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Article

Association of Vitamin A and Iron Supplementation on Major Clinical of Infectious Diseases in Ghanaian Children: A Cross-Sectional Study

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Abstract: Background: The utilization of nutrient supplementation as a tool to address issues such as iron and vitamin A deficiencies among children in Sub-Saharan Africa (SSA) has been beneficial. Nevertheless, there is a dearth of evidence on the effect of vitamin A and iron supplementation on infectious diseases in pre-school children in SSA. We aimed to assess the association of vitamin A and iron supplementation on the incidence of major clinical features of infectious diseases (diarrhea, cough, and fever) in children aged 6–59 months in Ghana. Methods: A cross-sectional study that utilized data from the Ghana Demographic and Health Survey, involving a sample size of 2,408 children. The collected data was analyzed using binomial logistic regression. Results: Dual supplementation with vitamin A and iron was associated with an increased risk of fever (OR: 1.68 95% CI: 1.22–2.32) and cough (OR: 1.43, 95% CI: 1.02–1.99) in children 6–59 months. Iron supplementation alone was associated with an increased risk of fever (OR: 1.32, 95% CI: 1.01–1.72) while Vitamin A alone significantly increased the risk of diarrhea (OR: 1.73, 95% CI: 1.03–2.92). Conclusions: Dual supplementation of vitamin A and iron is associated with an increase in childhood morbidity, endorsement of combined supplementation should be done with caution.

Keywords: iron; vitamin A; supplementation; infectious disease; preschool children

1. Introduction

Micronutrients are essential for the attainment of optimum health and disease prevention. Vitamin A and Iron are keys to the course of several biological processes including vision, growth and development as well as reproduction and immunity [1]. Vitamin A deficiencies (VAD) and iron deficiencies (ID) are the most common micronutrient deficiencies within the children population in developing countries. These two deficiencies are also regarded as key influencers of morbidity and mortality [2,3]. Globally, an estimated one-third of children aged 6 to 59 months were reported to be affected by VAD with Sub-Saharan Africa (SSA) recording the most incidences, thus 48 % [4]. In 2017, the Ghana micronutrient survey reported a 20.8 % incidence of VAD and a 21.5 % of ID among children in preschool [5]. In Africa, VAD and ID are common among children due to poor nutrition, high level of poverty as well as infection-related conditions like diarrhea and malaria. The detrimental effects of VAD and ID on the health and development of children have prompted recommendations for supplementation to reduce the load of morbidities in children [6].

In children under the age of five, inadequate levels of micronutrients have been linked to an increased risk of infection-related illnesses and account for 61% of global deaths resulting from diarrhea diseases [7]. Vitamin A supplementation (VAS) has been shown to improve immunity, reduce mortality and severity of infectious diseases in childhood [8,9], whilst supplementation with iron has been demonstrated to prevent iron-deficiency anemia and has the propensity of decreasing cognitive impairment related to anemia [10,11]. Also, there are suggestions that depending on the

nature of the disease condition, the effect of vitamin A and iron may present variedly. However, the effect of Vitamin A and iron supplementation on diarrhea and respiratory infections remain equivocal.

A substantial correlation between VAS and diarrhea has been demonstrated using meta-analysis from developing countries. Earlier studies reported that VAS exhibited an overall reduction in all-cause mortality by 23–30% [12–17] as well as diarrhea-specific mortality in children by 32% [17]. Mayo et al. found a 15% reduction in the incidence of diarrhea [12], however, Grotto et al. indicated that VAS had no coherent protective effect on the incidence of diarrhea and that it slightly increased the incidence of respiratory tract infections [17]. According to an analysis conducted in SSA, VAS was modestly associated with increased odds of fever, diarrhea and acute respiratory infection [18]. Despite the modest effect of iron supplementation in increasing the likelihood of the development of diarrhea by a previous systematic review, no evident detrimental impact was exhibited on the overall incidence of infectious illnesses in children [19]. Furthermore, ID and VAD often exist together [20] and the interaction between ID and VAD may aggravate each deficiency [21,22]. However, there are few studies that assess the synergistic effects of vitamin A and iron on infectious diseases. Existing evidence suggests that dual supplementation of vitamin A and iron can significantly decrease the rate of diarrhea-related illness and shorten the duration of respiratory and diarrhea-related illnesses [23].

Presently, in SSA, there are limited articles aimed at assessing the association of vitamin A and iron supplementation in children. To the best of our knowledge, this is the first study conducted in SSA that explores the synergistic effects of vitamin A and iron on diarrhea, fever and cough. Hence filling this research gap will provide valuable insights for designing vitamin A and iron supplementation programs targeted at specific groups of children bound to benefit the most. Thus, this study aimed to assess the association of vitamin A and iron supplementation on diarrhea, fever and cough in children aged 6–59 months based on data from demographic and health surveys (DHSs) conducted in Ghana.

2. Materials and Methods

2.1. Data Source, Study Setting and Population

A cross-sectional study was carried out with Ghana as its setting, using data from the 2014 Ghana Demographic and Health Survey (GDHS) [24], which is part of a global survey conducted by MEASURE DHSs in 85 low- and middle-income countries (LMICs) worldwide. The DHS is easily accessible online and aims to gather information on various population, health, and nutrition indicators such as childhood mortality, maternal and child health, family planning methods, women and children's nutritional status, and household socioeconomic factors. The DHS utilizes a two-stage cluster sampling technique to obtain nationally representative data for different residential areas and regions. The selection of enumeration areas (EAs) takes into account both rural and urban locations in Ghana, and households are chosen from these areas. The detailed sampling procedure can be found in the final report of the 2014 GDHS. Specifically, we utilized data from the birth recode and individual recode files, focusing on children aged 6–59 months in our study.

2.2. Exposure Variables

The study used iron and vitamin A intake as the key explanatory variable. We got the response “In the past seven days or two weeks was your child given iron pills, sprinkles with iron, or iron syrup like [this/any of these]?” and “In the last six months, was your child given a vitamin A dose like [this/any of these]?” respectively. We then divided the study subjects into four groups according to whether they had taken iron and vitamin A, with group I receiving neither iron nor vitamin A, group II receiving only iron, group III receiving only vitamin A, and group IV receiving both iron and vitamin A.

2.3. Outcome Variable

In this study, three components of infectious diseases, diarrhea, cough, and fever, were considered as the outcome variables. The incidence of diarrhea was determined by asking the mother the question; "Has your child experienced diarrhea in the past seven days or two weeks?" Similarly, the incidence of cough was established by asking the mother the question; "Has your child had an illness accompanied by coughing in the past seven days or two weeks?" Similarly, the occurrence of fever was determined by asking the mother, "Has your child been sick with a fever in the past seven days or two weeks?" The responses to these questions were regarded as the dependent variable, which is a dichotomous variable indicating whether a child had the stated illness. All the responses were recorded as "yes" (coded as 1) or "no" (coded as 0).

2.4. Covariates

We also controlled for the following variables, age in months (6–12 months; 12–24 months; 24–36 months; 36–59 months), sex (boys; girls), the mother's level of education (no education; primary; secondary; higher), the family's wealth index (poorest; poorer; middle; richer; richest), stunting (yes; no), wasting (wasted; non-wasted; overweight), and the type of residential area (urban; rural).

2.5. Statistical Analysis

The analysis was carried out in two stages. Firstly, the basic characteristics of the study participants were described according to groups of iron and vitamin A intake and the different clinical features of infectious diseases. Secondly, a series of binomial logistic regressions were performed to assess the effects of characteristic variables on the likelihood of having diarrhea, cough, and fever, respectively. The odds ratios (OR) with 95% confidence intervals (CI) were calculated to determine the significance of the variable being studied. Statistical significance was pegged at $P < 0.05$. The analysis of the data was conducted using Stata version 16.0.

3. Results

3.1. Baseline Characteristics

A total of 2,451 children aged 6 to 59 months were included in GDHS, and 2,408 were included in our study after removing the missing key variables (32 missing for stunting, 11 missing data for wasting). The majority of children were between 36–59 months of age (41.03 %, $n=988$). The boy–girl ratio was 1:1 (1258 vs. 1150). Nearly two–thirds of the children (1,430/2,408) were from rural areas. The number of children receiving vitamin A ($n=1,495$) far exceeds the number of children receiving iron ($n=544$). Stunting was observed in about a quarter of children (502/2,408), while only 104 children reported wasting. In terms of maternal education, more than one–third (864/2,408) had no education, and only 77 had mothers with higher education. About 85% of household wealth index were middle, poorer and poorest category. During the survey, there was a relatively small difference in the number of children whose mothers reported diarrhea, fever and cough, with 317 (13.16%) children having diarrhea, 401 (16.65%) children having fever and 351(14.58%) children having cough, respectively. Table 1 shows the basic characteristics of children based on whether they had symptoms of diarrhea, fever and cough.

Table 1. Characteristics of study subjects according to different disease conditions.

	Diarrhea		Fever		Cough	
	No.(%)	yes	No.(%)	yes	No.(%)	yes
	2091(86.84)	317(13.16)	2007(84.35)	401(16.65)	2057(85.42)	351(14.58)
Age in months						
6-12 months	250(11.95)	38(11.99)	250(12.46)	38(9.48)	240(11.67)	48(13.67)
12-24 months	469(22.43)	111(35.02)	474(23.62)	106(26.43)	484(23.53)	96(27.35)
24-36 months	456(21.81)	96(30.28)	444(22.12)	108(26.93)	467(22.70)	85(24.22)

36-59 months	916(43.81)	72(22.71)	839(41.80)	149(37.16)	866(42.10)	122(34.76)
Sex						
male	1067(51.03)	191(60.25)	1032(51.42)	226(56.36)	1083(52.65)	175(49.86)
female	1024(48.97)	126(39.75)	975(48.58)	175(43.64)	974(47.35)	176(50.14)
Type of place of residence						
urban	847(40.51)	131(41.32)	834(41.55)	144(35.91)	823(40.01)	155(44.16)
rural	1244(59.49)	186(58.68)	1173(58.45)	257(64.09)	1234(59.99)	196(55.84)
Iron intake						
no	1636(78.24)	228(71.92)	1570(78.23)	294(73.32)	1603(77.93)	261(74.36)
yes	455(21.76)	89(28.08)	437(21.77)	107(26.68)	454(22.07)	90(25.64)
Vitamin A intake						
no	802(38.35)	111(35.02)	783(39.01)	130(32.42)	802(38.99)	111(31.62)
yes	1289(61.65)	206(64.98)	1224(60.99)	271(67.58)	1255(61.01)	240(68.38)
Highest educational level of mother						
no education	739(35.34)	125(39.43)	699(34.83)	165(41.15)	756(36.75)	108(30.77)
primary	427(20.42)	70(22.09)	410(20.43)	87(21.69)	421(20.47)	76(21.65)
secondary	854(40.84)	116(36.59)	828(41.25)	142(35.41)	813(39.52)	157(44.73)
higher	71(3.40)	6(1.89)	70(3.49)	7(1.75)	67(3.26)	10(2.85)
Stunting						
no	1655(79.15)	251(79.18)	1592(79.32)	314(78.30)	1614(78.46)	292(83.19)
yes	436(20.85)	66(20.82)	415(20.68)	87(21.70)	443(21.54)	59(16.81)
Wasting						
wasted	85(4.06)	19(5.99)	90(4.48)	14(3.49)	89(4.33)	15(4.27)
non-wasted	1955(93.50)	293(92.43)	1866(92.98)	382(95.26)	1918(93.24)	330(94.02)
overweight	51(2.44)	5(1.58)	51(2.54)	5(1.25)	50(2.43)	6(1.71)
Wealth index of family						
poorest	671(32.09)	101(31.86)	622(30.99)	150(37.41)	671(32.62)	101(28.77)
poorer	450(21.52)	71(22.40)	438(21.82)	83(20.70)	463(22.51)	58(16.52)
middle	376(17.98)	78(24.61)	363(18.09)	91(22.69)	370(17.99)	84(23.93)
richer	319(15.26)	39(12.30)	311(15.50)	47(11.72)	300(14.58)	58(16.52)
richest	275(13.15)	28(8.83)	273(13.60)	30(7.48)	253(12.30)	50(14.26)

Table 2 describes the characteristics of children based on groupings by iron and vitamin A intake. Of these groups, Group I refer to children who did not receive iron or vitamin A, totaling 788 (32.72%) children. Group II represents children receiving iron only, a total of 1,076 (44.69%). Group III includes a total of 125 (5.19%) children who received vitamin A only. Finally, Group IV consisted of children who received both iron and vitamin A, a total of 419 (17.40%). Children aged 36 – 59 months (51.14% vs. 36.52% vs. 40% vs. 33.90%) and children from the poorest household wealth index category (40.61% vs. 30.02% vs. 23.20% vs. 23.87%) were significantly higher than the other categories.

Table 2. Characteristics of study subjects according to different groups.

	Group I	Group II	Group III	Group IV
	No.(%)	No.(%)	No.(%)	No.(%)
	788(32.72)	1076(44.69)	125(5.19)	419(17.40)
Age in months				
6-12 months	94(11.93)	134(12.45)	10(8.00)	50(11.93)
12-24 months	133(16.88)	312(29.00)	25(20.00)	110(26.25)
24-36 months	158(20.05)	237(22.03)	40(32.00)	117(27.92)
36-59 months	403(51.14)	393(36.52)	50(40.00)	142(33.90)
Sex				
male	422(53.55)	538(50.00)	67(53.60)	231(55.13)
female	366(46.45)	538(50.00)	58(46.40)	188(44.87)
Type of place of residence				
urban	313(39.72)	427(39.68)	46(36.80)	192(45.82)
rural	475(60.28)	649(60.32)	79(63.20)	227(54.18)
Highest educational level of mother				
no education	351(44.54)	369(34.29)	36(28.80)	108(25.78)
primary	153(19.42)	208(19.33)	33(26.40)	103(24.58)
secondary	266(33.76)	461(42.85)	51(40.80)	192(45.82)
higher	18(2.28)	38(3.53)	5(4.00)	16(3.82)
Stunting				
no	601(76.27)	863(80.20)	95(76.00)	347(82.82)
yes	187(23.73)	213(19.80)	30(24.00)	72(6.69)
Wasting				
wasted	29(3.68)	52(4.83)	5(4.00)	18(4.30)
non-wasted	742(94.16)	993(92.29)	119(95.2)	394(94.03)
overweight	17(2.16)	31(2.88)	1(0.80)	7(1.67)
Wealth index of family				
poorest	320(40.61)	323(30.02)	29(23.20)	100(23.87)
poorer	140(17.77)	260(24.16)	35(28.00)	86(20.53)
middle	133(16.88)	203(18.87)	22(17.60)	96(22.91)
richer	109(13.83)	162(15.06)	23(18.40)	64(15.27)
richest	86(10.91)	128(11.90)	16(12.80)	73(17.42)
Diarrhea				
no	700(88.83)	936(86.99)	102(81.60)	353(84.25)
yes	88(11.17)	140(13.01)	23(18.40)	66(15.75)
Fever				
no	678(86.04)	892(82.90)	105(84.00)	332(79.24)
yes	110(13.96)	184(17.10)	20(16.00)	87(20.76)
Cough				
no	689(87.44)	914(84.94)	113(90.40)	341(81.38)
yes	99(12.56)	162(15.06)	12(9.60)	78(18.62)

3.2. Morbidity

Table 3 presents the results of binary logistic regression (OR and its 95% confidence interval) based on whether there was diarrhea, fever, and cough, respectively. Iron alone (group II) significantly increased the risk of fever (OR: 1.32, 95% CI: 1.01–1.72) compared to children who received neither iron nor vitamin A (group I). In addition, children aged 24–36 months were at increased risk of fever (OR: 1.60, 95% CI: 1.06–2.41), while a higher household wealth index was a protective factor for the development of fever (OR: 0.48, 95% CI: 0.27–0.83). Vitamin A alone (group III) significantly increased the risk of diarrhea (OR: 1.73, 95% CI: 1.03–2.92) compared to children who received neither iron nor vitamin A (group I). After adjusting for covariates, a child who received vitamin A alone had 1.73 times higher risk of developing diarrhea than a child who received neither iron nor vitamin A. Boy sex was 0.49 times more likely to develop diarrhea than girls. Relative to children aged 6–12 months, children aged 12–24 months were more likely to develop diarrhea (OR: 1.67, 95% CI: 1.11–2.51). Conversely, children aged 36–59 months were less likely to develop diarrhea (OR: 0.54, 95% CI: 0.35–0.83). Finally, children who received both iron and vitamin A (group IV) had an increased risk of fever (OR: 1.68, 95% CI: 1.22–2.32) and cough (OR: 1.43, 95% CI: 1.02–1.99) compared with no intake.

Table 3. Results of a series of binomial logistic regressions according to different disease conditions.

	Diarrheal	Fever	Cough
Variables	odds ratio 95%CI	odds ratio 95%CI	odds ratio 95%CI
Age in months			
6-12 months	1 reference	1 reference	1 reference
12-24 months	1.67* (1.11,2.51)	1.46 (0.97,2.2)	1.02 (0.69,1.5)
24-36 months	1.46 (0.96,2.23)	1.60* (1.06,2.41)	0.96 (0.64,1.42)
36-59 months	0.54* (0.35,0.83)	1.18 (0.8,1.74)	0.74 (0.51,1.07)
sex(male=1)	1.50* (1.17,1.91)	1.24 (1,1.55)	0.90 (0.71,1.13)
type of place of residence(urban=1)	1.34 (0.97,1.85)	1.06 (0.79,1.43)	0.93 (0.68,1.28)
group			
I	1 reference	1 Reference	1 reference
II	1.07 (0.80,1.44)	1.32* (1.01,1.72)	1.17 (0.88,1.53)
III	1.73* (1.03,2.92)	1.23 (0.72,2.07)	0.71 (0.37,1.33)
IV	1.30 (0.91,1.86)	1.68* (1.22,2.32)	1.43* (1.02,1.99)
highest education level of mother			
no education	1	1	1

	reference	reference	reference
primary	0.89 (0.63,1.24)	0.91 (0.67,1.24)	1.18 (0.85,1.65)
secondary	0.73 (0.53,1.01)	0.81 (0.60,1.08)	1.12 (0.82,1.55)
higher	0.53 (0.21,1.35)	0.63 (0.26,1.51)	0.79 (0.37,1.7)
stunting	0.86 (0.63,1.17)	0.96 (0.73,1.25)	0.81 (0.59,1.1)
wasting			
wasted	1 reference	1 reference	1 reference
non-wasted	0.75 (0.44,1.27)	1.36 (0.76,2.45)	1.04 (0.59,1.84)
overweight	0.49 (0.17,1.43)	0.69 (0.23,2.06)	0.75 (0.27,2.07)
wealthy index of family			
poorest	1 reference	1 reference	1 reference
poorer	1.06 (0.75,1.50)	0.77 (0.57,1.06)	0.78 (0.54,1.12)
middle	1.36 (0.93,2.01)	1.03 (0.73,1.46)	1.41 (0.97,2.05)
richer	0.75 (0.46,1.25)	0.64 (0.41,1.01)	1.24 (0.79,1.96)
richest	0.61 (0.34,1.12)	0.48* (0.27,0.83)	1.29 (0.76,2.19)

* p<0.05.

4. Discussion

Micronutrient malnutrition remains predominant on the continent of Africa and its detrimental effects on the overall health and productivity of a country act as a barrier to socio-economic development. While there have been some improvements in reducing micronutrient deficiencies in Ghana over the past decade, anemia and VAD still pose significant public health concerns [5]. Our study found that iron supplementation was markedly associated with an increased probability of fever with no significant impact on the occurrence of diarrhea and cough. Notably, supplementation with vitamin A was associated with an increased risk of the occurrence of diarrhea but had no significant effect on the occurrence of fever and cough. The combined administration of vitamin A and iron significantly increased the occurrence of fever and cough. Our research study also noted more complex correlations between supplementation and children distinguished by age, sex and household wealth index.

4.1. Effect of Iron on Diarrhea, Fever and Cough

The role of iron has received considerable attention in the interaction between supplementation and infections [19,25], as studies have confirmed that supplementation adversely modifies the gut microbiome [26,27], cause damage to cells mediated through free radicals and increases the virulence of pathogenic enteric bacteria [28,29]. However, published results concerning the relationship between iron and infectious disease have shown considerable inconsistency [25]. Adverse outcomes associated with iron supplementation have been reported by several studies from developing

countries [30,31]. On the other hand, an earlier study found a desirable effect of iron supplementation with a reduced frequency of fever, respiratory infections, and diarrhea in children from the ages of 2 to 5 years [32]. According to Iannotti et al. the protective effect of iron supplementation against diarrhea, respiratory infections, or fever may be limited to children who are iron deficient [33]. Notwithstanding, Smith et al. in a study that had only anemic children at baseline, reported iron supplementation was associated with an increase in fever-associated severe malaria [31]. In our study, supplementation with iron alone had no significant effect on diarrhea and cough morbidities, and this finding supports previous studies, which found no protective effect of iron supplementation against diarrhea and acute lower Respiratory tract infection morbidity [23], however, there was an increased risk of fever in our study. Future prospective studies in broader populations are needed to explore possible mechanisms of iron supplementation and infectious diseases. We also found that age 24–36 months was associated with a high risk of fever with iron supplementation. The age-dependent outcomes presented by our study may be accounted for by differences in the physiological response to iron supplementation in 24–36 months children. Further research is necessary to better understand the underlying reasons. We also found a significant correlation between household wealth index and the incidence of fever. Children from the richest household wealth index had a lower risk of fever. The possible explanation might be that children from higher socioeconomic families were more likely to receive requisite healthcare services and nutritious diet, which improves their immune system and consequently lowers their susceptibility to infections [34]. These results underscore the profound impact of a family's wealth on the health of children.

4.2. Effect of Vitamin A on Diarrhea, Cough and Fever

VAS has consistently reduced overall infant mortality and diarrhea-associated infant mortality [16,35]. However, the effect of VAS on diarrhea has been inconsistent across research studies but may increase the incidence of respiratory tract infections [36,37]. The observed outcomes of our research align with previous studies regarding the influence of VAS on diarrhea disease [38]. Several theories have been proposed regarding the relationship between VAS and diarrhea. Firstly, the effect of vitamin A on the immune response of the individual against certain pathogens leads to variations in clinical outcomes for specific pathogen infections. A wide range of pathogens accounts for diarrhea-related illness, with each capable of triggering a unique mucosal immune response and pathogenic mechanisms [39,40]. VAS up-regulates the T helper subset 2 cell (Th2) lymphocyte response and down-regulates the activity of T helper type 1 cell (Th1), which is protective against intracellular infections like *Salmonella* spp [41]. These immunoregulatory effects of vitamin A suggest that the impact of supplementation on pathogen-specific clinical outcomes may rely on the role played by Th1–Th2 responses in these outcomes. The increased risk in the incidence of diarrhea in our study may be attributable to the relatively higher incidence of gastrointestinal pathogens such as *Salmonella* spp. In Ghana, *Salmonella* spp. is commonly associated with the incidence of diarrhea among preschool children [42] and tends to yield these symptoms through pathogenesis on the intestinal epithelial barrier [43]. Supplementation with vitamin A may lead to reduced protection for children against this pathogen since vitamin A down-regulates the Th1 response, which protects against infections by this pathogen [41]. Similarly, the lack of significant effect of VAS on cough and fever may be indicative of the impact of supplementation on the immune response to the pathogens that cause respiratory tract infections and fever. A study by Binka et al reported no difference in the incidence of fever and malaria parasitemia as observed between children receiving VAS and those who had received a placebo [44]. Nonetheless, intervention trials have yielded some conflicting findings. For instance, a decrease in the occurrence of diarrhea and cough with fever was observed to have resulted from VAS in an intervention trial conducted in Mexico [45]. The differential regulation of the pathogen-specific immune response by vitamin A, disparities in the prevalence of pathogens across various communities and study populations could account for the inconsistencies observed in the outcomes of VAS. Our study also showed that boy sex and age 12–24 months were associated with a higher risk of diarrhea compared to aged 6–12 months while aged 36–59 month was associated with less likelihood of developing diarrhea. A higher rate of diarrhea in children 12–24

months is likely due to weaning occurring from 12 months in most children in Ghana. In Ghana, the span of breastfeeding is generally prolonged, with 98% of children from ages 9 to 11 months remaining breastfed and a significant 50% still being breastfed at 20–23 months old [24]. Inappropriate weaning practices have been linked to an increased risk of diarrhea disease. Previous studies showed similar results with an increase in the incidence and duration of diarrhea after weaning in 1- and 2-year-old children [46]. Health education programs should be directed toward mothers, with a focus on improving weaning practices, enhancing food hygiene, and providing better childcare.

4.3. Effect of Vitamin A Combined with Iron on Diarrhea, Cough and Fever

Combined supplementation with vitamin A and iron resulted in a significant increase in the risk of fever and cough, in contrast to vitamin A or iron supplementation alone. Up until now, no definitive mechanism has been put forth to explain the synergistic interaction or combined effect of vitamin A and iron on immune function. We have deduced several explanations that may account for the afore-observed outcome. While vitamin A and iron are known to play a crucial role in ensuring the optimal functioning of the immune system, there is evidence indicating that excessive intake of vitamin A and iron through supplementation may increase the adverse effects of infections. VAS has been linked to downregulating the innate immune response as a result of epigenetic modifications [47,48]. Iron supplementation, on the other hand, has a detrimental effect on the immune system by generating free radicals, and contributes to enhanced bacterial and viral growth, potentially raising the susceptibility to infections [28,29]. Therefore, it is entirely conceivable that the simultaneous intake of both vitamin A and iron supplements could result in immune suppression and in effect increase the vulnerability of children to infectious diseases. The response of the body to high doses of vitamin A and iron depends on the underlying vitamin A and iron status of the child. There may also be interactions between vitamin A and iron that contribute to an increased susceptibility to infections. The interrelationship between vitamin A and iron has been widely acknowledged by several studies over the years [49]. Studies have demonstrated VAS significantly increases hemoglobin, hematocrit, and serum iron [50]. A dual increase in serum iron caused by vitamin A and iron supplementation might have the evolutionary function of increasing circulating iron fuel for pathogens with the result that pathogenic bacteria are no longer restricted in their supply of iron leading to an increase in infections. An excess of iron in the gut of a child who is not deficient in iron can be detrimental [26]. We assert that the increase in the risk of cough and fever observed in preschool children receiving a combined supplementation of iron and vitamin A might be a result of the exacerbative effects of vitamin A on iron status. It is recommended that the administration of vitamin A should be administered singularly or only when a deficiency is detected and the status of iron and vitamin A is known, since co-administration of vitamin A increases the adverse effect of iron for infectious disease susceptibility, as seen in this study. Governments or relevant authorities are advised to encourage the measurement of vitamin A and iron status in children as part of their strategic attempts to ensure optimum outcomes of supplementation in children. Iron supplementation ought to be administered in enriched foods where the detrimental interactions of vitamin A and iron may be reduced. Further trials on supplementation are necessary to corroborate the relationships between vitamin A and iron in children residing in areas endemic to infectious diseases.

Several study strengths must be acknowledged. This is the first study carried out in SSA to assess the association of vitamin A and iron supplementation on major clinical features of infectious diseases in children aged 6–59 months. This study is among the first to demonstrate the adverse effect of vitamin A and iron on cough and fever in children 6–59 months. Our study employed data from a national survey in Ghana, hence the study findings are generalizable to the entire country. Conversely, it is important to acknowledge some limitations associated with this study. First was the absence of clinical or laboratory assessments and the reliance on mothers' recall as the principal basis for the determination of exposure to vitamin A and iron supplementation. This renders the study liable to recall and skewed categorization bias, which can lead to an underestimation or exaggeration

of the true strength of the study. Secondly, we did not take into account the current vitamin A and iron status of children during the survey. Thirdly, the study is susceptible to systematic errors, since it is an observational one. Another significant limitation is the absence of standardized definitions for individual clinical morbidities. The use of standardized definitions and active surveillance would have improved the rigorousness of the study's conclusions. Finally, concerning the questionnaire, the period between exposure and the relevant outcomes was inconsistent.

5. Conclusion

In summary, our findings indicate that iron and vitamin A supplementation alone were associated with an increase in the occurrence of fever and diarrhea respectively, while dual supplementation of iron and vitamin A supplementation was associated with an increased risk of fever and cough. The likelihood of the occurrence of childhood morbidities was found to be independently influenced by factors such as age, sex, and household wealth index. Our study offers further understanding into the complex relationships between vitamin A and iron supplementation and infectious disease. The implication of this study would be the combined supplementation of vitamin A and iron may have more drawbacks than benefits, highlighting the need to avoid indiscriminate supplementation to all children and careful consideration when implementing such treatment approaches. The outcome of this study has significant policy implications, especially in countries such as Ghana where iron supplementation or fortification initiatives are not present as yet. Further studies are needed to better understand the synergy effect of vitamin A and iron on infectious diseases, particularly in areas with high infection rates. Such studies will also contribute to the implementation, monitoring, and evaluation of nationwide vitamin A and iron supplementation initiatives in Sub-Saharan Africa.

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Data Availability Statement: Data described in the manuscript are available through access to the Ghana Statistical Service Website, statsghana.gov.gh.

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References

1. Zhang, X.; Yang, K.; Chen, L.; Liao, X.; Deng, L.; Chen, S.; Ji Y. Vitamin A deficiency in critically ill children with sepsis. *Crit Care*. **2019**, 23(1), 267.
2. Smith, R.G. The vitamin cure for eye disease: how to prevent and treat eye disease using nutrition and vitamin supplementation. Laguna Beach, CA: *Basic Health Publications* **2012**.
3. Stevens, G.A; Bennett, J.E; Hennocq, Q; Lu, Y; De-Regil, L.M; Rogers, L.; et al. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. *Lancet Glob Health*. **2015**, 3(9),e528–36
4. UNICEF. Current status and progress. 2016 <https://data.unicef.org/topic/nutrition/vitamin-a-deficiency/#> (accessed May 2023).
5. Wisconsin-Madison/KEMRI-WellcomeTrust/UNICEF UoGGUo. Ghana Micronutrient Survey 2017. UNICEF-Accra Accra, Ghana. **2017**.

6. Stoltzfus, R.J.; Dreyfuss, M.L. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Washington, DC: *ILSI Press*. **1998**.
7. Bryce, J.; Boschi-Pinto, C.; Shibuya, K.; Black, R.E. WHO Child Health Epidemiology Reference Group. WHO estimates of the causes of death in children. *Lancet*. **2005**, 365(9465), pp. 1147–52.
8. Sommer, A. Vitamin A deficiency and clinical disease: an historical overview. *J Nutr*. **2008**, 138(10), 1835–9.
9. Gogia, S.; Sachdev, H.S. Neonatal vitamin A supplementation for prevention of mortality and morbidity in infancy: systematic review of randomized controlled trials. *BMJ*. **2009**, 338, b919.
10. Grantham-McGregor, S.; Ani, C. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr*. **2001**, 131(2S–2), 649S–666S.
11. de Silva, A.; Atukorala, S.; Weerasinghe, I.; Ahluwalia, N. Iron supplementation improves iron status and reduces morbidity in children with or without upper respiratory tract infections: a randomized controlled study in Colombo, Sri Lanka. *Am J Clin Nutr*. **2003**, 77(1), 234–41.
12. Mayo-Wilson, E.; Imdad, A.; Herzer, K.; Yakoob, M.Y.; Bhutta, Z.A. Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis. *BMJ*. **2011**, 343, d5094.
13. Imdad, A.; Herzer, K.; Mayo-Wilson, E.; Yakoob, M.Y.; Bhutta, Z.A. Vitamin A supplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. *Cochrane Database Syst Rev*. **2010**, (12), CD008524.
14. Fawzi, W.W.; Chalmers, T.C.; Herrera, M.G.; Mosteller, F. Vitamin A supplementation and child mortality. A meta-analysis. *JAMA*. **1993**, 269(7), 898–903.
15. Imdad, A.; Yakoob, M.Y.; Sudfeld, C.; Haider, B.A.; Black, R.E.; Bhutta, Z.A. Impact of vitamin A supplementation on infant and childhood mortality. *BMC Public Health*. **2011**, 11 suppl 3(suppl 3):S20.
16. Beaton, G.H.; Martorell, R.; Aronson, K.J.; Edmonston, B.; McCabe, G.; Ross, A.C.; et al. Effectiveness of Vitamin A Supplementation in the Control of Young Child Morbidity and Mortality in Developing Countries: *Nutrition Policy Discussion Paper No. 13*, **1993**.
17. Grotto, I.; Mimouni, M.; Gdalevich, M.; Mimouni, D. Vitamin A supplementation and childhood morbidity from diarrhea and respiratory infections: a meta-analysis. *J Pediatr*. **2003**, 142(3), 297–304.
18. Gebremedhin, S. Vitamin A supplementation and childhood morbidity from diarrhea, fever, respiratory problems and anemia in sub-Saharan Africa. *Nutr Dietary Suppl*. **2017**, 9, 47–54.
19. Gera, T.; Sachdev, H.P. Effect of iron supplementation on incidence of infectious illness in children: systematic review. *BMJ*. **2002**, 325(7373), 1142.
20. Semba, R.; Bloem, M. The anemia of vitamin a deficiency: Epidemiology and pathogenesis. *Eur J Clin Nutr*. **2002**, 56(4), 271–81.
21. Sommer, A.; Tarwotjo, I.; Djunaedi, E.; West, K.P. Jr.; Loeden, A.A.; Tilden, R.; et al. Impact of vitamin A supplementation on childhood mortality: A randomised controlled community trial. *Lancet*. **1986**, 1(8491), 1169–73.
22. Jang, J.T.; Green, J.B.; Beard, J.L.; Green, M.H. Kinetic analysis shows that iron deficiency decreases liver vitamin A mobilization in rats. *J Nutr*. **2000**, 130(5), 1291–96.
23. Chen, K.; Chen, X.R.; Zhang, L.; Luo, H.Y.; Gao, N.; Wang, J.; et al. Effect of simultaneous supplementation of vitamin A and iron on diarrhea and respiratory tract infection in preschool children in Chengdu City, China. *Nutr*. **2013**, 29(10), 1197–203.
24. Ghana Statistical service. Ghana Demographic and Health Survey **2014**: doi:10.15171/ijhpm.2016.42
25. Fishbane, S. Review of issues relating to iron and infection. *Am J Kidney Dis*. **1999**, 34, S47–52.
26. Zimmermann, M.B.; Chassard, C.; Rohner, F.; N'goran, E.K.; Nindjin, C.; Dostal, A.; et al. The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Cote d'Ivoire. *Am J Clin Nutr*. **2010**, 92(6), 1406–15.
27. Dostal, A.; Baumgartner, J.; Riesen, N.; Chassard, C.; Smuts, C.M.; Zimmermann, M.B.; et al. Effects of iron supplementation on dominant bacterial groups in the gut, faecal SCFA and gut inflammation: a randomised, placebo-controlled intervention trial in South African children. *Br J Nutr*. **2014**, 112(4), 547–56.
28. Kortman, G.A.; Boleij, A.; Swinkels, D.W.; Tjalsma, H. Iron availability increases the pathogenic potential of *Salmonella typhimurium* and other enteric pathogens at the intestinal epithelial interface. *PLoS One*. **2012**, 7(1), e29968.
29. Dostal, A.; Gagnon, M.; Chassard, C.; Zimmermann, M.B.; O'Mahony, L.; Lacroix, C. *Salmonella* adhesion, invasion and cellular immune responses are differentially affected by iron concentrations in a combined in vitro gut fermentation-cell model. *PLoS One*. **2014**, 9(3), e93549.
30. Mitra, A.K.; Akramuzzaman, S.M.; Fuchs, G.J.; Rahman, M.M.; Mahalanabis, D. Long-term oral supplementation with iron is not harmful for young children in a poor community of Bangladesh. *J Nutr*. **1997**, 127(8), 1451–5.
31. Smith, A.W.; Hendrickse, R.G.; Harrison, C.; Hayes, R.J.; Greenwood, B.M. The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. *Ann Trop Paediatr*. **1989**, 9(1), 17–23.

32. Angeles, I.T.; Schultink, W.J.; Matulessi, P.; Gross, R.; Sastroamidjojo, S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. *Am J Clin Nutr.* **1993**,58(3),339–42.
33. Iannotti, L.L.; Tielsch, J.M.; Black, M.M.; Black, R.E. Iron supplementation in early childhood: health benefits and risks. *Am J Clin Nutr.* **2006**,84(6):1261–76
34. Cohen, S. Social status and susceptibility to respiratory infections. *Ann N Y Acad Sci.* **1999**,896,246–53.
35. Ghana VAST Study Team. Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions, and child mortality. *Lancet.* **1993**,342(8862),7–12.
36. Dibley, M.J.; Sadjimin, T.; Kjolhede, C.L.; Moulton, L.H. Vitamin A supplementation fails to reduce incidence of acute respiratory illness and diarrhea in preschool-age Indonesian children. *J Nutr.* **1996**,126(2),434–42.
37. Barreto, M.L.; Santos, L.M.; Assis, A.M.; Araújo, M.P.; Farenzena, G.G.; Santos, P.A.; et al. Effect of vitamin A supplementation on diarrhoea and acute lower-respiratory-tract infections in young children in Brazil. *Lancet.* **1994**,344(8917),228–31.
38. Long, K.Z.; Montoya, Y.; Hertzmark, E.; Santos, J.I.; Rosado, J.L. A doubleblind, randomized, clinical trial of the effect of vitamin A and zinc supplementation on diarrhea disease and respiratory tract infections in children in Mexico City, Mexico. *Am J Clin Nutr.* **2006**, 83(3),693–700.
39. Evans, A.S.; Brachman, P.S. editors. Bacterial infections of humans: Epidemiology and control (2nd edition). New York: Plenum Medical Book Company. **1991**.
40. Balows, A.; Hausler Jr, W.J.; Hermann, K.L.; Isenberg, H.D.; Shadomy, H.J. editors. Manual of Clinical Microbiology, 5th edition. Washington, American Society for Microbiology, **1991**.
41. Mizuno, Y.; Takada, H.; Nomura, A.; Jin, C.H.; Hattori, H.; Ihara, K.; et al. Th1 and Th1-inducing cytokines in Salmonella infection. *Clin Exp Immunol.* **2003**,131(1),111–7.
42. Afum, T.; Asandem, D.A.; Asare, P.; Asante-Poku, A.; Mensah, G.I.; Musah, A.B.; et al. Diarrhea-Causing Bacteria and Their Antibiotic Resistance Patterns Among Diarrhea Patients From Ghana. *Front Microbiol.* **2022**,13,894319.
43. Bertelsen, L.S.; Paesold, G.; Marcus, S.L.; Finlay, B.B.; Eckmann, L.; Barrett, K.E. Modulation of chloride secretory responses and barrier function of intestinal epithelial cells by the Salmonella effector protein SigD. *Am J Physiol Cell Physiol.* **2004**,287(4),C939–48.
44. Binka, F.N.; Ross, D.A.; Morris, S.S.; Kirkwood, B.R.; Arthur, P.; Dollimore, N.; et al. Vitamin A supplementation and childhood malaria in northern Ghana. *Am J Clin Nutr.* **1995**,61(4),853–9.
45. Long, K.Z.; Rosado, J.L.; DuPont, H.L.; Hertzmark, E.; Santos, J.I. Supplementation with vitamin A reduces watery diarrhoea and respiratory infections in Mexican children. *Br J Nutr.* **2007**,97(2),337–43.
46. Mølbak, K.; Gottschau, A.; Aaby, P.; Højlyng, N.; Ingholt, L.; da Silva, A.P. Prolonged breast feeding, diarrhoeal disease, and survival of children in Guinea-Bissau. *BMJ.* **1994**,308(6941),1403–6.
47. Arts, R.J.; Blok, B.A.; van Crevel, R.; Joosten, L.A.; Aaby, P.; Benn, C.S.; et al. Vitamin A induces inhibitory histone methylation modifications and down-regulates trained immunity in human monocytes. *J Leukoc Biol.* **2015**,98(1),129–36.
48. Arts, R.J.; Benn, C.S. Vitamin A induces long-term epigenetic modification in the innate immune system. *Sight Life* **2015**,29,17–20
49. Ramakrishnan, U.; Grant, F.K.; Goldenberg, T.; Bui, V.; Imdad, A.; Bhutta, Z.A. Effect of multiple micronutrient supplementation on pregnancy and infant outcomes: a systematic review. *Paediatr Perinat Epidemiol.* **2012**,26,153–67.
50. Al-Mekhlafi, H.M.; Al-Zabedi, E.M.; Al-Maktari, M.T.; Atroosh, W.M.; Al-Delaimy, A.K.; Moktar, N.; et al. Effects of vitamin A supplementation on iron status indices and iron deficiency anaemia: a randomized controlled trial. *Nutrients.* **2013**,6(1),190–20

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