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The role of multi-micronutrient supplementation in paediatric HIV management in Nigeria: A randomized controlled study

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Abstract

Background: To compare the immunologic and hematologic effects of three micronutrient supplements in HIV positive children in Lagos, Nigeria.

Methods: This double blind randomized controlled study included one hundred and ninety children, aged 5 – 12 years, in Lagos, Nigeria. 64, 63 and 63 participants were assigned to multi-micronutrient supplement A, B and C respectively for 6 months. Supplements A, B and C contained 7 micronutrients at recommended daily allowance (RDA) (comparable to standard of care multivitamin), 22 micronutrients at RDA, and 22 micronutrients at 3RDA respectively. Using paired sample t tests and factorial repeat measures ANOVA, within and between group changes in CD4 count and haemoglobin (Hb) levels were evaluated after six months.

Results: After 6 months of supplementation, paired sample t test showed CD4 count did not significantly differ from baseline for all 3 groups. Between subject effect also did not significantly differ in the three groups after 6 months (factorial repeat measures ANOVA, $F(2, 187) = 0.846$, $p = 0.436$, Partial Eta Squared = 0.009). Hb levels were significantly increased after supplementation in all 3 supplement groups. Increases were not significantly different between groups (factorial repeat measures ANOVA, $F(2, 187) = 0.549$, $p = 0.591$, Partial Eta Squared = 0.006).

Conclusion: Equivalent effects were observed. After 6 months of supplementation, mean CD4 count was not significantly different between groups. Hb concentration was significantly increased in all three groups, but increase did not differ between groups.

Keywords: HIV; Micronutrients; Paediatric; Nigeria; Randomized Controlled Study

The study is registered with the Clinical Trial Registry at https://clinicaltrials.gov/. Unique identifier number assigned to this study is NCT02552602.

Background

Multiple micronutrient deficiencies have been documented in HIV positive persons [1], [2]. Deficiencies correspond with low circulating antioxidants to act as free radical scavengers [1], [3]. The resulting increased levels of reactive oxidative species reportedly have a direct effect in progressing HIV disease [4], [5]. HIV disease progression is characterized by CD4 count decline, and consequential deterioration in immune functioning [6]. With significant positive correlation between CD4 count and antioxidant levels [7], multi-micronutrients could potentially address existing oxidative stress and limit HIV disease progression [8]. Low Hb levels (anaemia) have also been associated with progressing HIV disease [9], [10]. The strong relationship existing between Hb levels and oxidative stress [11] would infer potential benefits of multi-micronutrients in preventing/ treating anaemia in people living with HIV (PLHIV) [12], [13].

Efficacy trials evaluating immunological and haematological effects of supplementation in PLHIV have been inconclusive [12]–[20]. Differences in supplement composition and strength could explain the conflicting results. By comparing three supplements (A, B, C), this study evaluated the effect of supplementation in HIV positive Nigerian children. Supplement A contained 7 micronutrients at RDA, B contained 22 micronutrients at RDA, and C contained 22 micronutrients at 3RDA. Outcomes of interest were CD4 count and haemoglobin levels. Immune deficiency (CD4 count <500cells/mm^3) and anaemia (Hb concentration <11.5g/dL) at baseline and after 6 months of supplementation were also compared.

Methods

Study design, participants, and location

This double blind randomized controlled study was conducted in HIV positive children, aged 5 -12 years, who received treatment at the Nigerian Institute of Medical Research (NIMR) Lagos, and the Lagos State University Teaching Hospital (LASUTH), Lagos, Nigeria. Recruitment was between 1st May 2015 and 30th September 2015. Participants were randomly assigned to one of three supplement groups. Multi-micronutrient tablets were used twice daily (one tablet in the morning, and one in the evening) for six months.

Inclusion/ exclusion criteria

Included; a) Children aged 5 – 12 years, who had previously tested positive to HIV, and attended the outpatient clinic at the HIV treatment centres; b) Children with guardians who were willing and able to return for follow up assessments and supplement refills; and c) Children with guardians who could give informed consent. Excluded; a) Children involved in other studies; b) Guardians with children who were relocating to another state; and c) Children receiving immunosuppressive therapy.

Randomization

Randomization of participant identity (ID) numbers was conducted prior to participant enrolment on randomizer.org. Randomization list was kept off-site and research team members were not aware of multi-micronutrient group assignment of ID numbers. Please see figure 1 for trial profile, showing participants group assignment.

Supplements

Multi-micronutrient supplements were manufactured specifically for the study by Brunel Healthcare Limited, United Kingdom. RDA was based on the 9-12 age group. Multi-micronutrient tablets were identical and could not be physically differentiated. Supplements (A, B and C) were packaged in identical containers and labelled with study ID numbers. Micronutrient composition of supplements A, B and C are shown in table 1.

To ensure supplement safety, concentrations of micronutrients were within tolerated upper limits (TUL). For group C supplement, TUL was the maximum allowance applied if 3RDA exceeded the limit.

Sample size calculation

After 6 months, a minimum 43cells/mm^3 greater CD4 count increase was anticipated for group C participants compared to groups A and B. This was estimated from the Kaiser et al study [15] that reported mean CD4 count increase of 65cells/mm^3 for participants assigned to the high strength group. As immunological reconstitution can differ in children compared to adults [23], anticipated mean CD4 count increase among group C participants in this present study was cautiously estimated to be 43cells/mm^3, a 33% reduction from 65cells/mm^3. At 43cells/mm^3, calculated effect size of 0.20, alpha error probability of 0.05, 85% power, and estimated 15% loss to follow up, the calculated sample size was 216.

A nationwide hospital strike meant limited time for participant recruitment. An achieved sample size of one hundred and ninety (190) reduced study power to detect changes in CD4 count. Computed power from post hoc power analysis was 81.4%.

Statistical analysis

Paired sample t test compared baseline CD4 count and Hb concentration data to endpoint data. Factorial repeat measures ANOVA compared supplements effects on participant outcomes at multiple time points. McNemar's test determined changes in presence of immune deficiency and anaemia after 6 months of supplementation.

Ethics

Ethics approvals were obtained from the Institutional Review Board (IRB) of the Nigerian Institute of Medical Research (NIMR), Health Research Ethics Committee (HREC) of the Lagos State University Teaching Hospital (LASUTH) and the Ethics Committee of the School of Health, Nursing and Midwifery of the University of the West of Scotland (UWS). All approvals were obtained prior to study commencement.

Results

One hundred and ninety (190) participants were recruited into the study; 135 from NIMR and 55 from LASUTH. 105/190 (55.3%) were male, 85/190 (44.7%) were female, 180/190 (94.7%) were on HAART. 64, 63 and 63 participants were randomly assigned to groups A, B and C respectively. Baseline comparability information is on table 2. Baseline CD4 count and Hb concentration for groups A, B and C were statistically analysed for comparability. P values of 0.293 and 0.972 respectively indicated no statistical significance. Other variables of interest were also comparable. All supplements were well tolerated. Ten participants reported adverse effects; four in group A, four in group B and two in group C.

Loss to follow-up

Twenty participants (20/190, 10.5%) were lost to follow up; 5 (7.8%), 7(11.1%) and 8 (12.7%) in groups A, B and C respectively. Losses between groups were comparable (X^2 = 2.76, p=0.838).

Intention to treat (ITT) analysis was applied. Multiple imputation generated missing data, with five (5) imputations generated for the dataset. Pooled parameter estimates from the five imputations were used in the analysis.

Endpoint evaluations

Mean CD4 count after 6 months supplementation was not significantly different from baseline for all three supplement groups. P values > 0.05 (table 3). CD4 count between subject effect was also not significantly different after 6 months (Factorial repeat measures ANOVA, F (2, 187) = 0.846, p = 0.436, Partial Eta Squared = 0.009). Immune deficiency was significantly reduced from 17.4% (33/190) at baseline to 11.6% (22/190) after 6 months (McNemar test, P= 0.009). See table 4.

Mean Hb concentration was significantly increased after 6 months supplementation in all 3 groups, p values < 0.001 (table 5). Between group effect was not significantly different (factorial repeat measures ANOVA, F (2, 187) = 0.549, p = 0.591, Partial Eta Squared = 0.006). Anaemia was significantly reduced from 55.3% (105/190) at baseline to 32.6% (62/190) after 6 months (McNemar test, p <0.001). See table 6.

Comparability of outcomes between groups, after 6 months of supplementation was indicative of equivalent effects of the supplements compared (table 7).

Discussion

Immunological and haematological benefits of multi-micronutrient supplements in HIV positive children (5- 12 years) were evaluated in this study. Three multi-micronutrient supplements which differed in strength and/ or composition were compared, with CD4 count and Hb concentration as outcomes of interest.

Previous efficacy studies reported conflicting effects of supplementation on these outcomes [13], [18]–[20], [25]. Differences in composition and concentration of supplements may have influenced results. By comparing three supplements, a better understanding of the benefits of supplementation within this population was anticipated.

Mean CD4 count was not significantly changed after 6 months of supplementation, even when high strength supplement was administered. Similar results were obtained in previous studies [18]–[20], [25], but not in others [14]–[16]. However, immune deficiency was significantly reduced 5.8% after 6 months, suggesting possible benefits of supplementation in PLHIV with CD4 count <500cells/mm^3. This warrants further investigation. Reduction in immune deficiency was not dependent on participant group assignment, indicating equivalent effect.

Equivalent effect of supplementation suggests that factors other than supplement composition and strength may have influenced differing results previously reported [14]–[16], [18], [20], [25]. Important study design differences including use of active controls may have blurred the effect of supplementation on CD4 count. Participants HAART status could also have affected results, as effectiveness of supplementation in the context of HAART is unclear [18]. Antiretroviral drugs promote oxidative stress [26]. Hence CD4 count response to

supplementation in HAART naïve and HAART experienced PLHIV may be dissimilar; possibly via changes to micronutrient profile after HAART initiation [27].

The cycle between micronutrient deficiencies, oxidative stress and HIV disease progression [28] suggests future multi-micronutrient studies in persons with high levels of ROS are warranted. Well conducted RCTs within that population would clarify the effectiveness of supplementation on oxidative stress, and its' possible translation to immune sufficiency.

Mean Hb concentration was significantly increased after six months supplementation in all three groups, but mean increase did not differ between groups. Hence, Hb concentration increase was not influenced by differences in supplements strength and/or composition in this population. Mechanisms such as haem synthesis, erythropoiesis modulation, protection against erythrocytes and oxidative damage may have been involved in increasing Hb levels [29].

Significantly increased Hb concentration was previously reported in another study [13], but was not demonstrated elsewhere [15], [18]. Differences in participant characteristics including HAART status [30], stage of HIV disease [31], and physiological status [32] may have influenced levels of anaemia in those populations, and contributed to the differing results. Furthermore, differences in anaemia predisposing factors in the different study populations may have also influenced demand for, and response to supplementation.

Micronutrient deficiency is a predisposing factor to developing anaemia [33]. Oxidative damage of erythrocytes, reduced globin production, capillary haemorrhage, and reduced iron mobilization are some mechanisms for micronutrient deficiency related anaemias [29]. In study populations with micronutrient deficiencies, supplementation may have translated to better haemoglobin response [34].

Another anaemia predisposing factor is zidovudine (AZT) use, via mechanisms including increased oxidative stress [35] and myelosuppression [36]. Micronutrients provide anti-oxidative [37] and myelo-protective effects [38]. Hence supplementation could overcome AZT induced hematologic toxicity [27], translating to increased Hb levels. As majority of participants in this present study were on zidovudine (AZT) based HAART regimen, the high percentage of participants with anaemia at baseline was not unexpected [36], [39]. In turn, their need for, and response to multi-micronutrient supplementation may have been increased.

It is important to mention that the significantly increased Hb concentration after supplementation occurred even though none of the supplements contained iron. This again suggests that anaemia in this population was likely secondary to the predominantly AZT based ART regimen, and not iron deficiency. However further studies may be needed to clarify this.

A 22.7% reduction in participants with anaemia after 6 months reflected the statistically significant increase in Hb concentrations with supplementation. Equivalent effects were again demonstrated here as reduction in anaemia was not influenced by participant group assignment.

Limitations to the generalizability of these findings exist. 94.7% (180/190) of participants in this study were on HAART. Oxidative stress in the context of HAART may be different in the absence of HAART. Hence response of supplementation in HAART naïve person is uncertain. Response in developed countries may also differ from findings in this study. Dissimilarities in diets suggest higher levels of micronutrient malnutrition in the developing world [40]. Therefore reproducibility of these study findings in developed countries is debatable. Lastly, the presence and prevalence of micronutrient deficiencies in this study

population was not determined. Establishing micronutrient deficiencies may have been beneficial in assessing PLHIV more likely respond to supplementation. This is an area for future research.

Conclusion

Equivalent effects of multi-micronutrient supplements of different strengths and composition was demonstrated in this present study. Mean CD4 count was not significantly changed regardless of multi-micronutrient group assignment of participants. A significant reduction in number of participants with immune-deficiency after 6 months, suggested possible benefits of supplementation in participants with CD4 count < 500cells/mm^3. Mean Hb level was significantly increased in all three supplement groups, with similar increases across groups. High anaemia presence in this population may warrant supplementation to improve haematological profile.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 11: Micronutrient composition of groups A, B and C multi-micronutrient supplements

Micronutrient	Group A	Group B	Group C
Vitamin A	600 µg	600µg	1700 µg*
Vitamin B1	0.9mg	0.9mg	2.7mg
Vitamin B2	0.9mg	0.9mg	2.7mg
Niacin	12mg	12mg	20mg*
Vitamin B6	1.0mg	1.0mg	3.0mg
Vitamin B12	-	1.8µg	5.4 µg
Folic acid	-	300µg	600 µg*
Vitamin C	45mg	45mg	135mg
Vitamin D	15 µg	15µg	25 µg*
Vitamin E	-	11 mg	33mg
Selenium	-	40µg	120 µg
Iodine	-	120µg	360 µg
Chromium	-	25µg	75µg

Zinc	-	5mg**	5mg**
Copper	-	440µg**	440 µg**
Manganese	-	1.9mg	1.9mg****
Inositol	-	60mg***	60mg***
Potassium	-	33mg***	33mg***
Calcium	-	134mg***	134mg***
Magnesium	-	50mg***	50mg***
Choline	-	30mg***	30mg***
Glutamine	-	40mg***	40mg***

*: Tolerated upper limits (TUL) and not 3RDA used

**: 1 RDA for 4-8 years incorporated into groups 2 and 3 supplements, due to possible negative effects of high serum concentrations in a HIV [21]

***: Doses lower than the RDA incorporated into groups 2 and 3 supplements to achieve acceptable sized tablets.

****: 1RDA incorporated due to increased vulnerability of anaemic individuals to manganese toxicity [22].

Table 2: Comparison of baseline categorical data in the three micronutrient groups

Baseline characteristic	Group A (SD/number)	Group B (SD/number)	Group C (SD/number)
CD4 count	975.44 (453.07)	908.36 (373.05)	1034.18 (665.77)
Hb concentration	11.05 (1.36)	10.91 (1.44)	11.06 (1.57)
Age	8.08 (1.99)	7.75 (1.94)	8.08 (1.94)
Male	50.8% (32/64)	54.0% (34/63)	61.9% (39/63)
Prior multivitamin use	82.8% (53/64)	82.5% (52/63)	76.2% (48/63)
Co-trimoxazole prophylaxis	43.8% (28/64)	52.4% (33/63)	47.6% (30/63)
Existing morbidities	25.0% (16/64)	19.0% (12/63)	27.0% (17/63)
HAART naïve	7.8% (5/64)	4.8% (3/63)	3.2% (2/63)
Zidovudine based HAART regimen	76.6% (49/64)	74.6% (47/63)	77.8% (49/63)
Mean height	125.81 (12.00)	125.31 (11.09)	128.76 (10.27)
Mean weight	25.09 (6.40)	24.81 (6.73)	25.18 (5.39)

Table 32: Mean CD4 count at baseline and endpoint			
Group	Baseline CD4 count (SD)	Endpoint CD4 count (SD)	P value
A	975.44cells/mm^3(453.07)	973.61cells/mm^3 (662.95)	0.984
B	908.36cells/mm^3 (373.05)	946.20cells/mm^3 (399.80)	0.536
C	1034.18cells/mm^3 (665.77)	967.69cells/mm^3 (406.58)	0.310
Total	972.66cells/mm^3 (526.22)	962.50cells/mm^3 (487.69)	0.821

Table 4: Mean CD4 count and immune status at baseline and after 6 months multi-micronutrient use (Based on WHO classification system [6]			
Reference value	Group	Number (%)	Mean CD4 count(SD)
< 200 cells/ mm^3	Severe immune deficiency	Baseline: 3 (1.6)	61.33 (53.68)
		Endpoint: 4 (2.1)	118.15 (51.50)
200-499 cells/ mm^3	Mild – advanced immune deficiency	Baseline: 30 (15.8)	400.70 (81.53)
		Endpoint: 18 (9.5)	384.49 (76.85)
≥ 500 cells/ mm^3	No significant immune deficiency	Baseline: 157 (82.6)	1117.34 (485.13)
		Endpoint: 168 (88.4)	1049.94 (474.48)

Table 5: Hb concentration at baseline and endpoint			
Group	Baseline Hb conc. (SD)	Endpoint Hb conc. (SD)	P value
A	11.05g/dL (1.36)	12.32g/dL (1.55)	< 0.001
B	10.91g/dL (1.44)	11.82g/dL (1.37)	< 0.001
C	11.06g/dL (1.57)	12.27g/dL (1.46)	< 0.001
Total	11.01g/dL (1.45)	12.14 g/dL (1.47)	< 0.001

Table 6. Mean haemoglobin concentration and anaemia status at baseline and after 6 months of supplementation (Based on WHO recommendation for diagnosis of anaemia for children aged 5-11 years [24])			
Haemoglobin concentration	Group	Number (%)	Mean g/dL (SD)
< 8.0 g/dL	Severe anaemia	Baseline: 2 (1.1)	6.80 (0.42)
		Endpoint: 1 (0.5)	7.67 (0.18)
8.0 –11.4g/dL	Mild – moderate anaemia	Baseline: 103 (54.2)	10.08 (0.90)
		Endpoint: 61 (32.1)	10.51 (0.77)

≥ 11.5g/dL	No anaemia	Baseline: 85 (44.7)	12.29 (0.74)
		Endpoint: 128 (67.4)	12.88 (1.04)

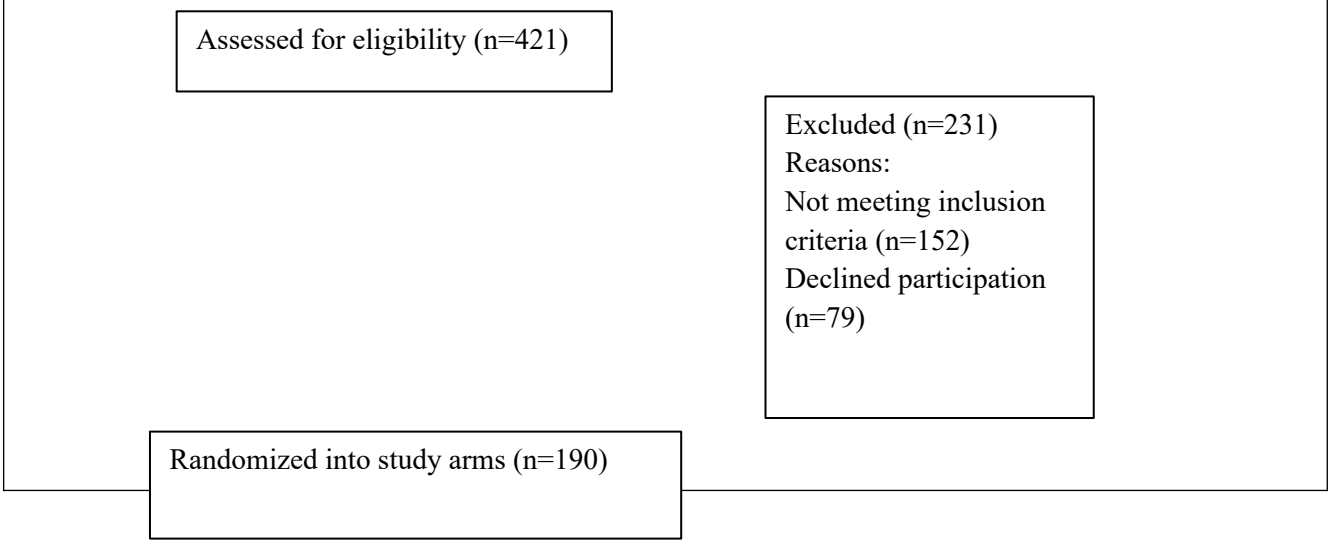
Table 7: Endpoint comparability between groups demonstrating equivalent effects

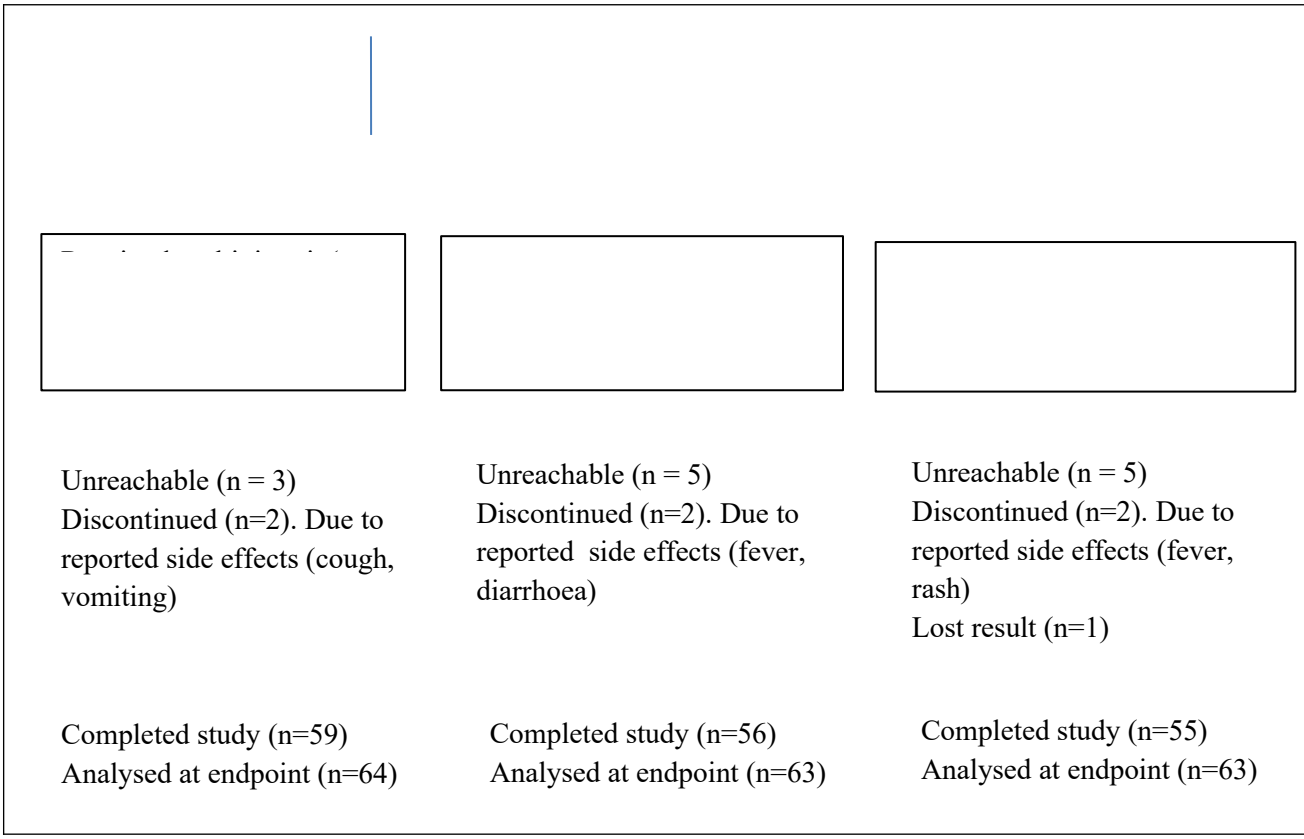
Variable	Group A	Group B	Group C	P value
CD4 count	973.61cells/mm^3	946.20cells/mm^3	967.69cells/mm^3	0.436*
Immune deficiency	12.5% (8/64)	9.5% (6/63)	12.7% (8/63)	0.800**
Hb level	12.32g/dL	11.82g/dL	12.27g/dL	0.591*
Anaemia	26.6% (17/64)	44.4% (28/63)	27.0% (17/63)	0.114**
Lost to follow-up	5	7	8	0.838**

*Factorial repeat measures ANOVA

**Chi Squared test

Figure 1: Trial Profile




Unreachable (n = 3)
Discontinued (n=2). Due to reported side effects (cough, vomiting)
Completed study (n=59)
Analysed at endpoint (n=64)
Unreachable (n = 5)
Discontinued (n=2). Due to reported side effects (fever, diarrhoea)
Completed study (n=56)
Analysed at endpoint (n=63)
Unreachable (n = 5)
Discontinued (n=2). Due to reported side effects (fever, rash)
Lost result (n=1)
Completed study (n=55)
Analysed at endpoint (n=63)