated in weekly personnel meetings, usually held at the end of the day on Fridays, and addressed personnel problem when I had the capacity to. I submitted weekly reports on my activities to Dr. Florence Bodeau-Livinec and Dr. Michel Cot and discussed the solution to problems we encountered on the field.

I gained field experience from my presence in Benin and also understood the complexities involved in following up participants in this area. When I returned from the field, I continued coordination through emails and telephone conversations and also continued with checking the validity of the data entered. Data entry was completed in June 2013.

2.5.2 Study Population and Inclusion Criteria

The study consisted of one-year-old children who were born to the first 1005 pregnant women recruited into the MiPPAD clinical trial (specifically, of the pregnant women who were followed until delivery). Detailed inclusion and exclusion criteria of the clinical trial have been discussed earlier in this chapter. All singleton children who were alive at age one year old were eligible to be included in the TOVI study.

2.5.3 Assessments during the TOVI Study

This section describes the assessments that were made during the TOVI study with specific focus on the assessments that are essential to this thesis. Categorically, I will explain:

- how the cognitive and motor functions of children were assessed by using the MSEL,
- the assessment of symptoms of maternal depression after delivery using the Edinburgh Postnatal Depression Scale (EPDS),

- maternal non-verbal intelligent quotient (IQ) assessment using the Raven's Progressive Matrices (RPM),
- the Home Observatory Measurement of the Environment (HOME) inventory that was used to assess the quality of the home environment including parent-child interactions.

These four different assessments were carried out when the child was one-year-old. The cognitive assessment of children using the American Guidance Service (AGS) edition of MSEL²⁶ was performed during their 12 month-scheduled maternity visit. This version was translated and adapted for use in this particular setting. Home visits of mothers were scheduled, based on their availability within three days after cognitive assessments, for the other assessments. Assessments made during home visits were administered by a research nurse different from the one who had earlier administered the MSEL assessments during the maternity visit. During the home visits, additional socioeconomic and nutritional data were also taken to assess family possession or wealth.

Aside the above four assessments, the TOVI study also assessed infant blood lead levels at age one year. Whole blood samples collected from one-year-old children were assessed for lead level at the University of Laval in Canada. Blood lead levels were measured using inductively coupled plasma mass spectrometry. The plasma mass spectrometry detects metals at a very low concentration (one part per trillion).

2.5.4 Detailed Description of Assessment Tools used in the TOVI Study

Mullen Scales of Early Learning

The MSEL²⁶ is an untimed and individually administered diagnostic battery that is designed to measure the cognitive and motor functions of children from birth through 68 months. Only trained professionals are permitted to administer the MSEL tasks.²⁶

The MSEL consists of five scales: Gross Motor (GM) scale, Fine Motor (FM) scale, Receptive Language (RL) scale, Expressive Language (EL) scale and Visual Perception (VP) scale. Each of the scales is made of at least thirty tasks. Because basic mobility and control such as throwing and standing play a significant role in the developmental learning abilities of children before 3

years of age, the MSEL's gross motor scale is designed for children 1 month through 33 months-old. The other four scales are administered for children from birth to 68 months. Since the children in our study were assessed for cognitive function at age one year, all the five scales of MSEL were administered.

The GM scale of the MSEL measures the balance and mobility of one-year-old children using tasks such as the ability to shift and reach for objects in prone position (lying down on the stomach), sitting with arms free and standing with hands held. The FM scale measures the fine coordination of movement involving the use of fingers and demonstrates the expression of visual organization. The FM scale for one-year-old children includes tasks of visual discrimination and motor control such as picking up a cube and displacing it from one point to the other. The RL scale measures the child's ability to respond to speech and sources of sound. The tasks involve understanding familiar language and auditory-spatial stimulus such as recognition of the mention of his/her name and the names or titles of other close family members and turning head to the direction of sound. The EL scale measures the voluntary ability to use speech appropriately and the use of gestures associated with of auditory sounds. The EL scale is indicative of a child's ability to express ideas using speech communication. The tasks of the VP scale for one-year-old children starts with the search for fallen objects by the child. The VP scale reveals the visual processing skills and spatial organization of the child. The tasks of each of the five scales are ordered to suit the developmental milestones for each age group. The starting task for each scale depends on the age of the child.

Prior to the TOVI study, the MSEL had never been used as cognitive assessment tool in Benin and it only existed in English. As part of the TOVI study, the MSEL was translated and adapted for the study setting in Benin. The translation and validation of the MSEL to be used in Benin was achieved in four steps:

- a. A psychologist translated the original version of the MSEL into French.
- b. Another psychologist then independently back translated the French version of the MSEL into English.

- c. The co-investigator and pediatric epidemiologist, Leslie Davidson, performed a comparison between the original MSEL and the back-translated English version for errors in the back-translated version that may have arisen from the French version.
- d. Another pediatric epidemiologist, Florence Bodeau-Livinec, completed the final corrections of the final translated French version of the MSEL.

After these four steps, the final French version of the MSEL was submitted to Pearson clinical Assessments (holder of the copyright for the MSEL) to be validated.

Using the validated French version of the MSEL²⁵⁰, with the instructions translated to Fon, the local language in the study site, trained research nurses administered the MSEL tasks to one-year-old children during their visit at the maternity clinic. During the administration of the tasks, unless otherwise requested by the administrator, the child sat on the lap of the mother or guardian who was in a seated position in a chair. The research scored the performances of the children on all five MSEL scales.

The raw scores obtained by each child for each MSEL scale were transformed into normalized scores called the *T*-scores by using a standardized table with the child's chronological age at assessment. *T*-scores of the FM, EL, RL and VP were combined to form the ELC score. The ELC score is representative of the average child performance on the four scales. The ELC score is indicative of the cognitive function of the child.

A psychologist, Michael Boivin, who is specialized in child development in sub Saharan Africa, trained research nurses who administered the MSEL to the children for two weeks. After the theoretical training, the research nurses practiced their training on fifteen children under the supervision of the psychologist and the PI for TOVI. Thirty-two children were followed afterwards in the following month in a pilot study after the MSEL was reviewed.

During and after the two-week training, the PI (Florence Bodeau-Livinec), the psychologist (Michael Boivin), a Beninese doctoral student and physician (Ghislain Koura), a Beninese pediatrician (Maroufou Alao) and the research nurses reviewed the administration of the tests. Two changes were made to the tasks to make it adaptable to the study setting. The following changes were made:

- Instead of cheerio, a potato chip was used in order to make use of existing local materials.
- If a child handed the ball to his or her mother instead of throwing the ball underhand, he or she still scored a point.

The research nurses and F. Bodeau-Livinec, two months after the commencement of the TOVI study, conducted the first quality assessment of the administration of the MSEL tests. During the quality assessment, research nurses were retrained on the administration of MSEL. Weekly meetings were conducted between the field coordinator and the other research nurses to review the progress of the study. Where necessary, difficulties and challenges in administering the MSEL were discussed with F. Bodeau-Livinec and M. Boivin.

Inter assessor reliability performed on a small number of children assessed for cognitive function revealed a strong reliability of assessors using the research nurse with most children assessed as the gold standard. Correlation scores between the gold standard research nurse and the other assessors ranged from 96% to 100% with a mean correlation score of 98%.

Edinburgh Postnatal Depression Scale (EPDS)

The EPDS is a 10 item self-rating screening questionnaire that was designed in 1987²⁸ to identify women with postnatal depression. The 10 items in the EPDS are developed to reveal symptoms of clinical depression such as sleep disturbance, guilt and suicidal thoughts among mothers in the year following the birth of their children. The response to each item is scored from 0 to 3 in ascending order of severity. Hence the possible sum of scores ranges from 0 to 30. Total EPDS score greater than 10 indicates possible postnatal depression.²⁸

The EPDS has been adapted and validated in several languages including French.²⁵¹ In the TOVI study, during the administration of the EPDS, research nurses translated the French version into Fon. A different research nurse did the administration of the EPDS within three days after the administration of the MSEL test.

Raven's Progressive Matrices (RPM)

The RPM is a non-verbal test that was developed to measure the IQ of an individual. In our study, we used it to measure the non-verbal IQ of mothers, one year after delivery. Each of the 60

items of the RPM is made up of a number of items with one missing part. The role of the mother was to identify from 6 possible options, the correct missing piece. Each item is scored differently. In the TOVI study, women responded to items orderly until they had six consecutive wrong answers. The scores obtained per item were summed to produce the RPM score. Like the EPDS, maternal non-verbal IQ using the RPM was assessed during the home visit.

Home Observatory Measurement of the Environment (HOME) Inventory

The HOME inventory was developed by Caldwell and Bradley²⁷ to assess the degree of support, stimulation and learning opportunities available for a child in his or her home environment. It focuses on the child in his or her environment and as a recipient of items and events occurring in the family surroundings. The HOME inventory uses a set of items that generally capture parental education and the interaction between the mother and the child. The items of the HOME inventory are clustered into 6 categories, namely; Parental Responsivity, Acceptance of Child, Organization of the Environment, Learning Materials, Parental Involvement, and Variety in Experience²⁷.

The HOME inventory was adapted in French for use in Allada, Benin. The adaptation and training of research nurses to administer the HOME was achieved in the following steps:

- Formal training of was given to research nurses in a classroom setting.
- Pilot study was conducted in the field.
- Items of the HOME inventory that were not usable in Allada were adapted following the pilot study.
- General review with the TOVI team on the field was conducted when needed.

After the training and piloting, changes were made to six items in the HOME inventory to suit the study setting. For example, it was discovered that only one family owned a toy after 10 home visits. The Learning Material category was therefore reduced to 4 items. Two items relating to books were changed to: “At least 2 books are present or can be seen” and “The child owns one or more books”. One item concerning how long the child spends outdoors was removed as the children in this study setting spend most of the day outside.

In addition to the four assessments that were taken during the TOVI study, mothers were also asked to respond to a questionnaire assessing family wealth or possessions. A score was generated for family possessions, which was the sum of individual scores given to specific items owned by the family of the pregnant woman thus whether the home had electricity or car (each scoring 3), a motorcycle or television (each scoring 2) and a radio, bicycle, motorcycle, or cattle (each scoring 1). Consideration given the score of amenities possessed by the family depended on its value within this community.

2.6 Statistical Packages

In part of the analysis presented in this thesis (but not in the published articles), the ELC and GM scores were categorized to represent poor function (less than mean score) and normal function (mean score or more). We also used the mean minus SD to represent poor function in other exploratory analysis. Where either of the cut-offs was used, it was clearly labeled to avoid confusion.

Generally, double data entry was performed using EpiData 3.1(Denmark) and verified for coherence. When incoherent, the originally filled questionnaire (hardcopy) was obtained for the correct information. Statistical analyses were conducted using Stata IC/11.2 for Windows (StataCorp Lp, College station, TX) in the early stages of the thesis and Stata IC/14.1 for Mac (StataCorp Lp, College station, TX) in later analysis. Unless otherwise indicated in a particular manuscript or article, statistical significance was defined as p-value less than 0.05.

2.7 Ethical Consideration

The Ethics Committee of the Hospital Clinic of Barcelona in Spain, the Research Institute for Development's (IRD) Consultative Ethics Committee in France, and the regulatory authorities and the National Ethics Review Committee of Benin approved the MiPPAD study protocol and informed consent. Prior to inclusion into the MiPPAD study, voluntary consent was obtained

from each enrolled woman after the study was explained in the preferred local language. For enrolled minors, informed consents were obtained from one parent or legal guardian.

Ethical approval for the TOVI study was sought from the institutional review boards of the University of Abomey-Calavi (Benin), New York University (USA), Michigan State University (USA) and the Research Institute for Development's (IRD) Consultative Ethics Committee (France). Prior to enrollment, we obtained informed consent from all the women who participated in this study for themselves and offspring.

In both studies, women were free to discontinue their participation at any stage of the study.

Chapter 3. Helminth Infection during Pregnancy and Early Cognitive and Motor Functions of Children

This chapter of my thesis presents the results of the analysis of the impact of soil-transmitted helminth (STH) infection on the cognitive and motor functions of one-year-old children. The chapter starts with brief recap of the importance of helminth infections in public health. The chapter also presents the conceptual framework for the impact of prenatal STH infections on infant cognitive development as well as the problem statement and hypothesis that prenatal helminth infection could have detrimental consequences on infant cognitive development. Summary of the published article including what is already known on the subject and what the article adds is also presented in this chapter. Further, the published article in *Plos Neglected Tropical Diseases* is also included in the chapter.

NB: List of references, figures and tables that appear in the article are independent and therefore do not follow the order presented in the entire thesis.

3.1 Recap: Importance of Helminth Infections in Public Health

STHs are the most prevalent helminths in our study population. STHs refer to parasitic worms that infect humans through a contaminated soil usually through ingestion of eggs or larvae or by cutaneous penetration. As stated in Chapter 2, helminths are among the top 5 leading causes of anemia in sub-Saharan Africa. Hookworms (*Ancylostoma duodenale* and *Necator Americanus*), Roundworms (*Ascaris lumbricoides*) and whipworms (*Trichuris trichiura*) are the commonest known STHs.

STH is estimated to affect more than one-fifth of the world's population with more than 25% of the global burden borne by the sub-Saharan African region. Although helminth infection rarely leads to death, it has an immense impact on the quality of life of infected individuals. The burden of helminth infection is estimated to be 1.14 YLD/1000 people in sub-Saharan Africa¹⁴⁴. Women of childbearing age, preschool-aged and school-aged children are the population at risk of helminth infection in endemic regions. Helminth infection has been associated with but not limited to reduced hemoglobin concentration^{10,252}, anemia^{253,254}, IDA^{255,256} and poor cognitive performance²⁵⁷.

3.2 Helminth Infection and Cognitive Development

Considering the susceptibility of the perinatal period to the risk of anemia, helminths are of major public concern among pregnant women living in endemic regions. Aside the anemia-related evidence among infected people, some studies have shown that helminth infection in children is associated with poor cognitive function including verbal fluency and learning^{258,259}. Indeed, the evidence that anthelmintic treatment of pregnant women have other effect than reducing STH infection rate is weak and only few trials exist on the benefits of prenatal anthelmintic chemotherapy.²⁶⁰ Recent evidence from a systematic review investigating the impact of anthelmintic treatment in children in clinical trials and cognitive function however reports of a probable limited or no impact of anthelmintics.²⁶¹ The conclusion of the systematic review came from 7 existing clinical trials on the subject, which used several different tools to assess cognitive function.²⁶¹ The inconclusiveness of the findings of the systematic review therefore supported the

need for further studies but with clearly marked out timeline of the exposure, helminths and a more detailed cognitive battery that takes into account several independent tests. Further, a meta-analysis on the impact of anthelmintics on maternal and child health showed reduced risk of very low birth weight (<1500 g) among women who had anthelmintics.²⁶² Considering that the prenatal phases of human development (including brain development) are most susceptible to insults such as that of helminths, we sought to investigate if helminths infection during pregnancy is associated with poor cognitive development, later on after birth.

3.3 Conceptual Framework: Prenatal Helminth Infection and Infant Cognition

To better understand how helminths infections during pregnancy could have an impact of the cognitive function of children, later after birth, and to understand the role of other factors, we developed a conceptual framework that addresses possible mechanisms of the hypothesized relationship.

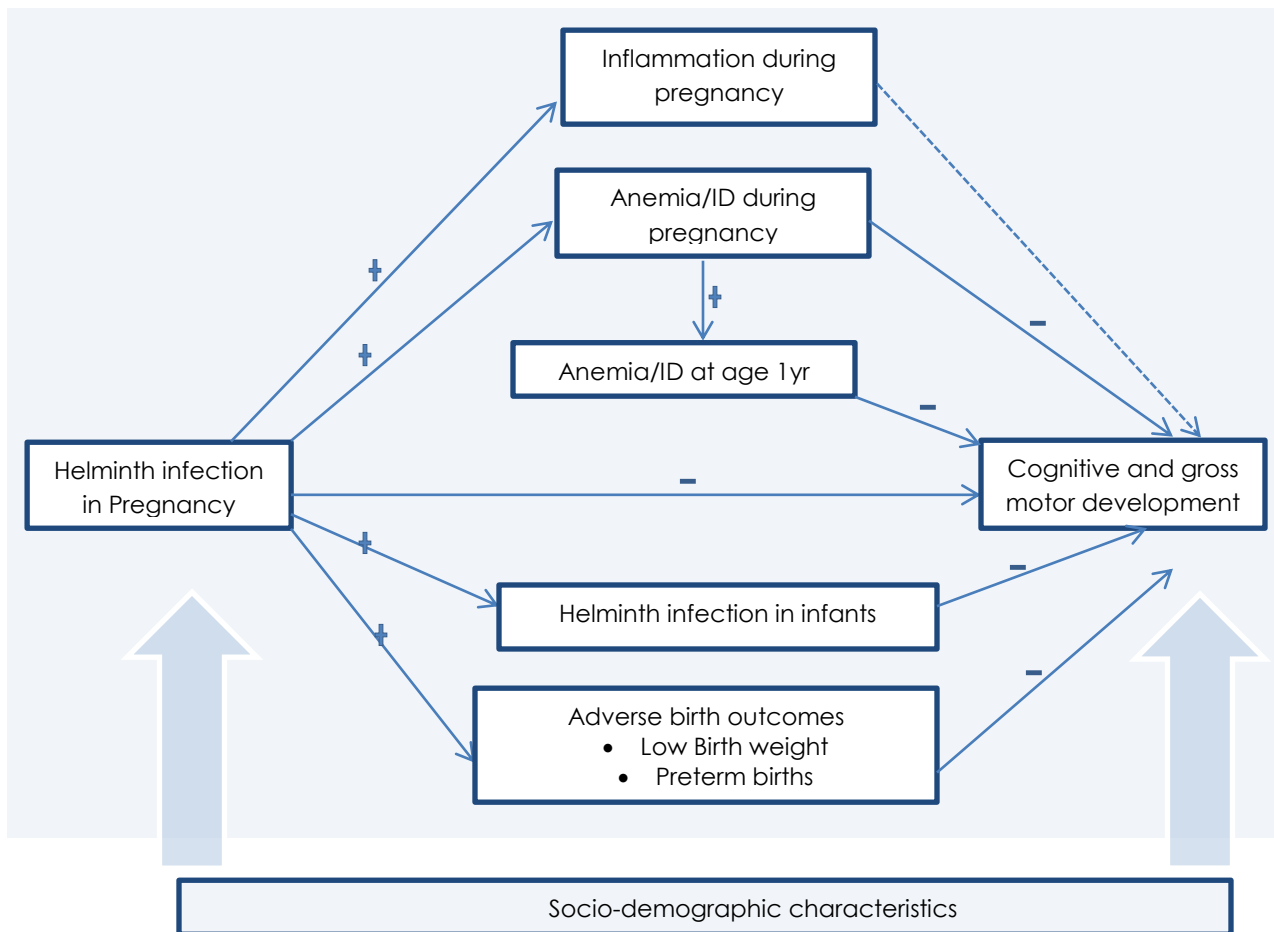


Figure 3.1 Conceptual framework of the relationship between prenatal helminth infection and infant cognitive and motor development

The conceptual model developed indicates that there are several ways in which helminth infection could impact on infant cognitive and motor development. Possible factors that could mediate the association included adverse birth outcomes, inflammation during pregnancy, anemia or ID during pregnancy and helminth infection in infants but in the article that follows, only the latter was tested.

What is known on the subject: Although inconclusive, helminth infections in children have been reported, by some studies, to be associated with poor cognitive performance. Children and women of childbearing age (including pregnant women) are most at risk of the consequences of helminth infection. The WHO recommends anthelmintics for pregnant women after their first trimester but the benefits remain ambivalent. Considering that the fetal stages of life are the most vulnerable periods of development, it is essential that we investigate if helminth infection in pregnancy on the cognitive development but prior to the first submission of our manuscript for publication, only one of such a study existed²³ indicating that the impact of prenatal helminth infection on offspring development has been understudied. From the aforementioned study, the findings concluded that helminth infection in pregnancy could have negative consequences on infant cognition although their results reflected that hookworm infection during pregnancy is not associated with poor infant cognitive development.

What the article adds: Prenatal intestinal helminth infection is associated with poor cognitive and gross motor functions of children at age one year. Measures to prevent helminth infections during pregnancy should be reinforced.

3.4 Article I: Helminth Infection in Pregnancy and Infant Cognition

RESEARCH ARTICLE

Impact of Helminth Infection during Pregnancy on Cognitive and Motor Functions of One-Year-Old Children

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Data Availability Statement: The authors confirm that all relevant data are within the present article and its Supporting Information files, and in an article published in *Plos Medicine* [González R, Mombona G, Ouédraogo S, Kakolwa MA, Abdulla S, et al. (2014) Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multicentre Randomized Controlled Trial. *PLoS Med* 11(9): e1001733. doi:10.1371/journal.pmed.1001733]. We cannot provide data on pregnant women from Clinical Trial as this will breach compliance with the protocol approved by

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Abstract

Objective

To determine the effect of helminth infection during pregnancy on the cognitive and motor functions of one-year-old children.

Methods

Six hundred and thirty five singletons born to pregnant women enrolled before 29 weeks of gestation in a trial comparing two intermittent preventive treatments for malaria were assessed for cognitive and motor functions using the Mullen Scales of Early Learning, in the TOVI study, at twelve months of age in the district of Allada in Benin. Stool samples of pregnant women were collected at recruitment, second antenatal care (ANC) visit (at least one month after recruitment) and just before delivery, and were tested for helminths using the Kato-Katz technique. All pregnant women were administered a total of 600 mg of mebendazole (100 mg two times daily for 3 days) to be taken after the first ANC visit. The intake was not directly observed.

Results

Prevalence of helminth infection was 11.5%, 7.5% and 3.0% at first ANC visit, second ANC visit and at delivery, respectively. Children of mothers who were infected with hookworms at

the research ethics board. Access to this data from the MiPPAD study may be available upon request through The MiPPAD Executive Committee (Dr. Raquel Gonzalez at +34-932-275-400 or raquel.gonzalez@eresib.cat).

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Competing Interests: The authors have declared that no competing interests exist.

the first ANC visit had 4.9 (95% CI: 1.3–8.6) lower mean gross motor scores compared to those whose mothers were not infected with hookworms at the first ANC visit, in the adjusted model. Helminth infection at least once during pregnancy was associated with infant cognitive and gross motor functions after adjusting for maternal education, gravidity, child sex, family possessions, and quality of the home stimulation.

Conclusion

Helminth infection during pregnancy is associated with poor cognitive and gross motor outcomes in infants. Measures to prevent helminth infection during pregnancy should be reinforced.

Author Summary

The WHO recommends anthelmintics for pregnant women after their first trimester but the benefits remain unequivocal. Although the consequences of helminth infection during pregnancy on the health of pregnant women have been well studied, the impact on the early development of offspring has been understudied. Studies suggest that helminth infection in children may be associated with poor cognitive development, but very little is known whether a similar consequence exists for offspring of women infected with helminths during pregnancy. From our study, we found that women who had intestinal worm infection at least once during pregnancy, had children with lower cognitive and motor scores. Moreover, hookworm infection in pregnant women prior to receiving anthelmintic treatment was associated with poor gross motor functions of children at one-year of age. Based on the findings of this study, measures to prevent helminth infections during pregnancy should be reinforced.

Introduction

Intestinal helminths infect more than two billion of the world's population, with the highest prevalence in Asia and sub-Saharan Africa.[1] The burden of intestinal helminth infection is estimated to be five million disability-adjusted life years (DALYs).[2] Helminth infections are rarely directly associated with increased mortality but are related to increased morbidity arising from the chronicity and consequences of infection.[3] Although the World Health Organization (WHO) highly recommends anthelmintic therapy for pregnant women in their second trimester[4], the benefits on anemia, congenital anomalies and perinatal mortality remains unequivocal[5]. In sub-Saharan Africa, it is estimated that one-third of pregnant women are infected with soil-transmitted helminths[6] although several studies have shown wide variation in prevalence across different countries, 11.1% in Benin[7], 25.7% in Ghana[8] and 49% in Gabon[9]. In Benin, anthelmintics are a component of the routine antenatal care (ANC) package given to pregnant women after their first trimester.[10]

A recent systematic review found little evidence that deworming in children is associated with better cognitive function, though most trials included were of poor quality.[11] A cross-sectional study revealed that compared to 7 to 18 year-old-children who were not infected with *Ascaris lumbricoides* and *Trichuris trichiura*, children who were infected with either of these species of helminth performed poorly on tests of memory and verbal fluency, respectively.[12]

Over the past decades, many studies have confirmed helminth infection during pregnancy as a risk factor for maternal iron deficiency (ID) and anemia [3,13,14]. However, evidence remains limited on the effects on adverse birth outcomes such as low birth weight (LBW) [15] which is known to be associated with poorer cognitive function in children. [16] Additionally, ID and anemia during pregnancy may be associated with poor cognitive function of infants as shown in a study in rural China which revealed that children of iron deficiency anemic (IDA) women performed significantly lower than those of non-IDA women in cognitive assessment tests. [17] The rapid rate of development of fetal organs makes them particularly susceptible to prenatal insults that are injurious to fetal development, and which could influence their development persisting even after birth. The early onset of delayed cognitive development could negatively influence several aspects of child development including preparedness for school. [18] Notwithstanding the evidence that helminths are associated with these indirect threats, very little is known about the impact of helminth infection during pregnancy on actual infant cognitive development. A study in Uganda concluded that *Mansonella perstans* and *Strongyloides stercoralis* infection during pregnancy may be associated with impaired executive function in children. [19]

The objective of this study was to determine whether maternal infection with helminths, both in general and with specific helminth species, during pregnancy, is associated with cognitive and gross motor functions of one-year-old children in Benin.

Methods

Population

Our prospective cohort included singletons born to pregnant women who were enrolled before 29 weeks of gestation in the *Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD)* clinical trial (NCT00811421) comparing sulfadoxine-pyrimethamine and mefloquine as intermittent preventive treatment of malaria in pregnancy (IPTp). The study was conducted in the district of Allada in Benin. One thousand and five HIV-negative pregnant women attending their first ANC visit in the health centers in each of the three sub districts of Allada (Sekou, Allada and Attogon) were recruited. Detailed inclusion and exclusion criteria in the *MiPPAD* trial are explained elsewhere. [7]

All live born children of recruited pregnant women who survived to 12 months were invited for neurocognitive assessment in the TOVI study (Fon language: *Tovi* means Child from the country).

Variables

Pregnant women. Socio-demographic and clinical data were collected at three ANC visits; at recruitment (first ANC visit), during the 2nd dose of IPTp (second ANC visit, at least one month after recruitment) and at delivery. At first ANC visit, information on gravidity, gestational age, anthropometric data and socio-demographic characteristics including age, education, and occupation were collected. Details of biological assessments for pregnant women and how prepregnancy BMI was calculated have been described in an earlier publication. [7] More specifically, data on maternal helminth infection comes from a parallel cohort study (Anemia in Pregnancy: Etiologies and Consequences “APEC”) which was nested within the *MiPPAD* trial. [20] At ANC visits, women were given IPTp, iron and folic acid as part of the ANC package in Benin. Women were also given a total of 600 mg of mebendazole (100 mg twice daily for 3 days) at the first ANC visit and again if they were tested positive for helminths at second ANC visit. Intake of mebendazole was not directly monitored. Containers were given to pregnant women to collect stool samples from the next morning after the ANC visits. Since it was

difficult to obtain stool samples when women were in labor, where possible, stool samples were collected 15 days prior to the expected date of delivery or within a week after delivery.

Thick smears of retrieved stool samples were immediately prepared and assessed using the Kato-Katz technique as described by the WHO[21]. One of the two slides prepared from each sample was systematically and independently examined by two laboratory technicians under a microscope. The mean of the two results was calculated and reported. To obtain the standardized fecal egg counts (FEC), in eggs per gram of stool (epg), for each individual stool sample, the counted number of eggs for each species of helminth was multiplied by twenty-four. *T. trichiura*, *A. lumbricoides* and *S. mansoni* were not independently considered as specific exposures in the univariate and multivariate analyses owing to their low prevalence throughout pregnancy. Also, due to the low intensity of hookworms in our study population, the WHO cut-off for classification[22] was not applicable. Instead, the intensity of helminth infection was categorized using the median as a cut-off value. In this article, helminth infection is defined as the presence of at least one egg of any species of helminth in the stool.

Gestational age was assessed according to fundal height at delivery.

Within three days after the cognitive assessments of children at age one year, a different nurse conducted home visits during which information on family possessions was collected and the Raven's Progressive Matrices (RPM) test was conducted. A score was generated for family possessions, which was the sum of individual scores given to specific items owned by the family of the pregnant woman thus whether the home had electricity or car (each scoring 3), a motorcycle or television (each scoring 2) and a radio, bicycle, motorcycle, or cattle (each scoring 1). The RPM uses an approach which does not require verbal proficiency to provide an estimate of intelligence quotient[23].

Infants. As part of the APEC study, stool samples were collected from a subsample of children ($N = 186$) at 6, 9 or 12 months for examination for helminths. At the health centers, trained research nurses individually assessed the cognitive function of 635 one-year-old infants using the Mullen Scales of Early Learning (MSEL) from April 2011 to November 2012. The MSEL which comprises of five scales, gross motor, fine motor, visual reception, receptive language and expressive language, was adapted to this setting prior to its use in this study.[24]

From the raw scores obtained by infants in each MSEL scale, normalized age specific (monthly) scores called the *t*-scores were generated. *T*-scores of the visual reception, fine motor, receptive language and expressive language scales were then combined to form the Early Learning Composite (ELC) score, which is indicative of early cognitive function.[25] Detailed quality assurance and reliability of assessment have already been published.[24] Briefly, five assessors were trained by MJB and FBL in the field and retrained three times. Difficulties to be discussed with FBL and MJB were reviewed during weekly meetings. Inter-rater reliability was checked.

The Home Observatory Measurement of the Environment (HOME) inventory was administered three days after MSEL assessment during the home visit. The HOME inventory, adapted for this setting, was used to assess the quality of the home environment including the parent-child interaction and the learning opportunities available to the child at home.[26] After training and piloting, changes were made to six items in the HOME inventory to suit our study setting. For example, one item regarding the child being outside of the house was removed as children spend most of the day outside. Description of how the HOME inventory was administered has been detailed in a previous publication. [24]

Statistical analyses

We first described and compared the baseline characteristics of women with singleton live births whose children were assessed and those whose children were not assessed for cognitive function. Secondly, we performed univariate analyses to assess crude associations between the ELC and the gross motor scores with helminth infection, helminth species, helminth density, co-infection with malaria, and covariates [maternal prepregnancy body mass index (BMI), family possessions, maternal occupation, education, the RPM and HOME scores]. These covariates were considered as potential confounding factors as they are known risk factors for poor cognitive development and may share common causes with helminth infection. Next, we conducted a multiple linear regression adjusting for covariates whose p-values were less than 0.20 in the univariate analysis. Finally, we performed stepwise removal of covariates from the model if they were found not to be statistically significant. From the final model, we evaluated the adjusted mean difference in ELC and gross motor scores. Infant characteristics at birth or age one-year including birth weight, preterm birth and infant helminth infection were hypothesized to be within the causal pathway (as mediators). All multivariate models were adjusted for infant sex. Although infant characteristics (preterm births, low birth weight, and weight-for-age at MSEL assessment) were hypothesized to be mediators in the association between prenatal helminth infection and infant cognitive function, we adjusted for these variables in a sensitivity analyses.

Statistical analyses were conducted using Stata IC/11.2 for Windows (StataCorp Lp, College station, TX). We used Pearson's correlation to assess the associations between the dependent variables and other continuous variables. The student *t*-test, Wilcoxon rank sum test and chi-squared test were used to compare means, medians and proportions, respectively. Statistical significance was defined as p-value less than 0.05.

Ethical considerations

The study was approved by the institutional review boards of the University of Abomey-Calavi in Benin, New York University and Michigan State University in USA and the Research Institute for Development's (IRD) Consultative Ethics Committee in France. At recruitment, we obtained written informed consent from all pregnant women and guardians of children who participated in this study in the presence of a witness. Women who could not read and write provided thumbprints to confirm their agreement to participate in the study after a nurse had explained the study.

Results

General characteristics of participants

As shown in Fig. 1, 863 live born singletons were enrolled into the birth cohort but 35 died before the age of one year leaving 828 eligible children. Of these, 635 (76.7%) were assessed for cognition using MSEL at approximately one year of age. The median age during MSEL assessments was 12.1 months (range: 11.3–15.3 months). Two children were not able to complete all of the MSEL subtests, leaving 633 children who were fully assessed. Maternal baseline characteristics were similar between women whose children were fully assessed for cognitive function and those whose children were not, as shown in Table 1. Also there was no significant difference between infant characteristics between children assessed and those not assessed.

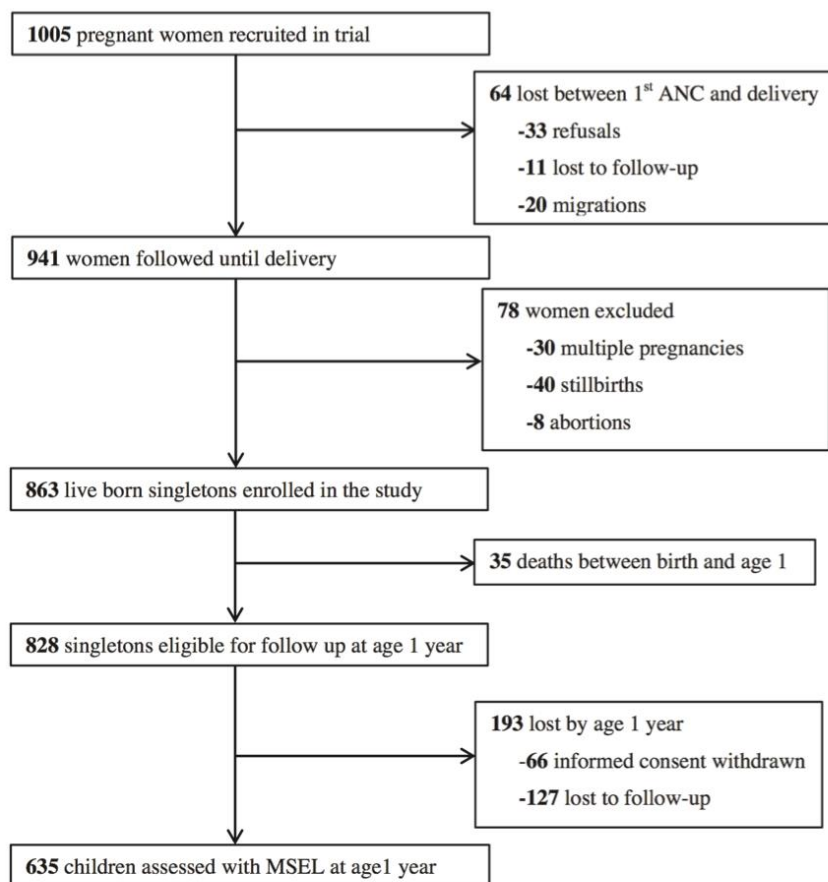


Fig 1. Flowchart outlining the follow-up of participating pregnant women until their children were one year old. The rate of follow-up of eligible children from birth till age one was 76.7%.

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Helminth infection among pregnant women and among children

At first ANC visit, the prevalence of helminth infection was 11.5% of which hookworm infections were the most prevalent (9.5%). Of the 52 women with hookworm infections at the second ANC visit, 12 were infected with the same species at first ANC (see Table 2 for prevalence and density of helminths). The prevalence (95% CI) of helminth infection among children by age one was 32.8% (26.0%-39.6%).

Maternal characteristics and ELC and gross motor scores

Maternal education, occupation, family possession, RPM and HOME scores and infant weight-for-age were associated with ELC and gross motor scores. Of note, maternal malarial infection was not statistically significantly associated with ELC and gross motor scores (see Table 3).

Table 1. Comparison of maternal baseline characteristics at first ANC visit and infant characteristics between children fully assessed and those not fully assessed for cognitive function.

Characteristics	Cognitive assessment status		P-value
	Fully assessed	Not/partially assessed	
	(N = 633)	(N = 230)	
Mothers			
Age in years [median (range)]	25 (17–45)	25 (13–42)	0.472
Gestational age in weeks [median (range)]	22 (10–28)	23 (8–28)	0.190
Gravidity			
Primigravidae	117 (18.5)	38 (16.5)	0.507
Multigravidae	516(81.5)	192 (83.5)	
Education			
Never schooled	418 (66.0)	156 (67.8)	0.622
Primary or more	215 (34.0)	74 (32.2)	
Occupation			
Housewives	313 (49.4)	107 (46.5)	0.447
Employed	320 (50.6)	125 (53.5)	
Prepregnancy BMI (kg/m²)			
Underweight (<18.5)	111 (17.5)	33 (14.4)	0.355
Normal (18.5–24.9)	456 (72.0)	167 (72.6)	
Overweight/Obese (≥25.0)	66 (10.4)	30 (13.0)	
Malaria			
Positive	101 (15.8)	31 (13.8)	0.371
Negative	532 (84.2)	199 (86.2)	
Infants			
Sex			
Male	310 (49.0)	104 (45.2)	0.329
Female	323 (51.0)	126 (54.8)	
Birth weight (kg)^a			
Low (<2.5)	56 (9.5)	28 (13.3)	0.124
Normal (≥2.5)	531 (90.5)	182 (86.7)	
Gestational age at birth (weeks)^b			
Preterm (<37)	43 (7.0)	17 (8.0)	0.631
Not Preterm (≥37)	572 (93.0)	196 (92.0)	
Weight at 1 year (kg) ^c	8.4	—	NA

Figures are number (percentage) unless otherwise indicated

^aN = 797

^bN = 828

^cN = 626

BMI-Body Mass Index

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Infant ELC and gross motor scores increased with increasing prepregnancy BMI class. As shown in Table 3, children born preterm and those with low birth weight had lower ELC and gross motor scores, respectively.

Table 2. Descriptive assessment of helminth infection in enrolled mother- infants pairs from Benin with follow-up from 29 weeks gestation through child's 1st year of life.

Characteristics	Infected N (%) or median (range)
Helminth infection	
At 1 st ANC visit	98 (11.5)
During 2 nd ANC visit	62 (7.5)
At delivery	21 (3.0)
Helminth species at 1 st ANC visit	
Hookworms	81 (9.5)
<i>Ascaris lumbricoides</i>	9 (1.1)
<i>Trichuris trichiura</i>	7 (0.8)
<i>Schistosoma mansoni</i>	2 (0.2)
Helminth species at 2 nd ANC visit	
Hookworms	52 (6.3)
<i>Ascaris lumbricoides</i>	7 (0.9)
<i>Trichuris trichiura</i>	5 (0.6)
<i>Schistosoma mansoni</i>	—
Helminth species at delivery	
Hookworms	19 (2.7)
<i>Ascaris lumbricoides</i>	2 (0.3)
<i>Trichuris trichiura</i>	—
<i>Schistosoma mansoni</i>	—
Helminth infection over the course of pregnancy (2 categories)	
Never infected	540 (77.8)
Infected at least once	154 (22.2)
Helminth infection over the course of pregnancy (3 categories)	
Never infected	540 (80.2)
Infected only once	108 (16.1)
Infected twice or more	25 (3.7)
Hookworm density at 1 st ANC visit (epg)	72 (24–2736)
Hookworm density at 2 nd ANC visit (epg)	72 (24–1416)
Hookworm density at delivery (epg)	120 (24–912)
Hookworm infection over the course of pregnancy (3 categories)	
Never infected	553 (82.5)
Infected only once	102 (15.2)
Infected twice or more	15 (2.2)
Helminth infection by age one year	61 (32.8)
Helminth species in Children by age one year (N = 186)	
Hookworms	8 (4.3)
<i>Trichuris trichiura</i>	39 (20.9)
<i>Ascaris lumbricoides</i>	8 (4.3)
<i>Schistosoma mansoni</i>	3 (1.6)
<i>Enterobius vermicularis</i>	3 (1.6)

epg- eggs per gram of stool

—indicates no egg found on laboratory examination

ANC- Antenatal Care

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Table 3. Association between maternal and infant socio-demographic and anthropometric characteristics and early learning composite (ELC) and gross motor scores.

Characteristics	ELC score		Gross motor score	
	Mean	P-value	Mean	P-value
N = 634				
Mothers				
Gravidity				
Primigravidae	99.8	0.323	48.2	0.021
Multigravidae	98.3		51.6	
Maternal Education				
None	96.4	<0.0001	49.4	<0.001
Some	102.9		54.1	
Maternal Occupation				
Housewives	96.3	<0.001	49.2	0.003
Employed	100.8		52.7	
Prepregnancy BMI				
Underweight	96.3	0.061	48.9	0.029
Normal	98.7		50.9	
Overweight/Obese	101.5		54.8	
Family Possession score ^a	0.1 ^b	0.004	0.2 ^b	<0.0001
RPM score ^a	0.1 ^b	0.035	0.1 ^b	0.002
HOME score ^a	0.2 ^b	<0.0001	0.2 ^b	<0.0001
Malaria at 1 st ANC				
Positive	100.4	0.162	51.2	0.362
Negative	98.2		49.8	
Malaria at 2 nd ANC				
Positive	100.7	0.465	56.1	0.086
Negative	98.5		50.9	
Infants				
Sex				
Male	97.9	0.227	51.9	0.101
Female	99.3		50.1	
Birth weight (kg) ^b				
Low (<2.5)	97.6	0.630	45.3	0.002
Normal (≥2.5)	98.5		51.7	
Gestational age at birth (weeks) ^c				
Preterm (<37)	95.9	0.007	51.0	0.951
Not Preterm (≥37)	99.5		51.1	
Weight for age at 1 year (kg) ^d	0.1 ^b	<0.001	0.2 ^b	<0.0001
Helminth infection by 1 year ^e				
Positive	99.4	0.539	52.8	0.337
Negative	100.8		50.6	

ANC- Antenatal Care

BMI-Body Mass Index

^a N = 629

^b N = 588

^c N = 594

^d N = 627

^e N = 182

^b Represented as Correlation coefficients not means

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Table 4. Association between helminth infection at ANC visits and maternal and infant characteristics.

	Helminth infection at 1 st ANC		Helminth infection at 2 nd ANC	
	N (%)	P-value	N (%)	P-value
Gravidity				
Primigravidae	20 (13.2)	0.484	11 (7.2)	0.889
Multigravidae	78 (11.2)		51 (7.6)	
Maternal Education				
Never schooled	72 (12.8)	0.109	51 (9.3)	0.005
Primary or more	26 (9.1)		11 (3.9)	
Maternal Occupation				
Housewives	53 (12.8)	0.242	40 (10.0)	0.009
Employed	45 (10.3)		22 (5.2)	
Prepregnancy BMI				
Underweight	20 (14.2)	0.498	14 (3.0)	0.156
Normal	69 (11.2)		45 (7.6)	
Overweight/Obese	9 (9.6)		3 (3.2)	
HOME score (median) ^a	27 (27)	0.642	27 (27)	0.411
Raven score (median) ^a	15 (15)	0.733	15 (15)	0.937
Family Possession (median) ^a	5 (5)	0.044	4 (5)	0.021
Malaria at 1st ANC visit				
Positive	18 (13.9)	0.366	12 (9.5)	0.366
Negative	80 (11.1)		50 (7.2)	
Malaria at 2nd ANC visit				
Positive	5 (15.2)	0.576	3 (8.8)	0.736
Negative	92 (11.6)		59 (7.5)	
Infant helminth infection				
At least once	5 (8.5)	0.336	6 (9.8)	0.573
Never	6 (4.8)		9 (7.3)	

BMI-Body Mass Index

^a Represented as median scores for women with helminth infection (median scores for uninfected women)

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Socio-demographic characteristics and maternal helminth infection

As shown in Table 4, maternal occupation and educational status were associated with helminth infection at second ANC visits. Family possessions score was associated with helminth infection at both ANC visits.

Prenatal helminth infection and infant cognitive and gross motor functions

The difference in mean ELC scores between children whose mothers were infected with helminths at first ANC visit and those whose mothers were not infected with any helminth remained significant after adjusting for maternal education, child sex and HOME score ($p = 0.013$). Pregnant women who were infected with helminths at least, once during pregnancy had children with poorer ELC scores, thus -4.4 (95% CI: -7.2 to -1.5) compared to those of mothers who were never infected during pregnancy after adjustment (see Table 5).

After adjusting for gravidity, maternal education, family possession, child sex and HOME score, helminth infection at first ANC visit was negatively associated with infant gross motor

Table 5. Relationship between helminth infection during pregnancy and mean scores of infant cognitive and gross motor function at age 1 year.

	Mean difference in ELC scores			Mean difference in gross motor scores	
	Crude beta [95%CI]	Adjusted beta [95%CI] ^a	Adjusted beta [95%CI] ^b	Adjusted beta [95%CI] ^c	Adjusted beta [95%CI] ^d
Helminth infection					
At 1st ANC visit	-4.2 [-7.6, -0.7]*	-4.3 [-7.6, -0.9]*	-4.3 [-7.7, -1.0]*	-3.9 [-7.3, -0.4]*	-4.4 [-7.9, -1.0]*
At 2nd ANC visit	-5.1 [-9.0, -1.1]*	-3.9 [-7.8, -0.0]*	-3.5 [-7.4, 0.3]	-2.2 [-6.1, 1.7]	-1.6 [-5.5, 2.4]
At delivery	-1.9 [-9.4, 5.6]	-0.2 [-7.5, 7.1]	-0.0 [-7.4, 7.4]	5.3 [-2.0, 12.6]	5.2 [-2.2, 12.9]
Hookworm					
At 1st ANC visit	-3.3[-7.1, 0.5]	-3.7 [-7.3, 0.0]	-4.0 [-7.7, -0.3]*	-4.9 [-8.6, -1.1]*	-5.5 [-9.3, -1.8]**
At 2nd ANC visit	-6.0 [-10.3, -1.6]**	-4.7 [-8.9, -0.5]*	-4.4 [-8.6, -0.2]*	-2.7 [-7.0, 1.5]	-2.2 [-6.5, 2.1]
At delivery	-1.1 [-8.9, 6.6]	0.6 [-7.0, 8.1]	1.1 [-6.6, 8.8]	6.9 [-0.7, 14.5]	7.2 [-0.6, 15.1]
Hookworm density at 1st ANC visit					
Not infected (ref)	0	0	0	0	0
Moderate (≤72, median)	-2.5 [-7.4, 2.5]	-3.2 [-8.1, 1.6]	-3.5 [-8.4, 1.4]	-5.4 [-10.3, -0.5]*	-6.5 [-11.4, -1.5]*
High (>72)	-4.4 [-9.9, 1.1]	-4.2 [-9.5, 1.2]	-4.6 [-9.9, 0.6]	-4.1 [-9.6, 1.2]	-4.4 [-9.7, 0.9]
Hookworm density at 2nd ANC visit					
Not infected (ref)	0	0	0	0	0
Moderate (≤72, median)	-5.2 [-10.4, 0.1]	-3.9 [-9.0, 1.2]	-3.7 [-8.9, 1.4]	-3.1 [-8.2, 2.0]	-2.6 [-7.8, 2.7]
High (>72)	-7.4 [-14.6, -0.2]*	-6.2 [-13.2, 0.9]	-5.7 [-12.9, 1.4]	-2.0 [-9.2, 5.2]	-2.1 [-9.3, 5.1]
Hookworm density at delivery					
Not infected (ref)	0	0	0	0	0
Moderate (≤120, median)	-2.8 [-13.3, 7.8]	-0.6 [-10.9, 9.6]	0.1 [-10.0, 10.2]	6.7 [-3.5, 17.0]	8.2 [-2.1, 18.5]
High (>120)	0.8 [-10.6, 12.1]	2.0 [-9.0, 13.0]	2.5 [-9.3, 14.4]	7.1 [-3.9, 18.2]	5.9 [-6.1, 18.0]
Occurrence of helminth infection over the course of pregnancy					
Never infected (ref)	0	0	0	0	0
At least once	-4.9 [-7.8, -2.1]**	-4.4 [-7.2, -1.5]**	-4.1 [-7.0, -1.3]**	-2.9 [-5.7, -0.0]*	-2.8 [-5.7, 0.1]
Malaria-helminth co-infection					
At 1st ANC visit	-4.0 [-12.1, 4.1]	-3.1 [-11.1, 4.9]	-2.2 [-10.1, 5.7]	-3.1 [-11.1, 4.9]	-2.0 [-9.9, 5.8]
At 2nd ANC visit ^e	8.6 [-18.9, 35.2]	9.5 [-17.2, 36.2]	10.2 [-16.1, 36.4]	25.3 [-2.1, 52.7]	26.7 [-0.4, 53.8]

Unless otherwise stated, reference group were children of women who were not infected at either 1st ANC visit, 2nd ANC visit or at delivery

ANC- Antenatal Care ref-reference category

*P-value <0.05

** P-value <0.01

*** P-value <0.001

^a Adjusted for maternal education, child sex and HOME score

^b Adjusted for maternal education, child sex, HOME score, preterm status and child weight-for-age

^c Adjusted for maternal education, gravidity, child sex, family possessions and HOME score

^d Adjusted for maternal education, gravidity, child sex, family possessions, HOME score, preterm status and child weight-for-age

^e Only one assessed child had a mother with malaria-helminth co-infection at 2nd ANC visit.

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function ($p = 0.028$). We observed that mothers who were infected with hookworms during the first ANC visit had children who scored less in the gross motor scale, -4.9 (95% CI: -8.6 to -1.1), compared to those whose mothers were never infected with hookworms at first ANC visit. With the exception of the association between gross motor scores and the occurrence of

helminth infection over the course of pregnancy, sensitivity analyses performed by further adjusting for infant preterm status and weight-for-age, yielded similar results in the association between infants gross motor function and prenatal helminth infection. Helminth infection at second ANC was no longer statistically significantly associated with infant ELC scores after sensitivity analyses, $p = 0.074$ (see Table 5). Further adjustment for LBW (not preterm birth) and weight-for-age showed similar conclusions in the sensitivity analyses. We performed multiple regression analysis further adjusting for research nurses and found little difference in the results.

Discussion

Our study has shown that intestinal helminth infection at first ANC visit is associated with poorer infant cognitive and gross motor functions at the age of one-year after adjusting for other known risk factors of cognitive and gross motor development. In our study population, prenatal hookworm infection was related to lower performance in gross motor tests. Our results also reveal that helminth infection at least once during pregnancy may have negative consequences on the cognitive and motor development of infants.

Our study is one of the few large prospective mother-child cohorts with relatively low attrition rate in francophone Africa[27] and including several assessments during pregnancy. To our knowledge, our study is the first to assess the impact of prenatal helminths on the psychomotor development of infants taking into account data from different stages of pregnancy. In addition, we used a comprehensive assessment for neurodevelopment carried out by research nurses specifically trained by an expert in cognitive assessment in African countries (co-author MJB). An additional strength of this study is the consideration of several potential confounding factors such as socio-economic status, maternal depression and RPM and HOME scores. Malaria has also been assessed several times during pregnancy allowing for the study of the impact of malaria-helminth co-infection on child development. Despite low power due to the low prevalence of co-infection, our results do not suggest a higher impact on child development of helminths when associated temporally with malaria. Maternal demographic and reproductive characteristics were also comparable between children lost to follow-up and those included in the study hence selection bias is unlikely.

Since pregnant women recruited in the trial had adequate antenatal care including at least, two ANC visits with treatment for helminth infection at first ANC visit (apart from emergency visits), our results are likely to underestimate the effect of prenatal helminth on infant cognitive function in the general population that may attend fewer ANC visits and receive fewer treatments. Also, the low sensitivity of the Kato-Katz technique for helminths[28] may have resulted in measurement error but since, in this prospective cohort, the assessment of helminth status was independent of the performance of infant in the MSEL at age 1 year, the misclassification would probably be non-differential of infant cognitive and motor scores thus the association may be biased towards the null. The low prevalence of *A. lumbricoides*, *T. trichiura* and *S. mansoni* did not permit us to study their independent impact on infant cognitive function. Given that treatment was given to women after their first ANC visit, the number of chronic infections was low in our study. Therefore, the effect of chronicity of untreated prenatal helminth infection on child development could not be evaluated. By definition, helminth infection is chronic until treatment. Women testing positive for helminths may have been chronically infected prior to their first ANC visit. However, testing positive for helminth infection at second ANC visit and/or at delivery after being infected with helminths at first ANC does not specifically indicate chronicity. Instead it could indicate reinfection after being treated following mebendazole administration at first ANC visit. Our study is also limited in the inability to

assess the presence of prenatal *S. haematobium* as urine samples were not examined for eggs of this species. Due to the low proportion of children assessed for helminth infection, we were not able to adjust for infants' infections in models. Species of helminth in mothers and infants were largely different, yet regardless of the species there was no correlation between helminth infection in mothers and children. This therefore suggests that the association between maternal helminths and child development may be independent of infants' helminths.

Apart from a cohort study that was nested within the Entebbe Mother and Baby Study in Uganda[19], we did not identify any published study on the impact of prenatal helminths on cognition in offspring. The negative relationship witnessed between maternal helminth infection and infant cognitive development in our study is consistent with the general conclusion in the aforementioned study. Converse to the findings of our studies, the authors found no association between maternal hookworm infection and infant neurocognitive development. One explanation may be that Nampijja et al.[19] excluded pregnant women presenting severe anemia (Hb concentration <80g/L). They also included some maternal and infant characteristics (such as maternal hemoglobin level and birth weight) in their final model. It is important to note that our study population had a low prevalence and a low intensity of helminth infection according to WHO classifications of the community endemic levels[22] and few cases of multiple infections with different helminth species.

The mechanism by which prenatal helminth infection influences infant cognitive function remains unknown. However, helminth infection especially with hookworms is a known risk factor for ID. When hookworms penetrate the intestinal mucosa of a host, they ingest the host's blood causing intestinal blood loss and erythrocytes lysis[29]. This could result in IDA [30] which may be disadvantageous during pregnancy because of the increased physiological demand for iron. Studies have shown that in very iron deficient mothers, maternal serum ferritin concentration is correlated with that of the neonate[31] while decreased concentration is associated with a decrease in brain iron concentration[32] which could in turn alter hippocampal development of the neonate[33]. A study among one-year-old children found that, those born with inadequate brain iron stores ($\leq 34\mu\text{g/L}$ cord ferritin) had lower psychomotor function and auditory recognition memory than those with adequate brain iron stores.[34]

Helminths may be associated with several adverse birth outcomes that could mediate the pathway between prenatal helminth infection and infant cognitive development. Although findings from clinical trials reveal no beneficial effect of anthelmintic treatment on LBW and preterm births[35], a large community study of about 5000 pregnancies in Nepal showed an increased risk of LBW and infant mortality among the children of women who did not receive antenatal anthelmintic treatment[36]. Notwithstanding the contradictory effects of prenatal helminth infection on birth outcomes, adverse birth outcomes have been confirmed by some studies to be associated with infant cognitive development.[37–39] Our results, after sensitivity analyses, however suggest that other plausible unmeasured factors could also account for the observed association between prenatal helminth infection and child development.

It is unlikely that increased susceptibility of children of infected mothers to helminth infection explains the decreased ELC and gross motor scores, as there was no association between prenatal helminth infection and infant helminth infection by age one. Moreover, the pattern of helminth species varied in the mothers compared to the children. *T. trichuris* was the most prevalent species of helminths in children (20.9%) contrary to high hookworm prevalence in pregnant women.

Mebendazole is a broad-spectrum anthelmintic drug that is effective against several intestinal helminths. However, it has lower cure rates and fecal egg reduction rates for hookworms than albendazole.[40] In our study, although infection by any helminths at second ANC visits was not associated with poor cognitive or gross motor function, hookworm infection remained

associated with ELC scores. This could be due to either the re-exposure of pregnant women to hookworms even after mebendazole administration or low cure rates against hookworms. Although we did not monitor the adherence to mebendazole treatment, the decline in parasite density at second ANC visit observed in the majority of pregnant women infected with the same species than at first ANC visit (see [S1 Table](#)) suggests good adherence.

Conclusion

This study provides evidence of an association between intestinal helminths and hookworms among pregnant women and poor cognitive and gross motor functions in their children at approximately 12 months of age. In view of these findings and as recommended by the WHO, measures to prevent helminth infections should be reinforced. Further studies are needed to corroborate our findings and explain the pathophysiological mechanisms of this relationship.

Supporting Information

S1 Table. Parasite density among 10 women infected with hookworm at first and second ANC visit
(DOCX)

S2 Table. Data on cognitive function of children.
(XLSX)

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Author Contributions

Conceived and designed the experiments: FBL MJB LLD MC MOM. Performed the experiments: FBL MOM SO GKK MJB MJA. Analyzed the data: MOM FBL. Wrote the paper: MOM FBL MC LLD. Coordinated the study: FBL MJB LLD MOM MJA AM SO GKK.

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Chapter 4. Prenatal Hemoglobin Concentrations and Infant Cognitive and Motor Functions

This chapter addresses the results of our analysis on the impact of prenatal anemia at the various stages of follow-up and infant cognitive development. Results of the relationship between hemoglobin levels during pregnancy and infant cognitive and motor scores are also described. As presented in chapter 4, this chapter begins with a brief recap of the relationship between hemoglobin concentration during pregnancy and adverse birth outcomes.

The chapter also presents the conceptual framework for the impact of prenatal anemia on infant cognitive development, which was developed before the analysis was conducted so as to understand the plausible theoretical mechanisms that could underline such an association. Summary of the published article including what is already known on the subject and what the article adds is also presented in this chapter.

Further, the published article in *Pediatrics* is also included in the chapter. In addition to this publication, supplementary results on Hb levels (categorized) and MSEL scores (categorized) are also presented. These descriptive and inferential results that are not included in the published article are supplementary and therefore do not contribute any extra information to what was presented in the article. Instead, they show extra exploratory analyses that were performed and confirms in addition to the U-shaped relationship, the risk of poor GM function among different categories of prenatal Hb concentration. Hence no discussion is presented after the supplementary results.

NB: List of references, figures and tables that appear in the article are independent and therefore do not follow the order presented in the entire thesis.

4.1 Recap: Hb Concentration during Pregnancy, Birth Outcomes and Child Development

As detailed in Chapter 1, beginning in the second trimester of pregnancy, there is a physiologic disproportional increase in plasma volume relative to the increase in RBC that leads to hemodilution. Hemodilution is essential for reducing the viscosity of blood and enhancing blood flow from the mother to the growing fetus. Apart from the physiologic reduction in Hb concentration, other factors such as malaria, ID, helminth and HIV infections can account for the reduction in Hb concentration leading to anemia.

In the previous chapters, the effects of these causes of anemia on birth outcomes and child development have been mentioned. The adverse birth outcomes, LBW, preterm birth, SGA, IUGR that have been associated with low prenatal Hb concentration have also been identified as risk factors for poor cognitive development and behavioral problems.²⁶³⁻²⁶⁵ Apart from low prenatal hemoglobin concentration, abnormally high Hb levels have also been associated with adverse birth outcomes, giving rise to a U-shaped relationship as explained by Steer *et al.*^{186,266} Abnormally high hemoglobin concentration results in hemoconcentration and increased viscosity of blood thus impeding the transport of nutrients to the fetus. Hemoconcentration have also been associated with preeclampsia which is also associated with adverse birth consequences.¹⁰²

4.2 Conceptual Framework: Prenatal Anemia and Infant Cognition

To better understand how anemia during pregnancy could have an impact of the cognitive function of children, and to understand the role of other factors, we developed a conceptual framework that addresses possible mechanisms or pathways of the hypothesized relationship.

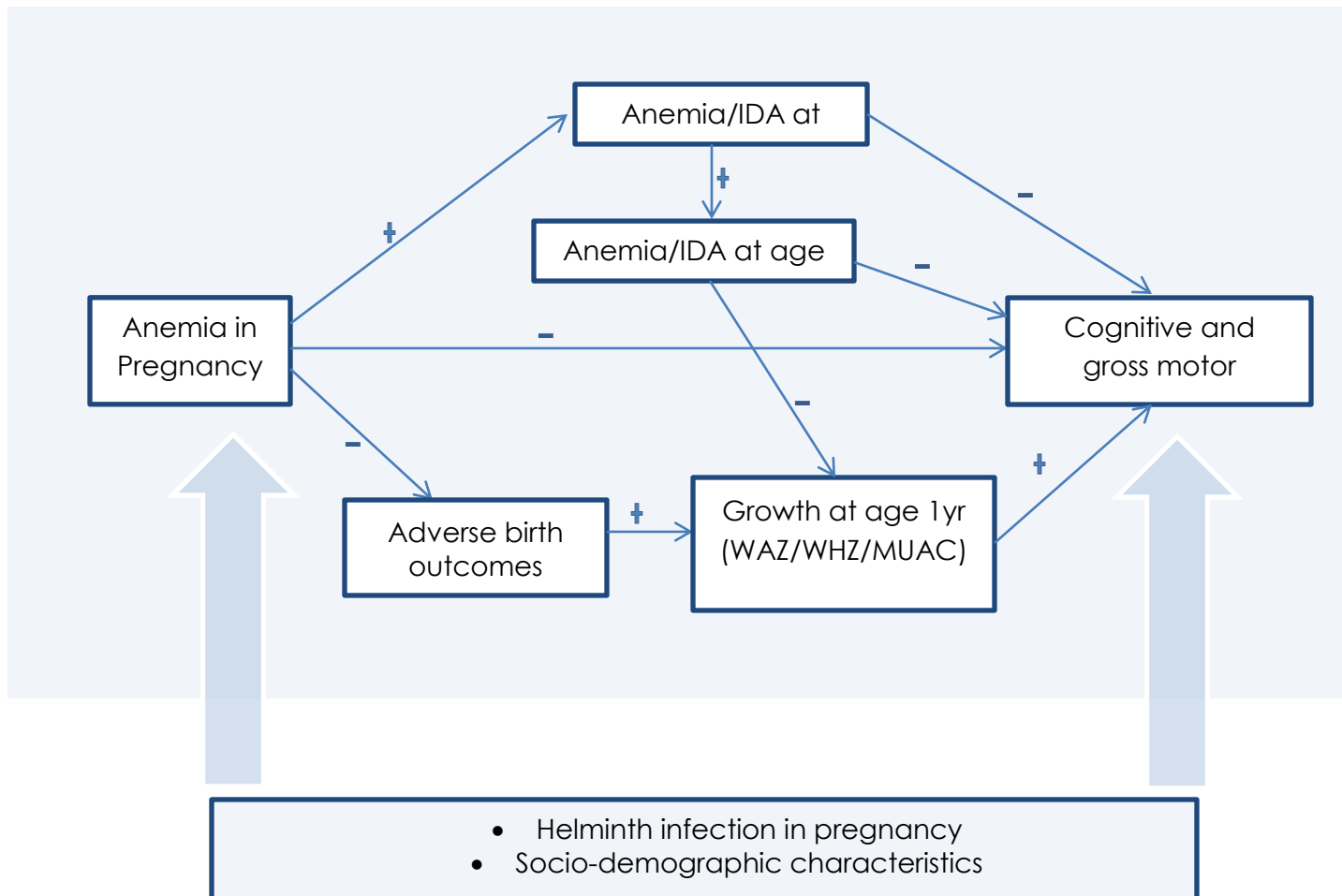


Figure 4.1 Conceptual framework of the relationship between anemia and cognitive development

4.3 Article II: Prenatal Hb Levels and Infant Cognition and Motor Functions

Prenatal Hemoglobin Levels and Early Cognitive and Motor Functions of One-Year-Old Children

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OBJECTIVE: To explore the relationship between prenatal hemoglobin (Hb) concentration and infant cognitive and motor functions.

abstract

METHODS: Our prospective cohort study included 1-year-old children born to women enrolled at their first antenatal care (ANC) visit in Allada, Benin, before 29 weeks of pregnancy, within a trial comparing the efficacy of sulfadoxine-pyrimethamine and mefloquine. Hb concentrations of pregnant women were determined from venous blood samples collected at first and second ANC visits of at least 1-month interval and at delivery. Women were prescribed oral iron, folic acid, and anthelmintics after the first ANC visit. A total of 636 children (76.8% of eligible children) were assessed by trained research nurses for cognitive and motor functions by using the Mullen Scales of Early Learning.

RESULTS: Prevalence of anemia (Hb < 110 g/L) decreased from 67.0% at first ANC visit (mean gestational age [SD], 22.1 [4.0] weeks) to 38.4% at delivery. Mean (SD) Hb concentrations increased from 103.7 (12.3) at first ANC visit to 112.4 (14.1) at delivery. We observed a significant negative quadratic relationship between infant gross motor (GM) function and Hb concentration at first and second ANC visits. Thus, infant GM scores increased sharply with increasing maternal Hb concentration until 90 g/L where increasing GM was mild, and began to decline after 110 g/L.

CONCLUSIONS: There appears to be an Hb concentration range that may be optimal for GM function of 1-year-old children. This may reflect the importance of physiologic hemodilution, which occurs after the second trimester until 34 weeks of gestation.



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Mr Mireku coordinated and supervised data collection of the TOVI study from the 3 study sites, performed and interpreted data analyses, drafted the initial manuscript, and revised the final manuscript; Dr Davidson designed the TOVI study and critically reviewed and revised the manuscript; Dr Koura supervised initial data collection of the TOVI study and critically reviewed and revised the manuscript; Drs Ouédraogo and Accrombessi designed, coordinated, and supervised data collection of the Malaria in Pregnancy Preventive Alternative Drugs (MIPPAD) clinical trial from the 3 study sites and reviewed the manuscript; Dr Boivin trained research nurses to use the Mullen Scales of Early Learning cognitive assessment, and critically reviewed and revised the manuscript;

WHAT'S KNOWN ON THIS SUBJECT: Studies on the consequences of abnormal prenatal hemoglobin (Hb) concentration have focused on maternal morbidities and adverse birth outcomes. To date, very little is known about the association between prenatal Hb concentration and infant cognitive and motor functions.

WHAT THIS STUDY ADDS: There is an inverted U-shaped relationship between maternal Hb concentration and infant gross motor function. Hb concentration between 90 and 110 g/L appears to be optimal for early gross motor function of children.

Early childhood, defined as the period from the prenatal phase until 8 years,¹ is the most critical developmental chapter of an individual's life trajectory. During the prenatal phase, the fetus undergoes extensive series of events involving neural migration, synaptogenesis, hippocampal development, and myelination,² which provide the structural foundation for brain development and function. This later becomes evident in the early developmental domains (social-emotional, fine-gross motor [GM], and language-cognition) of the growing infant. Due to the sensitivity of the fetal brain, its development could be altered by exposure to prenatal insults such as infections, stress, toxins, nutrient deficiencies and ischemia.^{3,4} Children with restricted prenatal development may potentially be mentally and socially impaired later in life.

A recent study in Benin suggests that maternal malnutrition, malaria infection, iron deficiency, helminth infection, and folate deficiency are the major risk factors for anemia in pregnant women at their first antenatal care (ANC) visits before treatments and micronutrient supplementation.⁵ Although access to iron and folic acid (IFA) supplements, intermittent preventive treatment of malaria in pregnancy (IPTp), and the use of anthelmintics during pregnancy are being achieved, the burden of anemia in least developed countries remain unacceptably high.⁶ In Africa, anemia in pregnancy (hemoglobin [Hb] concentration <110 g/L) is a severe public health problem estimated to be affecting over 17 million pregnant women.⁷ Significant increase, as well as decrease, in Hb concentration in pregnancy may imply deprivation of iron, oxygen, and other essential micronutrients to fetus for development. A study by Gonzales et al⁸ revealed a U-shaped relationship between prenatal Hb and

the risk of adverse birth outcomes. They observed that low prenatal Hb (<90 g/L) is associated with increased odds of stillbirths and preterm births and small-for-gestational age likewise Hb concentrations greater than 150 g/L.⁸ However, to date, little is known about the impact of prenatal Hb levels on early child psychomotor development.

Considering the evidence of adverse effects of low and high Hb levels during pregnancy, we hypothesized an inverted U-shaped relationship between infant cognitive and motor scores and maternal Hb during pregnancy. We therefore explored whether there was a nonlinear relationship between prenatal Hb at different periods of pregnancy and cognitive and GM development of 1-year-old children in Benin. We also compared the cognitive and motor functions of children of anemic and nonanemic women.

METHODS

Population

This prospective study (called TOVI) included 636 1-year-old surviving singletons born to women enrolled in the Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) study (NCT00811421). MiPPAD was a clinical trial comparing the efficacy of sulfadoxine pyrimethamine and mefloquine as IPTp. Inclusion and exclusion criteria for the clinical trial are fully explained elsewhere.⁹ Briefly, HIV-negative women who were at most 28 weeks pregnant and had not taken IPTp, IFA supplements, anthelmintics, or vitamin B12 since conception were recruited in maternity clinics in 3 subdistricts of Allada, Benin, during their first ANC visit.

Data and Hb Assessment During Pregnancy and at Birth

Maternal education, gravidity, gestational age, weight, and height

were determined at first ANC. Maternal prepregnancy BMI was calculated by using a method detailed in a previous publication.⁵

At 2 ANC visits, of at least 1-month interval, and at delivery, venous blood was obtained for analyses. Ten microliters of each blood sample was placed into a cuvette of the Hemo-Control photometer (EFK Diagnostics, Magdeburg, Germany) to measure the Hb concentration. Quality control was ensured by daily calibration of the Hemo-Control device by laboratory technicians and by crosschecking the Hb of 1 in 10 consecutive samples at the Allada Central Hospital laboratory by using a hematology analyzer (Erma Laboratory, Tokyo, Japan). Alkaline electrophoresis on cellulose acetate (Helena Laboratories, Beaumont, TX) was used to determine Hb genotypes.

Anemia was defined as Hb <110 g/L. After each assessment, pregnant women with Hb between 70 g/L and 110 g/L were treated with 400 mg of oral ferrous sulfate (200 mg twice daily) in accordance with the national guidelines. Those with Hb below 70 g/L were referred to a district tertiary hospital for blood transfusion. Per the guidelines of Beninese Ministry of Health, oral ferrous sulfate (200 mg daily) and folic acid (5 mg daily) supplements were provided for all pregnant women after the first ANC visit until 3 months after delivery. Additionally, 600 mg of mebendazole were given to pregnant woman in the second trimester. During the study period, drugs and nutrient supplements were given to women without charge.

Assessments at Age 1 Year

Research nurses were trained specifically to administer and score the cognitive and motor tests. Cognitive and motor functions of 636 1-year-old children were assessed by using the Mullen Scales of Early Learning (MSEL),¹⁰ which was translated and adapted for this

setting.¹¹ The MSEL encompasses the developmental domains of childhood and consists of 5 scales: GM, fine motor, language reception, language expression, and visual reception. After scoring each item, crude scores obtained from each of the 5 scales were transformed into normalized age-specific scores called *T* scores. Three children who obtained crude scores lower than 5 were given the least *T* score in the corresponding scale. *T* scores of all but GM scale were summed and converted to obtain the Early Learning Composite (ELC) scores.¹²

The quality of the home environment (using the Home Observatory Measurement of the Environment [HOME] inventory),¹³ postnatal depression (using the Edinburgh Postnatal Depression Scale),¹⁴ maternal nonverbal intelligent quotient (using the Raven's Progressive Matrices test),¹⁵ and infant anthropometric measurements were also assessed at 1 year of age.

Statistical Analyses

Summary statistics and distributional analyses were performed for all variables independently. The 2 dependent variables used in the analyses were the ELC and GM scores. The main independent variables were Hb concentrations (g/L) measured at first and second ANC visits and at delivery. The Hb concentrations were also categorized to reflect the anemia status of pregnant women.

We performed crude linear regression analyses between ELC and GM scores, and Hb concentration and anemia at each ANC visit and at delivery, separately. Bivariate analyses were performed to determine the correlation between covariates maternal and infant characteristics and ELC and GM scores. Direct acyclic graph was used to justify potential confounding.¹⁶ Covariates that were associated with the ELC and crude GM scores at $P < .20$ were selected for multiple

regression analyses. Stepwise removal of covariates was performed until all covariates in the models remained significant.¹⁷ Because Hb physiologically varies with gestational age, gestational age (trimesters) at time of Hb assessment was kept in the adjusted models even if it was not significant. Because of the physiologic increase in Hb toward the end of pregnancy,¹⁸ preterm births were excluded only when establishing the association between Hb concentration at delivery and infant ELC and GM scores (model II).

Afterward, we explored possible nonlinearity of the association between the ELC and GM scores and Hb at each ANC visit and at delivery by using Box-Cox right-hand-side only transformation with a maximum iteration of 10. We fixed the Box-Cox regression such that only Hb was transformable as specified in the equation:

$$y_j = \beta_0 + \beta_1 x_{1j}^{(\lambda)} + \gamma_1 z_{1j} + \gamma_2 z_{2j} \dots \gamma_k z_{kj} + \epsilon_j$$

where y is the ELC or GM score, x_1 is the Hb concentration (the only variable transformed by a Box-Cox transform parameter λ), and z_1, z_2, \dots, z_k are the covariates selected into the model and they were untransformed.¹⁹

The Box-Cox regression analyses were run for the association between GM and ELC scores and Hb concentrations at each ANC visit and at delivery. When nonlinearity was confirmed, a quadratic transformation of the Hb variable was used in the multiple regression models as we had hypothesized a plausible inverted U-shaped relationship (model III). Selected confounders were not in the causal pathway of prenatal Hb concentration and infant GM scores.

In a subsequent sensitivity analysis, we further adjusted model III for other known causes of anemia during pregnancy such as iron deficiency,

malaria, and helminth infection at the time of assessment while retaining the previous covariates in the model (model IV).

All statistical analyses were conducted by using Stata IC/12.1 (Stata Corp, College Station, TX).

Ethical Considerations

The study was approved by the institutional review boards of the University of Abomey-Calavi (Benin), New York University, Michigan State University, and the Research Institute for Development's Consultative Ethics Committee (France). At recruitment, we obtained informed consent from all pregnant women and guardians of children who participated in this study.

RESULTS

A total of 636 children (76.8% of 828 eligible) were followed until age 1 year. A flowchart of the follow-up of pregnant women and children has been published elsewhere.²⁰

Maternal baseline characteristics were similar between children not assessed and those assessed at age 1 year.²⁰ Mean maternal age (SD) at first ANC visit was 26.8 (5.6) years. The prevalence of malaria was 16.0% at first ANC visit, declined to 3.7% at second ANC visit, and then increased to 10.6% at delivery. Seven percent of children were born preterm, and 9.6% weighed <2500 g at birth (Supplemental Table 5). Mean (SD) Hb at first ANC visit was 103.7 (12.3), 105.4 (10.6) at second ANC visit, and 112.4 (14.1) at delivery. Anemia during pregnancy declined from 67.0% at first ANC visit to 38.4% at delivery (Table 1).

Table 2 shows the associations between maternal and infant characteristics and mean ELC and GM scores. Women who had at least primary education had children with significantly better cognitive and motor function than those who had never schooled ($P < .001$). Underweight women had children with the least GM scores. Family

TABLE 1 Gestational Age and Hb Distribution Among Women Whose Children Were Assessed for Cognitive and Motor Function at Age 1 y

	First ANC Visit, N = 636	Second ANC Visit, N = 627	Delivery, N = 609
Gestational age, wk			
Mean	22.0	28.8	38.3 ^a
SD	3.9	3.8	3.2
Range	10–28	18–40	23–55
Hb, g/L			
Mean	103.7	105.4 ^b	112.4
SD	12.3	10.6	14.1
Range	41–143	55–138	40–168
Anemia, Hb < 110 g/L			
n (%)	426 (67.0)	403 (64.4)	234 (38.4)

^a N = 596.

^b N = 626.

possession scores, Raven's Progressive Matrices, and HOME scores and infant weight at age 1 year were positively correlated with both ELC and GM scores.

As shown in Table 3, prenatal anemia at both ANC visits and at delivery were not associated to ELC scores even after adjusting for confounders. However, in the adjusted model, children of anemic mothers had better GM scores 2.3 (95% confidence interval [CI]: 0.1 to 4.6) than those of nonanemic mothers.

The HOME score, maternal education, and infant weight-for-age covariates that were strongly related to both GM and ELC scores were selected for the Box-Cox regression. The coefficients of the transformed Hb at all 3 assessments (β_1) were not significant for the models for ELC score and Hb concentrations. For the adjusted models involving GM score and Hb, we found that β_1 were significant for Hb at first and second ANC visits and in addition, the model rejected the hypothesis that the model is linear;

TABLE 2 Associations Between Characteristics of Pregnant Women and Children and ELC and GM Scores

Characteristics	ELC Score		GM Score	
	Mean	P	Mean	P
N = 636				
Gender				
Girl	99.2	.21	50.2	.14
Boy	97.8		51.8	
Weight-for-age z score ^a	0.2 ^c	<.001	0.3 ^c	<.001
Gravidity				
Primigravida	99.8	.28	48.2	.02
Multigravida	98.2		51.6	
Maternal education				
Never schooled	96.2	<.001	49.4	<.001
Primary or more	102.9		54.1	
Maternal occupation				
Housewives	96.1	<.001	49.2	.002
Employed	100.8		52.8	
Prepregnancy BMI				
Underweight	96.4	.07	48.9	.03
Normal	98.6		50.9	
Overweight/obese	101.5		54.8	
Family possession score ^b	0.1 ^c	.005	0.2 ^c	<.001
HOME score ^b	0.2 ^c	<.0001	0.2 ^c	<.001
RPM score ^b	0.1 ^c	.03	0.1 ^c	.002
EPDS score ^b	-0.0 ^c	.98	-0.0 ^c	.67

EPDS, Edinburgh Postnatal Depression Scale; RPM, Raven's Progressive Matrices.

^a N = 629.

^b N = 631.

^c Represented as correlation coefficients not means.

indicating that quadratic transformation of Hb at the first and second ANC could be used as hypothesized.

As shown in Table 4, the regression coefficient of the quadratic terms was negative and significant for the associations between Hb at first and second ANC visits and GM function at age 1 year. Diagrammatic representation of the nature of the association between prenatal Hb at first and second ANC visit and GM score are shown in Figs 1 and 2, respectively. In both figures, the GM for 1-year-old children increased sharply with increasing prenatal Hb until 90 g/L where the increase was steady then declined sharply over Hb level of 110 g/L. The peak for the quadratic curves was 98 g/L and 97 g/L at first and second ANC visits, respectively.

DISCUSSION

To our knowledge, this study is the first to investigate the relationship between Hb levels at different periods during pregnancy and infant cognitive development. This study reveals that there is an inverted U-shaped relationship between prenatal Hb at first and second ANC visits and infant GM function indicating that low and high Hb concentrations during pregnancy may be detrimental to the early motor functions of 1-year-old children. Peak infant GM scores were observed at prenatal Hb of 98 g/L and 97 g/L at first and second ANC visits, respectively. However, prenatal anemia was associated with high GM scores at second ANC visit.

An important strength of our study is that we assessed Hb at different times during pregnancy. Also, the follow-up of 76.4% of eligible children recorded in this study is appreciable because it compares well to other mother-child cohorts in Africa.²¹ Additionally, the MSEL used is a comprehensive tool for cognitive and motor assessment of children. Further, the consideration of several potential confounders in our

TABLE 3 Relationship Between Prenatal Anemia and ELC and GM Scores

Anemia	ELC Score		GM Score	
	Crude Mean Difference (95% CI)	Adjusted Mean Difference (95% CI) ^a	Crude Mean Difference (95% CI)	Adjusted Mean Difference (95% CI) ^b
First ANC visit				
No anemia	Reference	Reference	Reference	Reference
Anemia	-1.0 (-3.4 to 1.4)	-0.4 (-2.7 to 1.9)	0.4 (-2.0 to 2.7)	1.6 (-0.7 to 3.9)
Second ANC visit				
No anemia	Reference	Reference	Reference	Reference
Anemia	0.5 (-1.8 to 2.8)	0.6 (-1.6 to 2.9)	2.2 (-0.2 to 4.5)	2.3 (0.1 to 4.6)*
Delivery				
No anemia	Reference	Reference	Reference	Reference
Anemia	-0.7 (-3.1 to 1.7)	-0.6 (-2.9 to 1.8)	-1.1 (-3.5 to 1.4)	-0.6 (-3.0 to 1.8)

*P < 0.05

^a Adjusted for maternal education, infant weight-for-age z score, HOME score, and gestational age at blood assessment.

^b Adjusted for maternal education, gravidity, family possession, infant weight-for-age z score, HOME score, and gestational age at Hb assessment.

study such as socioeconomic factors, mother-child interaction, maternal postnatal depression, and maternal IQ based on statistical significance and direct acyclic graphs contributes to the strength of this study.

During pregnancy, there is a ~50% physiologic increase in plasma volume, peaking between 28 to 34 weeks of gestation, and a disproportionate 20% to 30% increase in red cell mass, which results in a decrease Hb concentration.²² This hemodilution during the second and third trimesters may be essential for fetal growth as the reduced thickness of the blood enhances effective flow of oxygen and other nutrients to the fetus.²² This could explain 2 observations in our results. Firstly, the observed high motor function

among children of anemic women at second ANC visit when more than 50% of women were between 28 and 34 weeks of gestation. Secondly, the optimum GM function observed among children of pregnant women with Hb levels between 90 and 110 g/L at first and second ANC visits even after adjusting for other known risk factors for low maternal Hb. This Hb range (90-100 g/L) for optimum GM function, considered anemic, raises questions about the cutoff for anemia.

Low GM function was observed at low Hb levels on the left end of the inverted U-shaped curve. The observed low levels of Hb among pregnant women at first ANC visit could be a result of pathologic anemia due to malaria, helminths, and iron deficiency among other unmeasured

micronutrient deficiencies that are risk factors. Low Hb levels at second ANC visit could be due to a combination of the aforementioned etiologies and physiologic increase in plasma volume.²³ Extremely low Hb concentration implies deficient oxygen transport to the fetus.²⁴ Although the fetus has mechanisms to adapt to decreased maternal oxygen delivery, the costs of such adaptation could be expensive, possibly leading to impaired fetal growth.²²

Low Hb levels during pregnancy could diminish the amount of accessible iron for the fetus. Iron availability is essential for effective fetal growth and function of several organ systems including the brain and skeletal muscle.²⁵ Prenatal iron deficiency is associated with decreased apical dendrite length and

TABLE 4 Linear and Quadratic Models for the Association Between Maternal Hb Concentration and Infant ELC and GM Scores

	Model I, β (95% CI)	Model II, β (95% CI)	Model III, β (95% CI) ^a	Model IV, β (95% CI) ^a
ELC score				
First ANC visit	0.05 (-0.04 to 0.14)	0.03 (-0.06 to 0.11)	NA	NA
Second ANC visit	0.02 (-0.09 to 0.12)	-0.00 (-0.10 to 0.10)	NA	NA
Delivery	0.03 (-0.06 to 0.12)	0.02 (-0.07 to 0.10)	NA	NA
GM score				
First ANC visit	0.02 (-0.07 to 0.11)	-0.04 (-0.13 to 0.05)	-5.5×10^{-3} (-9.5×10^{-3} to -1.4×10^{-3})**	-5.6×10^{-3} (-9.6×10^{-3} to -1.6×10^{-3})**
Second ANC visit	-0.06 (-0.17 to 0.04)	-0.09 (-0.19 to 0.01)	-6.6×10^{-3} (-1.2×10^{-2} to -9.2×10^{-4})**	-7.0×10^{-3} (-1.3×10^{-2} to -1.2×10^{-3})*
Delivery	-0.02 (-0.11 to 0.07)	-0.04 (-0.13 to 0.05)	NA	NA

Model I, crude (unadjusted); Model II, linear model adjusted for maternal education, infant weight-for-age z score, HOME score, and gestational age at blood assessment (for ELC score) and in addition, gravidity, family possession (for GM score); Model III, quadratic model adjusted for maternal education, gravidity, family possession, infant weight-for-age z score, HOME score, and gestational age at Hb assessment; Model IV, Model III + adjusting for iron deficiency, helminth, and malaria at respective ANC visit; NA, not applicable. *P < .05; **P < .01. ^a β (95% CI) for the quadratic term for Hb.

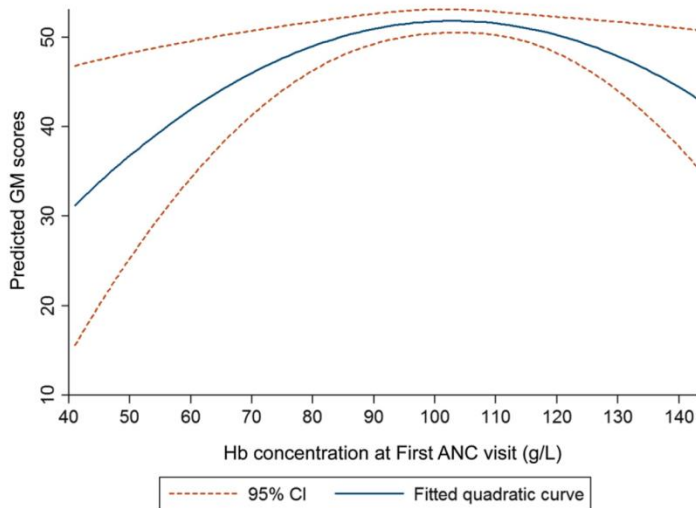


FIGURE 1 Relationship between Hb concentration at first ANC visit and infant GM scores. The solid line indicates the fitted model, whereas the dash lines represent 95% CIs.

could alter the hippocampal development even in the early stages of pregnancy.^{25,26} The apical dendrites in the hippocampus play a substantial role in memory and sensory functions. Studies in pregnant mice have also revealed that

there is a direct relationship between maternal iron levels and fetal brain iron.²⁷ Low fetal brain iron could be responsible for the poor neuromotor skills in infants as a study revealed that 1-year-old children with brain iron deficiency ($\leq 34 \mu\text{g/L}$) had

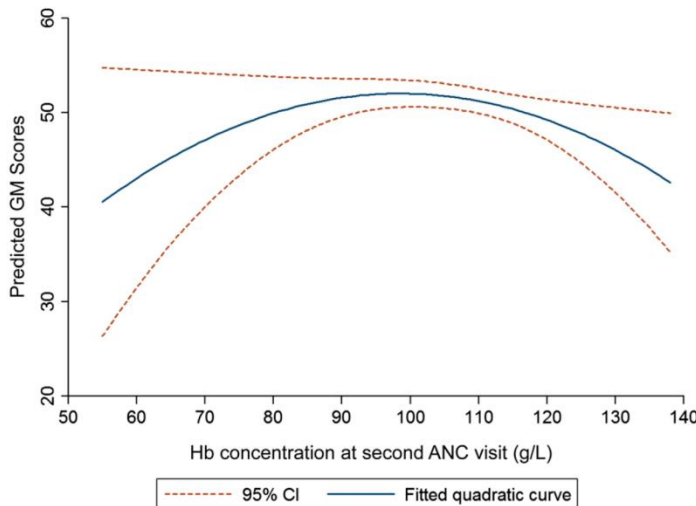


FIGURE 2 Relationship between Hb concentration at second ANC visit and infant GM scores. The solid line indicates the fitted model, whereas the dash lines represent 95% CIs.

slower motor development compared with those with adequate brain iron.^{27,28} However, the inverted U-shaped association persisted at second ANC visit after women were given IFA supplements and even after adjusting for iron deficiency, helminth, and malaria suggesting that we really measured the effect of Hb on motor function.

Conversely, higher than normal prenatal Hb could be due to nonexpansion of plasma and as a result, increased blood viscosity. Increased viscosity implies reduced blood flow to the intervillous space.²² This could lead to fetal stress due to placental-fetal perfusion and consequently impair the development of essential fetal organs.²² This condition of hemoconcentration could explain the right-hand-side of the quadratic curves where increasing Hb concentrations during pregnancy were associated with decreasing infant GM scores and may reflect the disadvantage of hemoconcentration during pregnancy.

The curvilinear nature of the observed relationship in our study is analogous to that between prenatal Hb and low birth weight.^{29,30} Maternal Hb concentration is a predictor of pregnancy complications and several adverse birth outcomes such as low birth weight, stillbirth, and preterm birth, which could mediate the pathway between prenatal Hb and infant GM function.³¹ These adverse birth outcomes have been identified to be risk factors for poor cognitive and motor development.^{32,33} A study of ~223 000 singleton pregnancies also revealed a U-shaped relationship between maternal Hb concentration and early neonatal mortality with the lowest perinatal mortality in the 90 to 110 g/L Hb range.³⁴

Chang et al³⁵ suggested that children born to women who were anemic at the third trimester performed significantly lower than those of nonanemic women in cognitive assessment tests, but the authors did not assess the relationship between

Hb concentration, as a continuous variable, and the cognitive scores. Contrary to the findings of Chang et al,³⁵ we observed no significant association between maternal anemia and infant cognitive function of our study. In the aforementioned study, the authors only assessed Hb in the third trimester with anemia prevalence of 45.2%. They also stated that anemia in the study population was mainly due to iron deficiency, which could explain the difference in findings. In our study population, prenatal anemia was multifactorial.⁵

Pregnant women were enrolled in a clinical trial and as a result received several interventions that are likely to improve maternal health, birth outcomes, and neonatal health and potentially influence the cognitive function of children. Hence, our study is likely to underestimate the real magnitude of the effect of low and high maternal Hb on offspring cognitive development in the population who may seek fewer than 2 ANC visits during pregnancy. Also, caution

should be taken in interpreting our results especially in the extreme ends of the curve as the CIs are wider due to the small number of observations.

CONCLUSIONS

This study reveals that there is an inverted U-shaped relationship between maternal Hb concentration during pregnancy and motor function of 1-year-old children. There appears to be an optimal range maternal Hb (90–110 g/L) that may be beneficial to infant GM function. It is the first study to report a quadratic relationship between prenatal Hb concentration and GM development of infants. Further studies are required to corroborate this curvilinear association. A follow-up study to investigate if this association persists even in the later life of the children is also necessary.

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ABBREVIATIONS

ANC: antenatal care visit
CI: confidence interval
ELC: Early Learning Composite
GM: gross motor
Hb: hemoglobin
HOME: Home Observatory
Measurement of the Environment
IFA: iron and folic acid
IPTp: intermittent preventive treatment of malaria in pregnancy
MiPPAD: Malaria in Pregnancy Preventive Alternative Drugs
MSEL: Mullen Scales of Early Learning

Dr Xiong designed the TOVI study, assisted in statistical analyses, and reviewed and revised the initial manuscript; Drs Massougbojji and Cot acquired funding for the MiPPAD study, conceptualized, designed, and supervised the MiPPAD trial, and reviewed and revised the initial manuscript; Dr Bodeau-Livinec conceptualized and designed the TOVI study, acquired funding for the TOVI study, trained research nurses to use the Mullen Scales of Early Learning cognitive assessment, supervised initial data collection, and reviewed and revised the initial manuscript, and all authors approved the final manuscript as submitted.

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4.4 Supplementary Statistical Analysis

Initial analysis conducted by categorizing Hb concentration traditionally into two groups (anemia or no anemia) or four groups (severe anemia, moderate anemia and mild anemia as defined by the WHO) showed no significant difference in relation to ELC or GM. It is for this reason that we considered using the continuous variable, Hb concentration, in our analysis.

Also in the preliminary analysis, we categorized GM score into two groups using the mean minus SD (37) as the cut-off since we expected scores below the cut-off an indication of cognitive or motor deficit. Another categorization of the GM scores was done using the mean GM score of 51 as the cut-off. The categories of lower scores were named *Poor GM function* and those of higher scores, *Good GM function*. Similar categorizations performed for the ELC score in its relationship with prenatal Hb levels yielded similar results of no significant relationship as shown in the article and as a result are not shown in the supplementary results section of this chapter of the thesis. We also categorized Hb as done in the study by Steer *et al*¹⁸⁶, thus taking the 10th percentile of Hb concentration (87 g/L) as the starting point, we created subsequent categories with an interval of 10 g/L. Hence our Hb categories were (<87 g/L, 87- 96 g/L, 97-106 g/L, 107-116 g/L, 117-126 g/L and >126 g/L).

In the univariate and multivariate analysis, where the expected number of any cell was less than 5, the category was collapsed with the adjacent category (most often at the extreme ends). Using the 97-106 g/L as the reference category (because it is more similar to the optimum range of 90-110 reported from the inverted U-shape), we performed a non-conditional logistic regression analysis of the relationship between Hb and the risk of poor GM score, adjusting for potential confounding variables. As we did in the sensitivity analysis using the continuous hemoglobin variable, we further adjusted for known causes of anemia at the time of Hb assessment (particularly, malaria, helminth infection and ID).

4.5 Supplementary Results

Table 5.1 shows the proportion of pregnant women who belonged to a particular category based on their hemoglobin concentration measured at the first or second ANC visit.

Table 4.1 Prenatal hemoglobin (Hb) categories at first and second ANC visit

Hb category (g/L)	First ANC visit, N=863 n (%)	Second ANC visit, N=841 n (%)
<87	81 (9.4)	32 (3.8)
87-96	156 (18.1)	133 (15.8)
97-106	264 (30.6)	285 (33.9)
107-116	260 (30.1)	283 (33.7)
117-126	83 (9.6)	90 (10.7)
>126	19 (2.2)	18 (2.1)

Using the mean gross motor (GM) score as the cut-off for infant gross motor function, a plot of the proportion of poor GM function (less than 51) by Hb concentration (g/L) at first and second ANC visits reveal a **U-shaped relationship** indicating that the proportion of poor GM function was highest at the extreme ends of Hb concentration (Hb < 87 g/L and Hb > 126 g/L) as shown in figures 4.2 and 4.3. Similar to figures 4.2 and 4.3, figure 4.4 also shows a U-shaped relation between the proportion of poor GM function in one-year-old children and hemoglobin concentration of their mothers at the first ANC visit. In figure 4.4, the last two groups were collapsed so that the new group represents Hb > 116 g/L.

Using the mean GM score (57) as the cut-off:

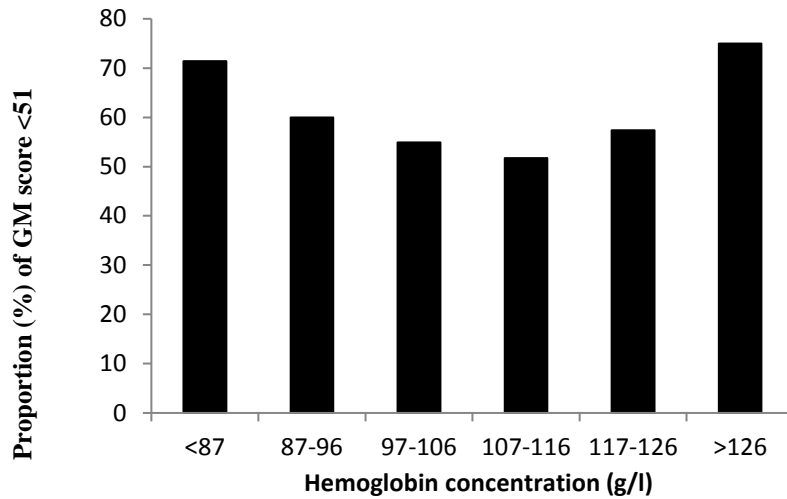


Figure 4.2 Risk of poor infant GM function by hemoglobin concentration at first ANC visit

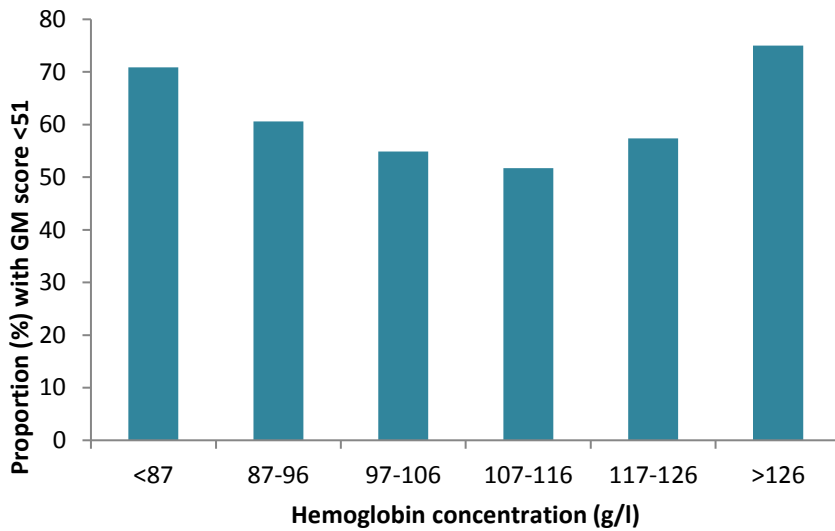


Figure 4.3 Risk of poor infant GM function by hemoglobin concentration at second ANC visit

Using the mean-SD GM score as the cut-off:

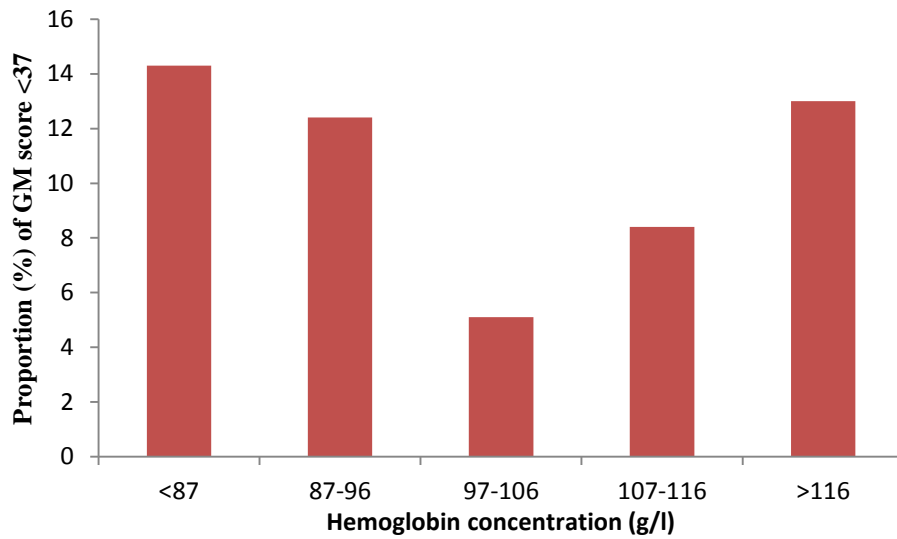


Figure 4.4 Risk of poor infant GM function by hemoglobin concentration (g/L) at first ANC visit

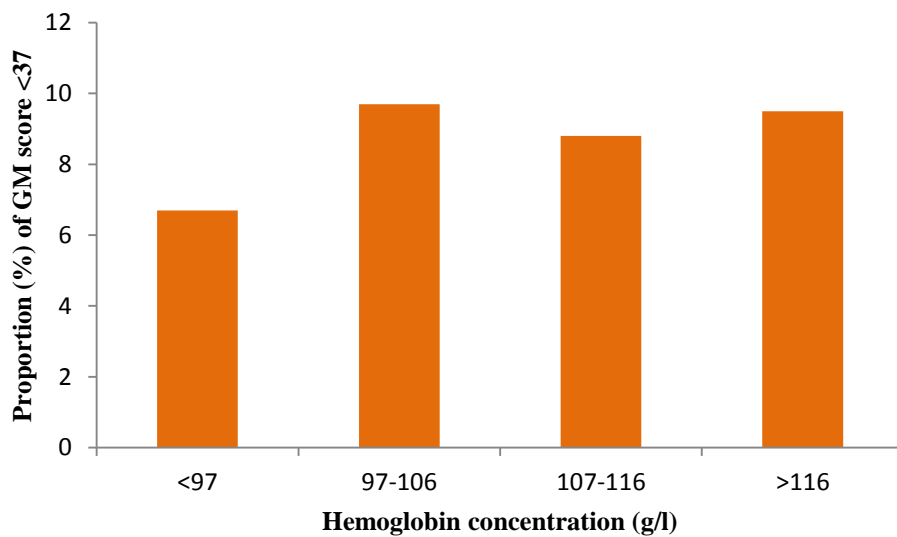


Figure 4.5 Risk of poor infant GM function by hemoglobin concentration (g/L) at second ANC visit

Table 4.2 shows the relationship between prenatal Hb concentrations at first and second ANC visits and infant GM function at age one year. Using Hb 97-106 g/L as the reference category, the odds of having a child with poor GM function generally was higher in Hb concentration categories less than 97 g/L and Hb concentration categories greater than 106g/L. Using the mean as the cut-off for GM function, children of mothers with Hb concentration greater than 126 g/L at first and second ANC visits were 3.8 (95% CI: 1.1-13.2) and 9.1 (95% CI: 1.7-48.3) times more likely to have poor GM function compared to those whose mothers Hb concentration at these ANC visits was between 97 g/L and 106 g/L.

Table 4.2 Relationship between prenatal Hb concentration and the odds of poor gross motor (GM) function in one-year-old children

	Using mean (GM function)	Using mean-sd (GM function)
	Adjusted OR (95% CI) [#]	Adjusted OR (95% CI) ^{##}
Hb at 1st ANC visit (g/L)		
<87	2.0 (1.0-4.2)	1.2 (0.4-4.0)
87-96	1.3 (0.8-2.2)	3.0 (1.2-7.3)*
97-106 (Reference)	1	1
107-116	0.9 (0.6-1.4)	2.4 (1.0-5.7)*
117-126	1.3 (0.7-2.4)	4.0 (1.5-10.9)†**
>126	3.8 (1.1-13.2)*	
Hb at 2nd ANC visit (g/L)		
<87	1.4 (0.6-3.5)	0.6 (0.3-1.5)‡
87-96	1.1 (0.7-1.8)	
97-106 (Reference)	1	1
107-116	1.4 (0.9-2.1)	0.9 (0.4-1.7)
117-126	1.3 (0.7-2.3)	
>126	9.1 (1.7-48.3)*	1.1 (0.5-2.8)‡

*P-value < 0.05

** P-value < 0.01

‡ Hb ≥117 g/L † Hb <97

#Adjusted for maternal education, gravidity, trimester at Hb assessment, RAVEN score, child sex, home score, weight-for-age Z-score ## Adjusted for gravidity, trimester at Hb assessment, family possession, child sex, home score, weight-for-age Z-score

Table 4.3 Relationship between prenatal Hb concentration and the odds of poor gross motor (GM) function in one-year-old children adjusting for known causes of anemia

	Using mean (GM function)	Using mean-sd (GM function)
	Adjusted OR (95% CI) [#]	Adjusted OR (95% CI) ^{##}
Hb at 1st ANC visit (g/L)		
<87	2.0 (1.0-4.2)	1.0 (0.3-3.8)
87-96	1.4 (0.8-2.3)	2.9 (1.1-7.4)*
97-106 (Reference)	1	1
107-116	1.0 (0.7-1.6)	2.5 (1.1-6.0)*
117-126	1.4 (0.7-2.6)	4.1 (1.5-11.2)†**
>126	4.3 (1.2-15.0)*	
Hb at 2nd ANC visit (g/L)		
<87	1.4 (0.5-3.5)	0.6 (0.3-1.5)‡
87-96	1.1 (0.7-1.8)	
97-106 (Reference)	1	1
107-116	1.4 (0.9-2.0)	0.8 (0.4-1.7)
117-126	1.6 (0.9-2.8)‡	0.8 (0.3-2.2)‡
>126		

**P*-value < 0.05

** *P*-value < 0.01

‡ Hb ≥117 g/L † Hb <97

All models were adjusted for iron deficiency, helminth infection and malaria at the time of Hb assessment and in addition, [#]Adjusted for maternal education, gravidity, trimester at Hb assessment, RAVEN score, child sex, home score, weight-for-age Z-score ^{##} Adjusted for gravidity, trimester at Hb assessment, family possession, child sex, home score, weight-for-age Z-score

The findings from this supplementary analysis confirm the evidence of a U-shaped relationship between the risk of poor GM function of one-year-old children and prenatal Hb concentration. These findings are similar to the quadratic relationship shown in the published article; however, these findings further show evidence of increased risk of poor GM score among children of mothers whose mothers had very low or high Hb concentration during pregnancy. When GM scores lower than 37 (mean-SD) was defined as poor GM function, mothers with hemoglobin concentration 87-96 g/L, 107-116 g/L and greater than 116g/L at the first ANC visit had children with increased odds of poor GM function [adjusted odds ratio, AOR: 3.0 (95% CI: 1.2-7.3), 2.4 (95% CI: 1.0-5.7) and 4.0 (95% CI: 1.5-110.9), respectively].

Evidence of the increased risk of poor GM function in other categories in comparison with the reference category (97-106 g/l), which is within the anemia category, may highlight the benefits of hemodilution during pregnancy. As explained in the attached article, the increased risk of

Chapter 5. Impact of Prenatal and Early Postnatal Iron Deficiency on Infant Cognition

This chapter is intended to report the results of the analysis on the relationship between prenatal and postnatal ID (at birth and age one year) and infant cognitive function. Although the aim of the thesis was to investigate the effect of prenatal causes of anemia on infant cognition, we decided to consider postnatal ID in this particular chapter because of the existing slight evidence of the impact of ID in children on their cognitive development. Also, considering postnatal results, we hoped to investigate if any observed association or the lack thereof between prenatal ID and infant cognition is not explained by infant ID as has been reported in other studies.

Although this thesis did not cover the impact of malaria, an important risk factor for anemia in pregnancy in endemic regions, on infant cognition, in this particular chapter we investigate the impact of malaria on the relationship between ID and infant cognition. The need to account for malaria arises from the controversy of the role iron sufficiency plays in malaria risk. These are detailed in the subsequent manuscript that has been submitted for publication in Pediatrics.

Summary of the unpublished article including what is already known on the subject and what the article adds is presented. The tables that correspond to the manuscript are shown after the references of the manuscript.

NB: List of references, figures and tables that appear in the article are independent and therefore do not follow the order presented in the entire thesis.

Effects of Prenatal Iron Deficiency on Ferritin Levels at Birth and Cognitive Function of Infants

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Abbreviations: ANC- Antenatal Care Visit; WHO- World Health Organization; LBW- Low Birth Weight; IPTp- Intermittent Preventive Treatment of malaria in pregnancy; MiPPAD- *Malaria in Pregnancy Preventive Alternative Drugs*; MSEL- Mullen Scale of Early Learning; ELC- Early Learning Composite; GM- Gross Motor; Hb- Hemoglobin; ID- Iron Deficiency; IDA- Iron Deficiency Anemia; CBSF- Cord Blood Serum Ferritin; HOME- Home Observatory Measurement of the Environment; EPDS - Edinburgh Postnatal Depression Scale; RPM- Raven's Progressive Matrices; BMI - Body Mass Index; SD - Standard deviation; CI - Confidence Interval

Key Words: cohort study, iron deficiency, anemia, pregnancy, cognition, child development, sub-Saharan Africa

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What's known on the subject: Iron deficiency (ID) in children is associated with poor cognitive development. However, the literature on the impact of prenatal ID on ferritin levels at birth and infant cognitive and motor functions are limited.

What This Study Adds: In a malaria-endemic region, ID in pregnancy in the context of iron supplementation is not associated with cord blood serum ferritin(CBSF) concentration and infant cognitive and motor development. Iron deficiency anemia at delivery is however associated with lower CBSF concentrations.

Contributor's Statement:

Michael O. Mireku: Mr. Mireku coordinated and supervised data collection of the TOVI study from the three study sites, performed and interpreted data analyses, drafted the initial manuscript, and revised and approved the final manuscript as submitted.

Michael J. Boivin: Dr. Boivin trained research nurses to use MSEL cognitive assessment, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

Leslie L. Davidson: Dr. Davidson designed the TOVI study, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

Romeo Zoumenou: Mr. Zoumenou supervised initial data collection of the TOVI study, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

Achille Massougbdji and Michel Cot: Drs. Massougbdji and Cot acquired funding for the MiPPAD study, conceptualized, designed and supervised the MiPPAD trial, reviewed and revised the initial manuscript and approved the final manuscript as submitted.

Florence Bodeau-Livinec: Dr. Bodeau-Livinec conceptualized and designed the TOVI study, acquired funding for the TOVI study, trained research nurses to use MSEL cognitive assessment, supervised initial data collection, reviewed and revised the initial manuscript and approved the final manuscript as submitted.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

Objective

To investigate the impact of prenatal maternal iron deficiency (ID) on cord blood serum ferritin (CBSF) concentration and infant cognitive and motor development.

Methods

Our prospective cohort study included 636 mother-singleton child pairs from 828 eligible pregnant women who were enrolled during their first antenatal care (ANC) visit in Allada, Benin, into a clinical trial comparing the efficacy of mefloquine and sulfadoxine-pyrimethamine. Venous blood samples of women were assessed for ferritin and hemoglobin concentrations at the first and second ANC visits (occurring at least one-month apart) and at delivery. Women were prescribed daily iron and folic acid supplements throughout pregnancy. Hematologic examinations were repeated for cord blood at birth. At age one year, cognitive and motor functions of children were assessed using the Mullen Scales of Early Learning (MSEL).

Results

The prevalence of prenatal ID at first and second ANC visits, and at delivery was 30.5%, 34.0% and 28.4%, respectively. CBSF concentrations were similar between ID and non-ID pregnant women. Neither prenatal ID nor CBSF concentration was associated with poor cognitive or gross motor function of children at one year of age. CBSF concentrations were lower among mothers who had iron deficiency anemia (IDA) at delivery compared to non-IDA pregnant women [adjusted mean difference: -0.2 (95% Confidence Interval: -0.4, -0.0)].

Conclusions

In a malaria-endemic region, ID in pregnancy in the context of iron supplementation is neither associated with CBSF concentration nor with infant cognitive and motor development. Prenatal IDA around the time of delivery is associated with lower CBSF concentrations.

Introduction

Iron deficiency (ID) remains the world's most prevalent micronutrient deficiency affecting about 2 billion people worldwide.¹ Pregnant women and children under five years especially, in less developed countries, are most at risk of ID.² During pregnancy, there is increased demand for iron to accommodate the needs of the fetal-placental unit. By the second trimester of pregnancy, the daily requirements increase to 6.8 mg which is three times that of a non-pregnant woman.³ This increased physiological demand for iron renders pregnant women highly vulnerable to ID in the absence of iron supplementation or proper nutrition. ID is assumed to account for more than half of the burden of anemia during pregnancy.⁴

One reason why iron is particularly important during pregnancy is the indispensable role it plays in fetal development and infant cognition, via biochemical processes involved in brain formation and function. Specifically, iron is involved in the appropriate myelination of the white matter of cerebellar folds, hippocampus development and neurotransmitter synthesis which are essential in fetal and child brain function.^{5,6}

ID during pregnancy could therefore have deleterious consequences for fetal development via deprived iron availability for fetal neurodevelopment. The precariousness of the fetus during this phase renders it vulnerable to insults including that caused by prenatal maternal micronutrient deficiencies which could have adverse consequence on fetal brain and cognitive function even after birth.⁷ Factors associated with delayed or sub-optimal infant cognitive functions have been shown to be indicative of child non-readiness for school.⁸

Evidence from some studies shows early postnatal exposure to iron deficiency in children to be a risk factor for poor cognitive and psychomotor development and in some cases, has been linked to long term behavioral consequences.^{9,10} In children, several studies have shown the importance

of iron sufficiency for cognitive development, appropriate behavioral development and gross motor function.¹¹⁻¹³ However low maternal prenatal iron levels measured as serum ferritin has not been consistently linked with low cord blood serum ferritin (CBSF) concentrations.^{9,10}

Despite the known detrimental consequences of early iron deficiency on cognition, studies on the impact of prenatal iron deficiency on neurocognitive outcomes in human subjects remain scanty. Hence, we set out to investigate the impact of iron deficiency at three periods of pregnancy and in early postnatal stages of infant development on the cognitive development of one-year-old children.

Methods

Study design and population

The prospective cohort study (called *Tovi*) which was principally set to investigate the relationship between hemoglobin concentration and infant cognitive function included 636 mother-singleton child pairs from 828 eligible pregnant women and their offspring.¹⁴ This paper is therefore a secondary analysis of that data. The mothers had all been enrolled earlier in the *Malaria in Pregnancy Preventive Alternative Drugs* (MiPPAD) clinical trial (NCT00811421) that compared the efficacy of mefloquine and sulfadoxine pyrimethamine as intermittent preventive treatment for pregnant women (IPTp).

HIV negative women of at most, 28 weeks of gestation who had no prior intake of IPTp, anthelmintics or iron and folic acid supplements were recruited into the MiPPAD clinical trial during their first antenatal care (ANC) visit to any of three maternity clinics in three sub-districts of Allada, Benin. Detailed inclusion criteria into the MiPPAD trial are explained elsewhere.¹⁵

The women were followed throughout pregnancy, at a second ANC of at least one-month after the first, and at delivery. At the baseline and during the periods of follow-up, anthropometric, socioeconomic and biological data were obtained from pregnant women. At delivery, infant birth weight and gestational age were determined and recorded. Infants were followed at age one year during which the quality of the home environment (using the Home Observatory Measurement of the Environment inventory, HOME)¹⁶, maternal postnatal depression (using the Edinburgh Postnatal Depression Scale, EPDS)¹⁷, maternal non-verbal intelligent quotient (using the Raven's Progressive Matrices test, RPM)¹⁸ and infant anthropometric measurements were assessed.

Prenatal hematologic and other biological assessments

At the first ANC visit, 4 ml venous blood samples of pregnant women was collected into an iron-free dry tube and stool samples were collected into a clean tube for biological assessments after which the pregnant women were administered IPTp, anthelmintics (if they were in their second trimester), and oral ferrous sulfate (200 mg daily) and folic acid (5 mg daily) as part of the ANC package in Benin. We provided daily iron and folic acid supplements, be taken at home, throughout pregnancy. Blood withdrawal and biological assessments were repeated at the second ANC visit and at delivery.

Serum ferritin concentrations were measured from 500µL of serum with an AxSym Immuno-Assay Analyzer (Abbot Laboratories, Abbot Park, IL). To correct for high levels of ferritin concentration resulting from inflammations, a rapid slide test was performed to determine the concentration of C-reactive protein (CRP) in the blood (CRP Latex; Cypress Diagnostics Inc., Campbellsville, Ontario, Canada). Inflammation was defined as CRP levels higher than 5 mg/L.

ID was defined as serum ferritin concentration less than 12 $\mu\text{g/l}$ or serum ferritin concentrations between 12 $\mu\text{g/l}$ and 70 $\mu\text{g/l}$ if CRP concentration in the presence of inflammation.

The Hemo-Control photometer (EKF Diagnostics, Barleben/Magdeburg, Germany) device was used to determine the concentration of hemoglobin. Hemoglobin (Hb) concentration was recorded in g/l. Hemoglobin concentration less than 110 g/l was considered anemia. A pregnant woman was deemed to have iron deficiency anemia (IDA) if she was iron deficient and anemic at the time of assessment. Malaria infection was determined by using the Lambaréné technique (detection threshold of 5 parasites/ μL).¹⁹

Hematologic and biological assessments were repeated for cord blood samples taken at delivery for a subset of 516 children. Anemia at birth was defined as cord blood Hb < 140 g/l.²⁰

Neurocognitive assessment

Of the 828 eligible singletons, 76.8% were evaluated for cognitive development at one year of age (from April 2011 to November, 2012) as shown in Figure 1.

Cognitive and motor developments of children were evaluated using the American Guidance Service edition of the Mullen Scales of Early Learning (MSEL).²¹ The MSEL comprises of five scales: gross motor (GM), fine motor, expressive language, receptive language and visual perception. The GM scale assessed central motor control and the ability of movement in several positions of the body. The sum of test scores obtained by the children in each MSEL scale was transformed into standardized age-specific (monthly) scores called the *T* scores, which are normally distributed. The standardized *T* scores of the fine motor, expressive language, receptive language and visual perception scales were combined to produce the Early Learning Composite

(ELC) score. The ELC is a measure of the general cognitive factor which is indicative of early cognitive performance.²¹

The MSEL were translated and adapted for this setting.²² Detailed quality assurance and reliability of assessments have been published.²² Assessors were blinded to prenatal maternal and neonatal iron levels and birth outcomes.

The sample size of 636 mother-infant pairs was enough to detect small differences of ≥ 0.16 SD in ELC or GM scores between children of ID mothers and those of non-ID mothers.

Statistical analysis

First, we compared maternal and infant characteristics between those who had CBSF assessments performed and those who did not. We also compared the maternal characteristics of children who were assessed for cognitive function and those who were not. Then we described separately, maternal and infant hematologic characteristics among the children who were assessed for cognitive and motor functions. We also conducted a univariate analysis between maternal and infant characteristics and CBSF concentration and ELC and GM scores.

Due to the non-normal distribution of CBSF concentration and the lack of homoscedasticity, we log transformed the CBSF concentration variable. The transformed variable was normally distributed from a visual inspection from the histogram. Using a multiple linear regression (MLR) analysis, we compared the mean log CBSF concentration, ELC and GM scores between iron deficient pregnant women and non-iron deficient pregnant women. Direct acyclic graphs, as shown in figure 2, were used in selecting confounders in the MLR analysis. All models were adjusted for maternal education and malaria infection at the time of iron assessment irrespective

of significance except malaria at birth, as there were only two cord blood samples positive with plasmodium parasites. To simplify models, stepwise removal of other covariates was performed until the *P*-values of covariates in the model were less than 0.05 with the exception of the HOME score, which is an indicator for infant development.

Statistical analyses were performed using Stata IC/14.1 for MAC (StataCorp Lp, College station, TX). Statistical significance was defined as *P*-value less than 0.05 from two-tailed tests.

Ethical Considerations

The institutional review boards of the University of Abomey-Calavi, Benin; New York University and Michigan State University, USA; and the Research Institute for Development's (IRD) Consultative Ethics Committee, France; approved the study. At recruitment, we obtained informed consent from all pregnant women and guardians of children who participated in this study.

Results

Maternal and infant characteristics of those who had CBSF assessed were comparable on all covariates to those who did not have CBSF assessed with the exception of gestational age at first ANC visit (small difference of one week), maternal occupation, prepregnancy BMI, and gestational age at birth. The prevalence of ID at first ANC and at delivery among pregnant women whose children were assessed for cognitive function was significantly less than the prevalence among those whose children were not assessed for cognitive function at age one year. (Supplemental Table 1) Women who had ID or IDA at baseline had a higher median gestational age compared to those who did not have ID or IDA, respectively. ID was common among

primigravida women compared to multigravida women as shown in Table 1. The median age (inter quartile range, IQR) of pregnant women at their first ANC visit was 25 (21-30) years. At baseline, the median gestational age (IQR) was 23 (19-26) weeks. The prevalence of ID among pregnant women at first and second ANC visits, and at delivery was 30.5%, 34.0% and 28.4%, respectively (Table 2).

CBSF concentration was significantly higher among women who were never schooled ($P=0.042$). Prenatal ID at any of the follow-up periods was not associated with CBSF concentration in the univariate analysis as shown in Table 3. Absent maternal education, lack of employment and fewer family possessions were all associated with lower ELC and GM scores.

As shown in Table 4, there were no statistically significant differences between ELC and GM scores for children whose mothers had prenatal ID or IDA at any of the follow-up visits and those whose mothers did not have ID or IDA at any of the follow-up visits. Likewise, anemia and ferritin levels at birth were not associated with ELC and GM scores at age one year. In the adjusted model, there was no difference in CBSF concentration between iron deficient and non-iron deficient pregnant women. However, compared to pregnant women with no iron deficiency anemia at delivery, women with iron deficiency anemia at delivery had significantly lower CBSF concentration [AMD (95% CI): -0.2 (-4.0, -0.0)] but as noted above this did not impact on the child's ELC or GM scores.

Discussion

In this current study, we found no relationship between prenatal ID or IDA or CBSF concentration and cognitive and motor functions of one-year-old children. Our study also revealed that iron deficiency during pregnancy is not associated with lower CBSF concentrations

in the context of iron supplementation. We however found that IDA around delivery was associated with low CBSF concentrations. To our knowledge, this is the first study to investigate the impact of prenatal iron deficiency on infant cognition in Benin in the context of prescribed iron supplementation and prophylaxis against malaria and helminthes.

Our findings are similar to a recent randomized clinical trial in Hebei, China, that reported no difference in gross motor function of children whose mothers were in the iron supplementation arm and those whose mothers were in the placebo arm during pregnancy.²³ Our results however contradicts the findings of another study conducted among a rural Chinese population, in which hemoglobin concentration was used as a marker for IDA in pregnancy. In the Chinese study, the authors reported that children of mothers with IDA had poorer mental development compared to those of non-IDA mother.²⁴ The contradictory findings may arise from the accurate marker of maternal iron stores, serum ferritin, use in our studies and the account for inflammation that may result in high serum ferritin levels. In our study population, the etiology anemia is multifactorial (helminth infections, malaria, other micronutrient deficiencies) and as such using hemoglobin concentration as a marker for ID may have been misleading. The relationship between prenatal hemoglobin concentration and infant cognitive and motor development in our study population has already been reported in an earlier publication.¹⁴

Considering the vulnerability of the fetus to prenatal insults and the essential role of iron in neurodevelopment, it is surprising that we found no association between prenatal ID and infant cognitive development. However, during pregnancy, the fetus has priority to iron supply over the pregnant woman's needs in order to ensure adequate iron stores within the first six months of life and thus leaving the mother deficient of iron in the absence of adequate supplementation.^{27,28} This could explain the absence of difference in CBSF between iron deficient and non-iron

deficient pregnant women although IDA at delivery was associated with lower CBSF concentrations in our study. Other studies among pregnant women have reported similar findings of fetal impassivity (in reference to ferritin supply) to the ID status of the mother.^{29,30}

In our study population, following the first ANC, all pregnant women were given daily oral iron and folic acid supplementations in addition to the IPTp they were randomized to, in the MiPPAD clinical trial. Pregnant women who remained iron deficient at the second ANC visit and at delivery may have had very low iron stores prior to pregnancy and as a result remained deficient following fetal demands even after supplementation or may not have taken the supplements, as the intake was not directly observed. The lack of association between prenatal ID and infant cognitive development may result because the fetal iron supply may have been adequate in iron deficient pregnant women who were offered supplementation as in non-iron deficient pregnant women.

In the recent past, a substantial number of researchers have shown interest in investigating the impact of ID in infancy on cognitive functions and behavior of children in human subjects as well as in animal models.^{31,32} However, few studies have been published assessing the impact of prenatal or fetal ID on early child cognitive development. Evidence emanating from randomized controlled trials comparing the cognitive function or behavior of children between pregnant women who had iron (iron and folic acid) supplementation to a placebo or iron and folic acid, to folic acid only treatments are irreconcilable. In a trial among the 4-year-old children of 430 women randomized to daily supplementation of iron or placebo, regardless of prior anemia or ID, from the 20th week of gestation to delivery in Adelaide, Australia, the authors found no difference in the cognitive function.³³ A follow-up of these children when they were 6 to 8-years-old further confirmed the previous findings of no difference in child behavior among children of iron

supplemented and placebo mothers.³⁴ Instead, the authors reported a higher proportion of abnormal behavior among children of iron-supplemented group. However, another trial in Nepal that later assessed cognitive function of 7 to 9-year-old children showed benefits of iron supplementation in pregnancy for better cognitive function compared to only vitamin A supplementation.³⁵

Although malaria and iron deficiency are both important risk factors of anemia in sub-Saharan Africa, the antagonistic role of iron metabolism and malaria remains a controversy among researchers. In a study in Kenya among children of 8 months to 8 years, the authors found a lower incidence of malaria among iron deficient children.³⁶ A similar association has also been reported among pregnant women by other researchers³⁷. It is hypothesized that the demand for iron by the *Plasmodium* parasite for growth contributes the disadvantage of iron sufficiency in the presence of malaria. It is for this reason that we adjusted for malaria at the time of ferritin assessment in our analysis in Table 4.

One limitation of this current study is that the intake of the daily iron supplementation among pregnant women was not monitored. Also, since it is unethical to not administer iron supplements to pregnant women, we cannot clearly report the impact of iron deficiency in pregnancy in the absence of iron supplementation, on the cognitive development of children. The inability to obtain hematologic data from all children in the study was another limitation to this study but there was little or no selection bias as maternal and infant characteristics were generally similar among children who had hematologic assessments and those who did not receive hematologic assessments and among those who had MSEL assessments and those who did not. Further, our study also provides an extensive analysis on the impact of iron deficiency from the prenatal phase

to age one year on the cognitive development of children adjusting for all the necessary confounders at each stage of follow-up.

Conclusion

Our study showed that in a malaria-endemic region, iron deficiency in pregnancy, in the context of iron supplementation, is not associated with CBSF concentration, and infant cognitive and motor development. Prenatal IDA around delivery is associated with lower CBSF concentrations. These findings are within a population that implemented current recommendations and we cannot report what could have occurred in the absence of iron supplementation. Hence, antenatal iron supplementation especially to iron deficient pregnant women as per international guidelines is encouraged as our findings are reported. Since active brain development continues even after age one year, follow-up studies are required to determine the long-term impact of perinatal IDA on infant cognitive development.

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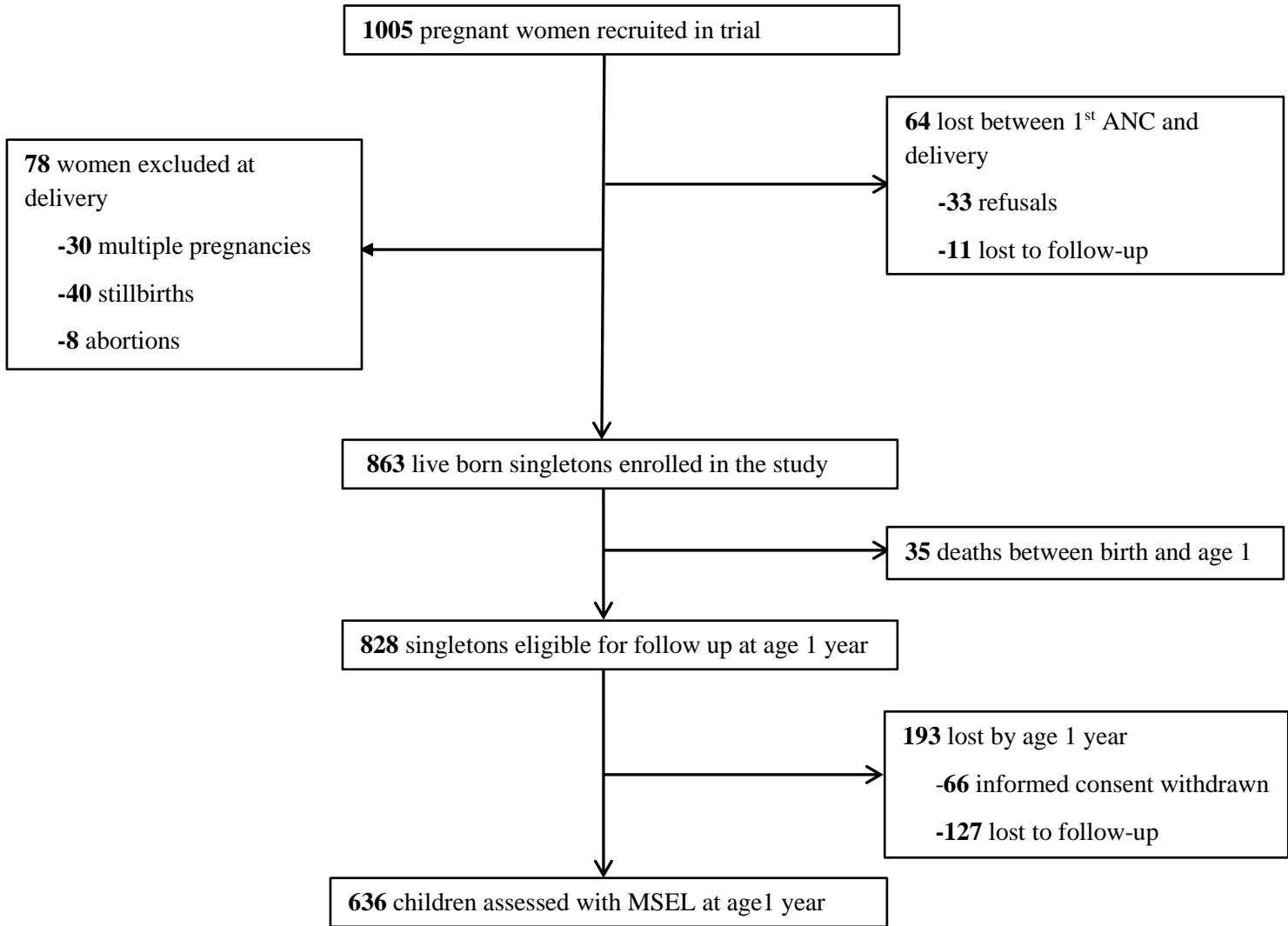


Figure 1. Flowchart of the follow-up of pregnant women and children

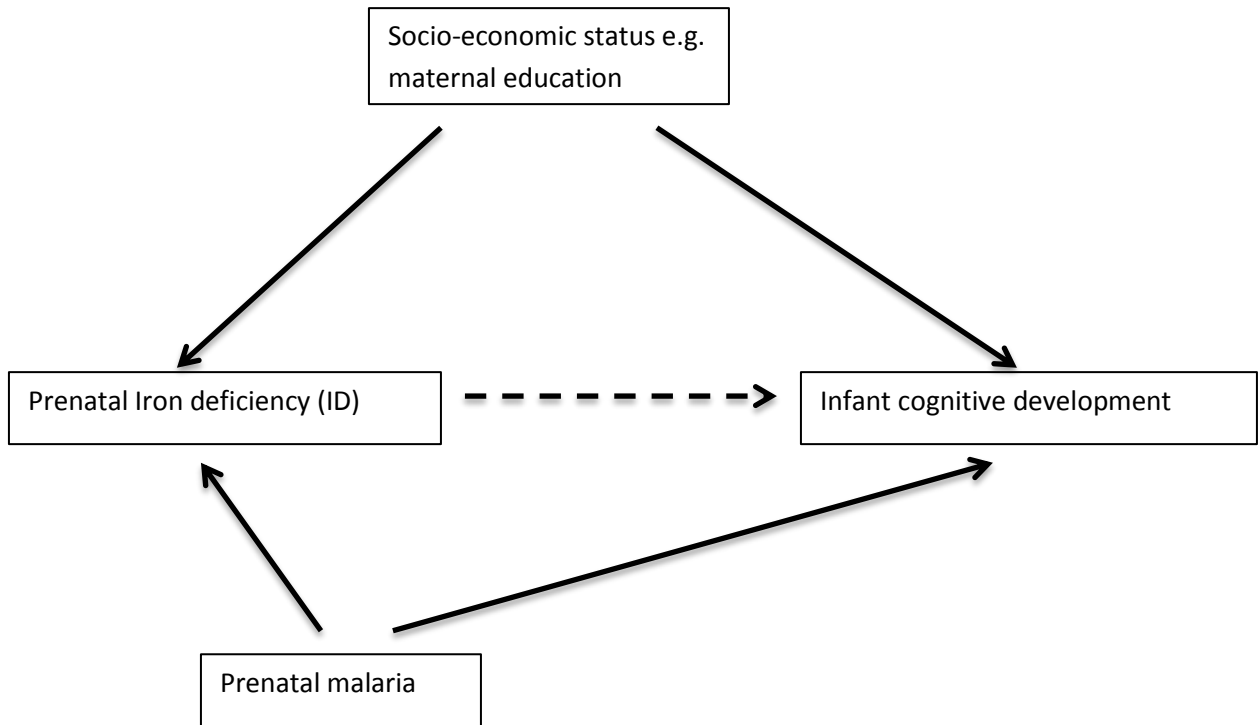


Figure 2. Direct acyclic graph of the relationship between prenatal ID and infant cognitive development.

Table1. Comparison of maternal and infants characteristics at baseline

Characteristics	Iron Deficiency (ID) at 1 st ANC			Iron Deficiency Anemia (IDA) at 1 st ANC		
	ID	No ID	<i>P</i> -value	IDA	No IDA	<i>P</i> -value
<i>Maternal</i>						
Maternal age (years) ^a	25 (20 -30)	25.0 (21-30)	0.621	25 (20-30)	25 (21-30)	0.790
GA at 1 st ANC (weeks) ^a	23 (20-26)	22 (19-25)	0.001	24 (20-26)	22 (19-25)	<0.001
Possessions score ^a	5 (3 -8)	5 (3-9)	0.362	5 (3-8)	5 (3-9)	0.528
Education						
Never schooled	129 (66.5)	292 (66.1)	0.916	93 (66.0)	328 (66.3)	0.946
Primary or more	65 (33.5)	150 (33.9)		48 (34.0)	167 (33.7)	
Gravidity						
Primigravida	24 (16.9)	93 (21.0)	0.009	19 (13.5)	98 (19.8)	0.087
Multigravida	170 (83.1)	349 (79.0)		122 (86.5)	397 (80.2)	
Occupation						
Housewives	103 (53.1)	212 (43.5)	0.234	75 (53.9)	240 (48.5)	0.324
Employed	91 (46.9)	230 (56.5)		66 (46.8)	255 (51.5)	
BMI (kg/m ²)						
Underweight (<18.5)	37 (19.1)	74 (16.7)	0.686	30 (21.3)	81 (16.4)	0.257
Normal (18.5-24.9)	139 (71.6)	320 (72.4)		100 (70.9)	359 (72.5)	
Overweight (≥25.0)	18 (9.3)	48 (10.9)		11 (7.8)	55 (11.1)	
<i>Infant</i>						
Birth weight (g)						
LBW (<2500)	13 (9.2)	48 (12.8)	0.107	12 (8.7)	49 (10.0)	0.657
Normal (≥2500)	178 (90.8)	391 (87.2)		126 (91.3)	443 (90.0)	
GA at birth (weeks)						
Preterm (<37)	27 (5.3)	14 (7.4)	0.771	10 (7.3)	33 (8.1)	0.859
Not-preterm (≥37)	485 (94.7)	175 (92.6)		127 (92.7)	448 (91.9)	
Sex						
Boys	95 (49.0)	216 (48.9)	0.535	71 (50.3)	240 (48.5)	0.695
Girls	99 (51.0)	226 (51.1)		70 (49.7)	255 (51.5)	
HOME score ^a	27 (25-28)	27 (25-28)	0.693	27 (25-28)	27 (25-28)	0.852

ANC - Antenatal Care Visit; LBW- Low Birth Weight; GA- Gestational Age; BMI - Body Mass Index; NA- Not Applicable

Table 2. Maternal and infant hematologic and clinical characteristics of children assessed for cognitive function using MSEL

	number (%) or mean \pm SD
<u>Maternal Characteristics</u>	
<i>At 1st ANC visit (N=636)</i>	
Anemia	426 (67.0)
Iron deficiency (ID)	191 (30.5)
Iron deficiency anemia (IDA)	141 (22.2)
Soil-transmitted helminth infection (N=627)	73 (11.6)
Malaria	102 (16.0)
<i>At 2nd ANC visit (N=627)</i>	
Anemia (N=626)	403 (64.4)
Iron deficiency	213 (34.0)
Iron deficiency anemia (N=626)	151 (24.1)
Soil-transmitted helminth infection (619)	54 (8.7)
Malaria	23 (3.7)
<i>At delivery</i>	
Anemia (N=609)	234 (38.4)
Iron deficiency (N=606)	172 (28.4)
Iron deficiency anemia (N=605)	69 (11.4)
Soil-transmitted helminth infection (N=538)	14 (2.6)
Malaria (N=610)	62 (10.2)
<u>Infant Characteristics</u>	
<i>At birth</i>	
Hemoglobin concentration (g/l) (N=582)	140.2 \pm 23.3
Anemia (Hb<140 g/l)	259 (44.5)
Ferritin concentration (μ g/l) (N=432)	176 \pm 171.56

ANC- Antenatal Care; Hb- Hemoglobin; SD- Standard deviation; MSEL- Mullen Scales of Early Learning

Table 3. Relationship between maternal and infant characteristics and cord blood ferritin concentration and infant cognitive development

Characteristics	Cord blood ferritin concentration		ELC score		GM score	
	Median (IQR)	<i>P</i> -value	Mean±SD	<i>P</i> -value	Mean±SD	<i>P</i> -value
<i>Maternal</i>						
Maternal age (years) ^a	-0.07	0.136	-0.03	0.407	0.05	0.190
GA at 1 st ANC (weeks) ^a	0.03	0.481	0.01	0.848	-0.00	0.95
Possessions score ^a	-0.03	0.542	0.12	0.003	0.14	<0.001
Education						
Never schooled	145.0 (96.6-202.6)	0.042	96.2±14.8	<0.001	49.4±13.9	<0.001
Primary or more	136.7 (63.4-203.8)		102.9±12.0		54.1±15.1	
Gravidity						
Primigravida	159.9 (69.6-228.5)	0.479	99.8±13.4	0.281	48.2±15.6	0.022
Multigravida	140.2 (86.3- 201.8)		98.2±14.5		51.6±14.1	
Occupation						
Housewives	138.6 (74.2- 200.2)	0.089	96.1±15.1	<0.001	49.2±13.7	0.002
Employed	145.3 (98.9-204.3)		100.8±13.1		52.8±15.0	
BMI (kg/m ²)						
Underweight	153.5 (90.8-218.5)	0.589	96.4±15.1	0.067	48.9±14.3	0.030
Normal	140.5 (81.7-201.0)		98.6±14.4		50.9±14.5	
Overweight	135.3 (82.4- 179.5)		101.5±11.6		54.8±14.1	
ID at 1 st ANC visit						
Yes	132.0 (78.0-210.2)	0.304	98.6±14.1	0.935	50.2±14.2	0.397
No	148.7 (93.1-202.9)		98.5±14.4		51.3±14.6	
ID at 2 nd ANC visit						
Yes	135.8 (90.1-201.2)	0.851	99.5±14.3	0.208	51.4±14.9	0.721
No	146.6 (81.8-202.6)		98.0±14.1		50.9±14.1	
ID at delivery						
Yes	127.7 (70.9-203.4)	0.261	98.2±14.2	0.801	51.2±13.3	0.896
No	146.3 (90.7- 202.7)		98.5±14.4		51.0±14.8	
<i>Infant</i>						
Birth weight (g)						
LBW (<2500)	154.1 (100.1-243.7)	0.267	97.8±14.9	0.705	45.6±15.0	0.002
Normal (≥2500)	140.3 (81.8-201.8)		98.6±14.3		51.6±14.3	
GA at birth (weeks)						

Preterm (<37)	138.1 (87.0-232.5)	0.930	95.7±15.5	0.141	49.3±14.11	0.350
Not preterm (≥37)	143.5 (81.7-202.9)		99.0±14.1		51.4±14.4	
Sex						
Boys	147.2 (90.5-205.1)	0.587	97.8±13.6	0.207	51.8±14.8	0.143
Girls	136.3 (78.5-201.0)		99.2±14.9		50.2±14.1	
HOME score ^a	-0.10	0.044	0.19	<0.001	0.19	<0.001
Cord blood ferritin concentration ^a	NA	NA	0.01	0.803	0.01	0.887

^aSpearman's rank correlation coefficients

ANC - Antenatal Care Visit; LBW- Low Birth Weight; GA- Gestational Age; BMI - Body Mass Index; NA- Not Applicable

Table 4. Relationship between maternal ID, IDA, cord blood ferritin levels and infant cognitive development

	Log Cord blood ferritin concentration AMD (95% CI)	Early Learning Composite score AMD (95% CI)‡	Gross Motor score AMD (95% CI)‡
<u>At 1st ANC visit</u>			
ID	-0.1 (-0.2, 0.1) ^a	0.4 (-1.9, 2.8) ^a	-1.1 (-3.5, 1.3) ^d
IDA	-0.0 (-0.2, 0.1) ^a	0.7 (-1.9, 3.3) ^a	-0.1 (-2.8, 2.5) ^d
<u>At 2nd ANC visit</u>			
ID	0.0 (-0.1, 0.2) NS	1.5 (-0.7, 3.8) ^a	-0.0 (-2.4 2.3) ^c
IDA	-0.1 (-0.2, 0.1) NS	1.3 (-1.2, 3.8) ^a	1.5 (-1.0, 4.1) ^c
<u>At Delivery</u>			
ID	-0.1 (-0.2, 0.1)	-0.7 (-3.1, 1.8)	-0.2 (-2.8, 2.3) ^c
IDA	-0.2 (-0.4,-0.0)*	-0.8 (-4.3, 2.7)	1.3 (-2.3, 4.8) ^c
<u>At birth</u>			
Log ferritin concentration	NA	0.8 (-1.0, 2.5) ^b	0.8 (-0.9, 2.5) ^e
Anemia (Hb< 140g/l)	NA	0.0 (-2.3, 2.3)	0.4 (-1.9, 2.7) ^c

**P*-value <0.05; ANC- Antenatal Care; ID- Iron Deficiency; IDA- Iron Deficiency Anemia;

NA- Not Applicable; NS- Not Significant model

All models were adjusted for maternal education and malaria at iron assessment and in addition, ^ahelminth infection at the time of hematologic assessment, ^bprepregnancy BMI, sex, ^cfamily possession, gravidity, ^d(a+c), ^eprepregnancy BMI, gravidity, and EPDS score.

‡Models further adjusted for the HOME score

Supplemental Table 1. Comparison of the maternal and infant characteristics between children with and without cord blood assessments and those with and without MSEL assessments at follow-up, age approximately one year

Characteristics	Cord blood ferritin			MSEL		
	Assessed	Not assessed	<i>P</i> -value	Assessed	Not assessed	<i>P</i> -value
<i>Maternal</i>						
Maternal age (years) ^a	25 (21 -30)	25.0 (20-30)	0.862	25 (21-30)	25 (20-30)	0.360
GA at 1 st ANC (weeks) ^a	22 (19-25)	23 (19-26)	0.008	22 (19-25)	23 (19-26)	0.130
Possessions score ^a	5 (3 -8)	6 (3-9)	0.163	5 (3.0-9.0)	4 (3-5)	0.291
Education						
Never schooled	354 (68.6)	220 (63.4)	0.112	421 (66.2)	153 (67.4)	0.741
Primary or more	162 (31.4)	127 (36.6)		215 (33.8)	74 (32.6)	
Gravidity						
Primigravida	87 (16.9)	68 (19.6)	0.305	117 (18.4)	38 (16.7)	0.577
Multigravida	429 (83.1)	279 (80.4)		189 (83.3)	519 (81.6)	
Occupation						
Housewives	269 (52.1)	151 (43.5)	0.013	315 (49.5)	105 (46.3)	0.397
Employed	247 (47.9)	196 (56.5)		321 (50.5)	122 (53.7)	
BMI (kg/m ²)						
Underweight (<18.5)	97 (18.8)	47 (13.5)	0.002	111 (17.4)	33 (14.5)	0.356
Normal (18.5-24.9)	376 (72.9)	247 (71.2)		459 (72.2)	164 (72.3)	
Overweight (≥25.0)	43 (8.3)	53 (15.3)		66 (10.4)	30 (13.2)	
ID at 1 st ANC visit						
Yes	178 (34.5)	109 (31.4)	0.346	194 (30.5)	93 (41.0)	0.004
No	338 (65.5)	238 (68.6)		442 (69.5)	134 (59.0)	
ID at 2 nd ANC visit						
Yes	190 (37.4)	106 (31.7)	0.092	213 (34.0)	83 (38.6)	0.219
No	318 (62.6)	228 (68.2)		414 (66.0)	132 (61.4)	
ID at delivery						
Yes	159 (31.1)	81 (29.0)	0.543	172 (28.4)	68 (37.0)	0.027
No	352 (68.9)	198 (71.0)		434 (71.6)	116 (63.0)	
<i>Infant</i>						
Birth weight (g)						
LBW (<2500)	47 (9.2)	62 (12.8)	0.070	61 (9.7)	28 (13.5)	0.120
Normal (≥2500)	465 (90.8)	424 (87.2)		569 (90.3)	179 (86.5)	
GA at birth (weeks)						
Preterm (<37)	27 (5.3)	33 (10.4)	0.005	43 (7.0)	17 (8.1)	0.583
Not-preterm (≥37)	485 (94.7)	283 (89.6)		575 (93.0)	193 (91.9)	
Sex						
Boys	252 (48.8)	162 (46.7)	0.535	311 (48.9)	103 (45.4)	0.361
Girls	264 (51.2)	185 (53.3)		325 (51.1)	124 (54.6)	
HOME score ^a	27 (25-28)	27 (26-28)	0.370	27 (25-28)	28 (27-29)	0.112
Cord blood ferritin concentration ^a	NA	NA	NA	139.9 (83.5-203.6)	156.3 (78.3-202.0)	0.472

^aMedian (Inter Quartile Range)

MSEL - Mullen Scales of Early Learning; ANC - Antenatal Care Visit; LBW- Low Birth Weight; GA- Gestational Age; BMI - Body Mass Index; NA- Not Applicable

Chapter 6. Discussion

The previous three chapters reported the findings of this thesis and of the TOVI project and as well included a discussion for each of our findings. To avoid repetition of what had already been discussed in the past chapters, the discussion chapter of this thesis will highlight a few strengths and limitations that may not have been mentioned in the articles and will also focus on the implications of our findings to the discourse concerning maternal anemia, iron supplementation interventions and treatment of helminths during pregnancy. This discussion will be centered on how the findings contribute or challenge current practice in connection with infant cognitive development presenting the perspective of what our findings bring to the table.

The chapter also highlights an ongoing systematic review on the impact of iron supplementation in pregnancy and infant cognitive development. This review was brought about by the findings of the TOVI study that prompted us to seek what literature exists on prenatal iron supplementation and cognitive development. The findings of the ongoing review also contributes to debate on iron supplementation on infant cognition, however due to time constraints, the detailed review could not be included as an independent paper in this thesis.

Based on the results of our study, within each sub-chapter of the discussion, recommendations are made where necessary.

6.1 Strengths and Limitations

This thesis comprehensively investigated the impact of anemia during pregnancy, and its risk factors on the early cognitive and motor developments of children. The use of the MSEL permitted us to properly capture infant cognitive function using the ELC score. Also, the assessment of other potential confounders including maternal IQ, the HOME and postnatal depression allowed us to account for other factors that could have potentially influenced infant cognition. The prospective continuation of the MiPPAD clinical trial with the TOVI study allowed for temporal inference on the impact of the exposures investigated and infant cognitive development. Further, as highlighted in the published articles, this study had a very little attrition as compared to other birth cohorts in the West Africa region.

Aside the limitations previously mentioned in the attached articles in Chapters 3, 4 and 5, we could not assess the impact of solely anemia, ID and helminths at different stages of pregnancy in the absence of treatment as this was not ethically appropriate in our study setting. Our study is further limited in not investigating the impact of malaria and helminth prophylaxis and micronutrient supplementation during pregnancy on the impact of cognitive function as all pregnant women received IPTp, anthelmintics and iron and folic acid supplements. Specifically, as part of the clinical trial, at least 98% and 87% took at least 1 dose and 2 doses of SP, respectively, as this was a directly observed treatment. The administration of anthelmintics and iron and folic acid supplements was not directly observed and an estimation of what proportion of pregnant women took the supplements and drugs is unknown and probably less.

6.2 Anemia Classification: The Challenges and Implications in Pregnancy

In this TOVI study we identified that Hb range 90-110 g/L at the first and second ANC visits were optimal for proper gross motor function when we used Hb concentration as a continuous variable. Also, we showed an increased risk of poor cognitive development at first ANC visit among children of pregnant women who had Hb concentration greater than 106 g/L compared to children of pregnant women within the Hb 97-106 g/L range. The Hb range reported to be optimal for gross motor function is within what would normally be classified as anemia

according to the thresholds set by the WHO. Considering that many of the women were in their second trimester at the time of enrolment into the MiPPAD study, a part of those with the optimal Hb range reported in our studies would have been considered non-anemic by the CDC ($Hb \geq 105$ g/L)⁵ highlighting the inconsistencies in anemia discrimination among pregnant women.

It is however worthy to note that ours is not the only study that showed normal child development or even better birth outcomes among mothers whose Hb range are often within the anemic, specifically, mildly anemic category. A meta-analysis of the relationship between anemia and SGA reported from the few existing studies that, severe and moderate anemia but not mild anemia were associated with increased risk of SGA births.²⁶⁷ Chumak and Grjibovski⁴⁶ also showed that Hb 90-99 g/L and 100-109 g/L is associated with a lower risk of preterm birth and LBW compared to prenatal hemoglobin of 120 g/L or more.

The origin of the current hemoglobin cut-offs as set by the WHO is the 1968 report²⁶⁸ which was a revision of the WHO study Group report in 1958²⁶⁹ that initially set the threshold at 100 g/L for pregnant women. The first threshold was chosen arbitrarily in the first report from global existing hematological data. Since then, many researchers and health guidelines have applied tweaks and adjustments to the WHO recommendations to generate their own. In the UK for example, $Hb < 110$ g/L is acceptable to define anemia in pregnant women until the 12th week of gestation, after which a cut-off of 105g/L is acceptable until delivery.²⁷⁰

From the evidences of the various studies mentioned and the findings of this thesis, and the flexibility in the acceptable cut-offs for defining anemia in pregnancy, the debate on whether the consequences of anemia should be used to identify anemia still continues. However, based on the findings of this thesis and the available literature on the subject, the threshold for anemia in pregnancy especially in the second and early third trimester could be reduced to 100g/l in the context of micronutrient (including iron) supplementation and malaria and helminth prevention and treatment in endemic regions.

6.3 Implications of High Hb Concentration during Pregnancy

Globally, trends in anemia prevalence is dwindling from an estimated prevalence of 43% in 1995 to 38% in 2011 and the decline is even evident in sub-Saharan Africa⁶⁸. As the global prevalence of anemia declines and the mean Hb concentration increases⁶⁸, it is particularly important for attention to be given to women with abnormally high Hb levels. As highlighted in the article on prenatal hemoglobin levels and infant motor function, abnormally high Hb concentration during pregnancy particularly in the second and third trimester is a reason for concern. Although pregnant women with abnormally high Hb concentration are non-anemic, studies have shown that such women exhibit similar adverse birth outcomes and infant development as those with abnormally low Hb levels.

Stephansson *et al* reported an increased risk of still birth among pregnant women with Hb concentration greater than 146 g/L compared to women with Hb concentration of 126 to 135 g/L during the first ANC visit⁴⁷. Steer *et al* also concluded that inability of the pregnant woman's Hb to fall below 105 g/L during pregnancy is associated with increased risk of LBW and preterm birth¹⁸⁶. Another study also showed that pregnant women with Hb between 90 g/L and 120 g/L, compared to, compared to women with Hb greater or equal to 120 g/L experienced better birth outcomes.⁴⁶ Although these studies and the findings of this thesis highlight the need for attention for women with abnormally high Hb levels during pregnancy, the question still remains; how high is an abnormally high Hb concentration during pregnancy?

6.4 Prenatal Iron Supplementation, Evolution of Hb concentration and Child Development

With general implementation of daily iron supplementation during pregnancy, as part of the ANC package during pregnancy, in many developing countries including Benin, it may be advisable that pregnant women be only administered iron supplements after results of hematologic assessments reveal low Hb and/or ferritin levels. This recommendation could be particularly important if further studies confirm our findings of poor GM function among children of pregnant women with high Hb levels during pregnancy. However whether or not iron supplementation only after evidence of anemia or ID deficiency is economically beneficial over

general daily iron supplementation is not known. Also, to what extent iron general iron supplementation improves maternal health, birth outcomes and child health is an important consideration.

As mentioned earlier, anemia is estimated to about 40 per cent of pregnant women worldwide with an even higher prevalence (about 50%) among pregnant women living in developing countries, particularly in Africa and South-East Asia.⁸ Considering that ID accounts for more than half of the burden of anemia⁶, the guidelines of the CDC and the WHO highly recommend iron supplementation during pregnancy. In fact, the guidelines of the CDC recommend universal iron supplementation to meet the iron needs of pregnant women.⁵⁷ The WHO guidelines also strongly recommend a daily intake of 30–60 mg of elemental iron throughout pregnancy with the higher end of the dose recommended for pregnant women irrespective of anemia or iron status in areas where the prevalence of anemia exceeds 40%.¹¹⁷

The strong recommendations of the CDC and WHO for iron supplementation in pregnancy lies in the evidence of improvement in maternal hematological indicators including higher hemoglobin and reduced ID, improvement in birth outcomes such as increased mean birth weight and reduced prevalence of LBW as published in systematic review.²⁷¹ The safety and efficacy of iron supplementation during pregnancy (either routine or intermittent) have been assessed in a number of clinical trials, the findings of which have been mostly inconsistent in terms of outcomes on maternal health and birth outcomes.

Some studies have reported a significant decrease in the incidence of LBW and an increase in mean birth weight among iron supplemented mothers in comparison to non-iron supplemented mothers.⁶³ Iron supplementation in iron sufficient pregnant women (ferritin concentration greater than 70 µg/L) has been shown to be non-beneficial relative to supplementation in iron deficient women.²⁷² Among non-anemic pregnant women with high Hb concentration (greater than 132 g/L) who took iron supplements of 50 mg elemental iron, the incidence of hypertension disorder and SGA births were higher compared to pregnant women in the placebo group (no iron supplementation).²⁷³ The findings of our study on prenatal ID and infant cognition revealed that children of iron deficient pregnant women performed similarly on cognitive and motor tests as children of non-iron deficient pregnant women. Iron supplementation therefore seems to be

essential for iron deficient pregnant women to provide adequate supply to the fetus. Findings of the three aforementioned studies and the findings of the TOVI study hints on the importance of iron supplementation during pregnancy and the its beneficial impact for iron deficient pregnant women but not for non-iron deficient pregnant women

Further, a recent update of the Cochrane Collaborative systematic review, that was used to advice the guidelines of the WHO, in 2015 however demonstrates no statistically significant beneficial effect of prenatal iron supplementation on LBW, premature birth, congenital abnormalities and neonatal mortality.¹⁹⁰ The absence of the beneficial effect of prenatal iron supplementation on LBW, preterm birth and other adverse birth outcomes as concluded in the aforementioned Cochrane review may less explain the effectiveness of prenatal iron supplementation and rather explain fetuses' priority access to nutrients including iron at the expense of maternal reserves. Meaning that, even in pregnant women who do not receive iron supplements during pregnancy but have some iron-rich diets, fetuses may still receive to some extent, the necessary iron for development. This explanation could also explain the lack of association between prenatal iron deficiency and infant cognitive development as shown in our paper on prenatal ID and infant cognition which also revealed there was no correlation between prenatal ID during the three periods of follow-up and cord blood ferritin.

The authors of the review further mentioned that pregnant women who received iron supplementation during pregnancy were at an increased of having hemoglobin concentrations greater than 130 g/L. High prenatal hemoglobin concentrations are potentially harmful to fetal development and could result in adverse birth outcomes such as preterm birth and LBW.^{45,274} In addition, evidence from TOVI revealed that gross motor function of one-year-old children was optimal for mothers with Hb between 90 and 110 g/L. Below 90 g/L and beyond 110 g/L, infant gross motor scores were lower.¹¹⁵ Implications of the impact of high prenatal hemoglobin concentration has been previously detailed in Chapter 6.3.

6.5 Prenatal Iron Supplementation and Infant Cognitive Development: The Review

Studies on possible mechanisms linking iron and brain function have also been conducted in several animal studies.^{275,276} There is quite an extensive literature on the impact and role of iron (deficiency or supplementation) in children on their cognitive development.^{203,233,277} Findings from such studies have revealed that iron supplementation in children have a positive impact in the neurocognitive function. In general, most researchers, gynecologists, nutritionists and pediatricians acknowledge that early exposure to ID and/or anemia could be detrimental to brain function. Some researchers have mentioned of a possible irreversibility of the impact of early ID on children's cognition even after iron supplementation later in life.^{232,278} Yet, very little work has been done to investigate the impact of prenatal ID and/or anemia on neurocognition and behavior of children.

Preliminary findings from an ongoing systematic review on the impact of anemia, ID and iron supplementation during pregnancy on the cognition and behavior of children reveals how little work has been done on this necessary topic.

In this ongoing review, 13 primary studies were identified of a 160 articles from PubMed search of published work in the last 15 years (from 01/01/2000 to 09/09/2015). Details of the selected articles are displayed in a table in Appendix 2 of this thesis. Of these, 5 studies were randomized controlled trials comparing iron (iron and folic acid) supplementation to a placebo or iron and folic acid, to folic acid only treatments. In a randomized control trial in Australia, among 4-year-old children of 430 women randomized to daily supplementation of iron or placebo from the 20th week of gestation to delivery, the authors found no difference in the cognitive function.²⁷⁹ A follow-up of these children when they were 6 to 8-years-old further confirmed the previous findings of no difference in child behavior among children of iron supplemented and placebo mothers.²⁸⁰ Instead, the authors reported a higher proportion of abnormal behavior among children of iron-supplemented group. In Nepal, authors showed that some aspects of intellectual functioning (fine motor, memory and inhibition control) were better in of 7-9 year olds of iron-folic acid supplemented mothers than children whose mothers had only vitamin A.²⁸¹ The remaining two clinical trials were conducted among the same sample of pregnant women whose children were followed at 3, 6 and 12 months in the first study and at 7-9 years in the second.

Both studies revealed iron supplementation during pregnancy was not associated with improved mental scores of children.^{282,283}

The remaining 8 out of 13 articles were all longitudinal observational studies of mother-child cohorts. Not all studies reported if women received iron supplements during pregnancy. However, all studies measured either ferritin or folic acid or hemoglobin or a genotype indicating ID during pregnancy and in addition, assessed the cognition, motor function or behavior of children at different ages. Cognitive assessment was performed for children at different ages, from 48 hours to 8 years. Among the studies that assessed maternal iron levels, maternal IDA during pregnancy was shown to have adverse consequences on infant cognition at 2-3 days²⁸⁴ and at 6 months^{285,286}. In other studies, maternal IDA showed no effect on infant cognition at 6 months²⁸⁷ and 8 years²⁸⁸. In the studies in which the relationship between prenatal Hb concentration and infant cognitive function were investigated, one found no significant association¹¹⁵ and two found a decreased IQ among children of anemic pregnant women^{24,286}. Two other papers showed an inverted U-shaped relationship between maternal Hb levels and infant IQ²⁸⁹ and motor function¹¹⁵, as shown in our study, indicating the possible harmful effects of high prenatal Hb levels.

In summary, evidence from the four out of five clinical trials comparing iron supplementation to placebo or iron and folic acid supplementation to folic acid only shows no evidence of a beneficial effect of iron supplementation on infant cognition and behavior. Further, observational studies remain inconclusive of the effect of ID and anemia during pregnancy on the neurocognitive function of children.

6.6 Anthelmintic Chemotherapy in Pregnancy and Child Development

Helminths are one of the major causes of anemia in the regions where they are endemic. Particularly, hookworms have been noted for their impact in inducing blood loss and ID in infected persons. During pregnancy, the WHO recommends that women in their second and third trimesters be given anthelmintics specifically, mebendazole or albendazole, as preventive therapy in regions of high endemicity of STHs.²⁹⁰ The teratogenic and embryotoxic fears associated with

the use of mebendazole and albendazole may be the reason for the restriction to these two trimesters although no such effects have been found in studies that have tested this hypothesis.^{147,291}

Evidence from the TOVI study as already mentioned in the previous three chapters reveal that helminth infection during pregnancy, particularly in the first ANC visit, prior to anthelmintic administration, was associated with poor cognitive and motor functions among one-year old children. Specifically, hookworms were the group of helminths that had significant negative impact on infant cognition and motor functions. Further, children of mothers who were infected with helminths at least once during pregnancy had poorer cognitive function compared to those whose mothers were never infected.

In the face of not administering anthelmintics to pregnant women in the first trimester of pregnancy or to women of reproductive age without knowledge of pregnancy, it is necessary to provide more public health education on prevention to women of childbearing age.

In our discussion of the paper published in *Plos NTDs* (Chapter 3), we hypothesized that the effect of prenatal hookworm infection on infant cognitive and motor functions may be via ID during pregnancy. On the contrary, we identified from the paper on ID that there was no difference in cord blood ferritin concentrations between iron deficient and non-iron deficient pregnant women; thus to say that there are mechanisms other than that via maternal ID that could lead to low cognitive development of children. Further research is therefore needed to ascertain the mechanisms by which helminth infection in pregnancy could influence the cognitive function of offspring.

6.7 The Absence of Association at Delivery: Physiologic or Evidence of Effective Intervention?

In all of the findings from the papers presented in this thesis, we found no association between anemia or its risk factors, helminth infection and ID, at delivery and infant cognitive or motor development. Neither did we find the inverted U-shaped relationship between hemoglobin

concentration and gross motor function as we had observed for Hb at first and second ANC visits. The failure to find any relationship raises the question of physiologic improvement by delivery or the effectiveness of the interventions that were rolled out during the MiPPAD trial.

Chapter 7. Conclusion and Perspectives

Tremendous improvements have been made over the past century in the prevention, management and treatment of anemia, particularly in pregnant women, the evidence of which is available in the global decline in anemia and increase in mean hemoglobin concentration. Notwithstanding these improvements, the prevalence of anemia in pregnancy in developing countries remains disproportionately high. Evidence of the impact of anemia in pregnancy and its risk factors on birth outcomes remain equivocal although the evidence is directed towards adverse birth consequences that are potential risk factors for poor cognitive development in children.

The scantiness of existing published work on the relationship between prenatal anemia, its risk factors and infant cognitive development makes our work one of the first to either investigate the relationship or report certain findings on the topic. The findings of this research reveal the significance of the consequences of anemia in pregnancy and its risk factors on the early cognitive development of one-year-old children. One important finding of our studies is the negative impact of intestinal helminths among pregnant women on infant neurocognitive development which is consistent with the conclusion of the only published work on the subject.²³ To our knowledge, our finding that children whose mothers were infected with hookworms prior to anthelmintic therapy performed poorly on cognitive and motor tests compared to those whose mothers were not infected with hookworms is the first to be reported. These results reinforce the recommendations by the WHO to administer anthelmintics to pregnant women living in endemic regions after their first trimester.

Our study showed that, in the presence of iron supplementation, ID during pregnancy in a malaria-endemic region is not associated with infant cognitive and motor development. The findings reflect the importance of iron supplementation in pregnancy as our findings further reveal that prenatal ID, in the presence of iron supplementation, is not associated with cord blood ferritin concentrations and infant cognitive development. Although we cannot envisage what would have happened by late pregnancy in the absence of iron supplementation, our findings reveal no impact of prenatal ID in early pregnancy (as in the first ANC visit when pregnant women had not received any supplements) on infant cognitive development.

Further, we found that there is an inverted U-shaped relationship between maternal hemoglobin concentration during pregnancy and motor function of one-year-old children, which persisted even after adjusting for common known causes of anemia in pregnancy. The inverted U-shape relationship is coherent with the U-shaped relationship between the risk of adverse birth outcomes and prenatal hemoglobin concentration as reported in some earlier publications.^{186,274} We identified an optimal range maternal Hb of 90-110 g/L that was beneficial to infant gross motor functions.

Considering that our studies are the first to report some of the findings or contributes to limited literature on the subject, further studies will be necessary to corroborate our findings. Follow-up study to investigate if this association persists even in the later life of the children is also necessary and will be performed through the EXPLORE project with a follow-up of the children at 6 years old. Furthermore, the mechanisms underlying the observed associations in our study are however not well understood although a number of hypotheses have been stipulated and could be explored using animal models or *in vitro* studies.

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Appendix

Appendix 1. Scoring Sheet for MSEL (Cover Page and Scoring Sheet for Gross Motor and Visual Perception Skills)



Eileen M. Mullen

FEUILLE DE NOTATION

	Année	Mois	Jour
Date de l'examen	_____	_____	_____
Date de naissance	_____	_____	_____
Âge chronologique	_____	_____	_____

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Echelle 1. Développement Moteur

	Item	Score
Stage 1	1-4 mo	1. Aime être porté, ajuste sa posture (V) 1 0
		2. Tourne sa tête (P) 1 0
		3. Agite les bras et les jambes vigoureusement (D) 1 0
		4. Tenu verticalement, tient sa tête de façon stable (V) 1 0
Stage 2	5-8 mo	5. Se tient sur les avant-bras (P) 1 0
	9-12 mo	6. Tient assis avec soutien, la tête stable (AsS) 1 0
		7. Se retourne (de P à D) 1 0
Stage 3	13-17 mo	8. Agrippe les doigts et se tire en position assise (de D à AsS) 1 0
		9. Change d'appui, tend la main pour atteindre un jouet (P) 1 0
		10. Tient debout les mains tenues, rebondit sur ses jambes 1 0
		11. Tient assis sans l'aide des bras (S) 1 0
Stage 4	18-20 mo	12. Se met debout (S) 1 0
		13. Passe de la position assise à quatre pattes (S) 1 0
		14. Marche une main tenue 1 0
		15. Tient debout tout seul (1-2 secondes) 1 0
		16. Marche seul (4-5 pas) 1 0
Stage 5	21-26 mo	17. Lance la balle devant lui, du bas vers l'avant 1 0
		18. Se met debout en roulant sur le côté (S à debout) 1 0
		19. Debout, accroupi, debout 1 0
		20. Monte les escaliers avec aide, sans alterner les pieds 1 0
		21. Court avec raideur 1 0
		22. Donne un coup de pied dans un ballon de 25 à 30 cm (2 essais sur 5) 1 0
Stage 6	27+ mo	23. Se tient sur un pied, avec aide 1 0
		24. Fait 4 ou 5 pas, un pied sur une ligne 1 0
		25. Monte seul l'escalier, sans alternance 1 0
		26. Descend d'un banc en sautant 2 1 0
① 1 pied , ② 2 pieds		
Stage 7		27. Saute sur place à pieds joints (un saut) 1 0
		28. Marche sur la pointe des pieds (4-5 pas) 1 0
		29. Marche sur une ligne en se servant des bras pour l'équilibre (6-7 pas) 1 0
		30. Descend seul l'escalier en alternant 1 0
Stage 8		31. Se met debout en s'asseyant, de face (S à debout) 1 0
		32. Tient en équilibre sur un pied (2-3 secondes) 1 0
		33. Court, tourne un coin, s'arrête 1 0
		34. Fait deux sauts à cloche pieds 1 0
		35. Marche sur une ligne, les bras le long du corps (6 pas) 1 0

NIVEAU de BASE = 3 items consécutifs avec un score d'au moins 1-point

NIVEAU PLAFOND = 3 items consécutifs avec un score de 0

Note Brute Développement Moteur

Echelle 2. Perception Visuelle

	Item	Score
1-4 mo	1. Fixe le triangle et suit sa trajectoire (D).....	2 1 0
	① Fixe ② Suit	
5-8 mo	2. Suit la trajectoire du visage schématisé sur 90 degrés (D).....	1 0
	3. Suit la trajectoire de l'œil de bœuf sur 180 degrés (AP).....	1 0
	4. Repère alternativement la balle rouge et le visage schématisé (AP).....	1 0
	5. Fixe le regard sur sa main (D).....	1 0
	6. Localise l'œil de bœuf de près et de loin (AsS).....	1 0
9-12 mo	7. Cherche la cuiller qu'on a fait tomber (AsS).....	1 0
	8. Tire la ficelle pour obtenir le disque.....	1 0
13-20 mo	9. Cherche l'anneau caché sous une serviette (S).....	2 1 0
	① partiellement caché, ② entièrement caché	
	10. Retourne la tasse à l'endroit (S).....	1 0
	11. Fait des associations entre objets.....	1 0
	___ brosse ___ cuillère ___ tasse ___ balle	[1]
	12. Cherche la voiture sous deux serviettes.....	1 0
	13. Est intéressé(e) par la charnière (ouverture/fermeture) d'un livre.....	1 0
	14. Prête attention à une image (A/V).....	1 0
	15. Cherche un jouet caché puis déplacé sous la serviette.....	1 0
21-32 mo	16. Distingue les formes sur la planche.....	4 3 2 1 0
	① ● ② ●● ③ ●●▲ ④ ●●▲+	
	17A. Apparie les objets après qu'ils soient nommés (A/V) (19 mois ou moins)	
	OU	
	17B. Apparie les objets sans nomination (20 mois ou plus)	
	___ chaussures ___ Voiture ___ Clés ___ Chevilles	
	① Un objet sans nomination.....	3 2 1 0
	② Deux objets sans nomination	
	③ Trois objets sans nomination	
	18. Emboîte les tasses gigogne.....	2 1 0
	① Emboîte trois tasses	
	② Emboîte quatre tasses	
33-44 mo	19. Classe les cuillères et les cubes par catégories.....	1 0
	(2 de chaque)	
	20. Associe des formes.....	1 0
	___ cercles ___ carrés ___ triangles	(2 de chaque)
	21. Associe des images.....	1 0
	___ chaussures ___ tasse ___ avion ___ différent du manuel	[2]
	22. Associe taille et couleur.....	1 0
	___ grands cercles rouges ___ petits cercles rouges	(2 de chaque)
	___ grands cercles jaunes ___ petits cercles jaunes	
	23. Mémoire d'une image.....	1 0
	24. Détails spatiaux I.....	1 0
45+ mo	25. Détails spatiaux II.....	2 1 0
	fleur ___ 1 ___ 2 ___ 3 ___ 4	[4] [3]
	26. Mémoire d'objets.....	1 0
	___ clé ___ balle ___ voiture	[2]
	27. Repère la position dans l'espace.....	1 0
	forme ___ 1 ___ 2 ___ 3 ___ 4	[4]
	28. Associe des lettres.....	1 0
	___ L ___ C ___ N ___ B ___ H ___ P	[6]
	29. Distingue gauche/droite.....	4 3 2 1 0
	___ lapin ___ marteau ___ enfant ___ wagon	[4] [3] [2] [1]
	30. Associe des lettres, des mots.....	4 3 2 1 0
	___ B ___ t ___ d ___ n ___ rt	[6] [5] [4] [2]
	___ be ___ bat ___ coat ___ chair (adapter, traduire)	
	31. Mémorise 3 images.....	1 0
	___ clé ___ livre ___ chaise	
	32. Détails spatiaux III.....	2 1 0
	___ chien ___ 1 ___ 2 ___ 3 ___ 4 ___ 5	[4] [3]
	33. Mémoire de la forme.....	2 1 0
	forme ___ 1 ___ 2 ___ 3 ___ 4	[3] [2]

Note Brute Perception Visuelle

Appendix 2. List of 13 selected articles on the impact of prenatal anemia, iron deficiency and iron supplementation on infant cognition and behavior

Authors - Country of study	Effect	No Effect	Conclusion	Comments
Chang <i>et al.</i> 2013 ¹ - China	<p>1. Prenatal IDA at 3rd trimester significantly associated with lower mental development of child at 12, 18, 24 months of age as well as lower psychomotor development at 24 months</p> <p>2. Association between prenatal ID and poor child mental health vanished after accounting for IFA supplementation</p>	<p>1. No impact of prenatal IDA on psychomotor development at 3 to 18 months</p> <p>2. No effect of prenatal IDA on child mental development at 3 and 6 months</p>	Maternal iron deficiency is associated with poor mental development of children. Early prenatal iron supplementation is beneficial to child mental development	<p>1. IDA was determined by Hb status < 110 g/l which indicates anemia rather than IDA</p> <p>2. Bayley scales of infant development (BSID) were used to assess mental development of children</p>
Christian <i>et al.</i> 2010 ² - Nepal	Significantly higher child cognitive and fine motor scores at 7-9 years among children of IFA supplemented pregnant women compared to women who received no IFA supplements	There was no difference in Movement Assessment Battery for Children scores between children of mothers in the IFA group and those in the placebo group	Evidence of the benefits of IFA supplementation during pregnancy on infant cognitive and motor functions later in life (in a region of high prenatal ID prevalence)	<p>1. Placebo group was vitamin A only and the IFA group received 400 µg folic acid and 60 mg of iron during pregnancy through 12 weeks after birth</p> <p>2. Universal Nonverbal Intelligence Test (UNIT) and the Movement Assessment Battery for Children (MABC) were the psychometric tests used</p>
Hernández-Martínez <i>et</i>	1. ID at first and second trimesters	N/A	Prenatal ID is associated with the	1. Neonatal behavior assessed

<i>al.</i> 2011 ³ - Spain	were significant predictors of neonatal behavior 2. Transferrin saturation at third trimester was a predictor of neonatal motor function		general autonomous response, motor performance and self-regulation capabilities of neonates	using the Neonatal Behavior Assessment Scale (NBAS).
Lewis et al. 2014 ⁴ – United Kingdom	Maternal genotypes at the single nucleotide polymorphisms in the genes <i>HFE</i> and <i>TMPRSS6</i> are strongly associated with prenatal hemoglobin levels	Maternal <i>HFE</i> and <i>TMPRSS6</i> genotypes (proxy for prenatal iron levels) were not associated with offspring IQ at 8 years	No evidence of the impact of prenatal iron levels on child cognitive functions	Cognition was assessed using the Wechsler Intelligence Scale for Children (WISC-III)
Li et al. 2009 ⁵ - China	Improved psychomotor development of one year old children of mothers in multimicronutrients arm in comparison to children of mothers in iron only or iron and folic acid arm	No significant difference mental development and psychomotor development between one-year-old children of women in iron and folic arm and children of mothers in only folic acid arm	In comparison to iron only or iron and folic acid supplementation, multimicronutrients in pregnancy improved the mental development of one-year-old offspring	Mental and psychomotor development of children were assessed using the Bayley Scales of Infant Development (BSID)
Li et al. 2015 ⁶ - China	N/A	No significant different in the scores of cognitive, verbal and reasoning tests between children of pregnant women in the IFA arm compared to those in the folic acid only arm.	No evidence of improved cognitive function among children whose mothers receive Prenatal iron supplementation	Wechsler Intelligence Scale for Children Fourth Edition (WISC-IV) was used to assess the cognitive function of children
Mireku et al. 2015 ⁷ - Benin	Inverted U-shaped relationship between prenatal anemia and gross motor scores of one-year-old offspring	No relationship between prenatal anemia and infant early learning composite scores	Optimal infant gross motor function observed among mothers with prenatal Hb range between	1. The Mullen Scales of Early Learning was used to assess motor and cognitive

			90g/L and 110 g/L	functions of children 2. Supplementation and prevention or treatment of malaria and helminth infections provided for enrolled pregnant women
Parsons <i>et al.</i> 2008 ⁸ - Australia	Prenatal iron supplementation was associated with abnormal teacher-rated peer problems among children of 6-8 years of age	Child behavior, temperament and abnormal total difficulties were not different between those whose mothers received prenatal iron supplements and those whose mothers did not.	Prenatal iron supplementation has no effect on improved child behavior at an early age	Strengths and Difficulties Questionnaire and parent-rated Short Temperament Scale for Children were used to assess child behavior.
Rioux <i>et al.</i> 2011 ⁹ - Canada	N/A	No significant relation was found between prenatal iron levels and infant cognitive development at 6 months.	Prenatal IDA was not associated with the cognitive development of children	Infant cognitive function was assessed using the Brunet–Lézine Scale of Psychomotor Development of Early Childhood and the Bayley Scales of Infant Development
Tran <i>et al.</i> 2013 ¹⁰ - Vietnam	Persistent IDA during pregnancy is associated with poor infant cognitive development at 6 months of age	N/A	Prenatal IDA has negative consequences on infant cognitive development	1. Bayley Scales of Infant and Toddler Development 3rd Ed, Cognitive Scale (BSID) 2. Prenatal IDA defined as Hb<110 g/L and serum ferritin <15 ng/mL
Tran <i>et al.</i>	Indirect effect of	No direct impact	Prenatal ID could	Infant motor

2014 ¹¹ - Vietnam	iron deficiency during early pregnancy on infant motor function at 6 months via anemia at late pregnancy	of iron deficiency in early or late pregnancy on infant motor functions	have negative consequences on infant motor development	function was assessed using the Bayley of Infant and Toddler Development Motor Scales (BSID-M)
Yang <i>et al.</i> 2010 - China	Pregnant women with in the highest Hb quintile (Hb \geq 124g/L) were 54% more likely to have children (4-6 year-olds) with poorer IQ compared to women in the moderate Hb quintile (110-116 g/L) at early gestation	There was no difference in child IQ between pregnant women with in the lowest Hb quintile (Hb < 103 g/L) compared to those in the moderate Hb quintile	High prenatal Hb concentrations may be detrimental to child cognitive function.	The Chinese version of the Wechsler Intelligence Scale for Children was used to assess child IQ
Zhou <i>et al.</i> 2006 ¹³ - Australia	Increased risk of abnormal behavior among children (4 years) of supplemented pregnant women (20 mg/d) compared to children of pregnant women in the placebo group	No significant difference in the mean IQ of children of women in the supplement group and those of women in the placebo group	Although iron supplementation during pregnancy reduces ID, it has no effect on offspring IQ	Child IQ was assessed by using the Stanford-Binet Intelligence Scale