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Original Research Article

A prospective, open-label, randomized, comparative clinical trial to assess the efficacy of NASO B12 or oral B12 supplementation along with standard of care in the treatment of anemia in women of reproductive age

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ABSTRACT

Background: Anemia is a prevalent health issue among women of reproductive age (WRA), often attributed to iron deficiency but also influenced by vitamin B12 deficiency. Despite widespread iron and folic acid (IFA) supplementation programs, anemia persists, necessitating comprehensive treatments. This study evaluated the efficacy of intranasal vitamin B12 supplementation (NASO B12 of Troikaa Pharmaceuticals Ltd.) with IFA compared to oral vitamin B12 with IFA and IFA alone, in WRA.
Methods: This open-label, randomized clinical trial enrolled 75 anemic WRA across three centers in Lucknow, India. Participants were randomized into three groups: NASO B12 + IFA, Oral B12 + IFA, and IFA alone. In the NASO B12 and oral B12 groups, B12 supplementation was administered on alternate days for two weeks, then weekly until day 42, while IFA was given daily in all groups for 84 days. Hemoglobin (Hb) and other hematological parameters were assessed at multiple intervals. Safety evaluations included vital signs and adverse events monitoring. ANOVA was used for statistical analysis, with $p<0.05$ indicating significance.
Results: Among 75 anemic women, NASO B12 demonstrated the fastest and most significant improvements in Hb and vitamin B12 levels, as well as a notably greater increase in RBC counts compared to both the Oral B12 and IFA groups. Hb levels increased significantly from baseline by 2.8 gm/dl at day 84. Vitamin B12 levels rose rapidly, exceeding 400 pg/mL by day 7 and sustaining through day 84. NASO B12 consistently improved RBC counts from day 7, reaching 4.53 mill/cmm by day 84. Changes in reticulocytes, ferritin, and folic acid supported hematopoietic normalization. The treatment was well-tolerated in all three groups, with no serious adverse events.
Conclusions: In conclusion, NASO B12, when combined with IFA, demonstrated superior and faster efficacy in improving hematological parameters compared to oral B12 + IFA and IFA alone, making it a promising treatment option for vitamin B12 deficiency and anemia in WRA.

Keywords: Hemoglobin, Vitamin B12, Anemia, Iron deficiency, Naso B12, Iron folic acid

INTRODUCTION

Anemia remains a widespread health issue, particularly affecting WRA in many parts of the world, especially in developing nations like India.[1] It is estimated that nearly 500 million women between the ages of 15 and 49 are impacted by this condition. In 2019, the world health organization (WHO) reported that around 30% of non-pregnant and 37% of pregnant women in this age group were living with anemia.[2] Moreover, the national family health survey (NFHS-5; 2019-21) statistics has highlighted the rising prevalence of anemia across all ages.[3]

While iron deficiency is widely recognized as the primary cause of anemia, a study examining various forms of anemia in children and adolescents found that approximately 25% of adolescents experienced anemia due to other factors, such as deficiencies in folate and vitamin B12.[4] Also, according to the WHO, various non-iron-related factors can also cause anemia, which includes deficiencies in vitamin B12.[2]

Adolescent girls are a nutritionally vulnerable population due to the unique demands of their growth and development, as well as their potential role in future generations' health.[5] In India, anemia among adolescent girls remains a widespread public health challenge, with prevalence rates reported to be 58.9%.[6]

Anemia is a significant health concern among pregnant women and is linked to various complications. Studies indicate that pregnant women with anemia face a heightened risk of conditions such as pre-eclampsia, preterm vaginal delivery, postpartum hemorrhage, puerperal sepsis, increased caesarean section rates, eclampsia, placenta previa, abortion, and abruption. Additionally, anemia in WRA has been associated with an elevated likelihood of dysmenorrhea, menorrhagia, puberty-related menorrhagia, ovarian cysts, and bleeding induced by medical termination of pregnancy pills.[7]

Research indicates that traditional iron supplementation alone is insufficient to address anemia comprehensively, as only about half of the affected individuals respond to such interventions.[8] This calls for a broader understanding of the nutritional factors influencing anemia beyond iron deficiency.

Studies have established a strong link between folic acid and vitamin B12 with hematopoietic function, with deficiencies in these nutrients being closely associated with the development of anemia.[9-12] Of these folic acid has been extensively studied and is used along with Iron in the prevention/ treatment of anemia.[13-15] Despite extensive efforts, including nationwide supplementation programs providing IFA, the burden of anemia persists at concerning levels.[5]

Vitamin B12 plays a vital role in DNA synthesis and it is well recognized that its deficiency is associated with hematologic disorders. Since a majority of the population in India is vegetarian, the deficiency of vitamin B12 is not scarce.[16] Vitamin B12 is a key nutrient for DNA synthesis and hematopoiesis, with its deficiency significantly impacting erythropoiesis. A deficiency in vitamin B12 can lead to heightened oxidative stress by diminishing reactive oxygen species (ROS) scavenging capacity, thereby disturbing the balance of antioxidant defences crucial for erythrocyte stability. This oxidative imbalance renders erythrocytes more susceptible to structural degradation.

Moreover, vitamin B12 functions as a cofactor for critical enzymes, including methionine synthase and methylmalonyl-CoA mutase, which are essential for DNA methylation and nucleotide synthesis. Insufficient levels of vitamin B12 impair DNA synthesis, effectively reducing erythrocyte maturation and resulting in low reticulocyte count.[17]

Incorporating vitamin B12 into anemia treatment has proven beneficial for many individuals. Many studies have assessed the impact of adding vitamin B12 to IFA therapy, with results varying significantly. Some studies demonstrate an improved response to anemia with vitamin B12 supplementation, while others show no noticeable effect. These differences may stem from the low and variable absorption of vitamin B12 via the oral route. Studies using oral vitamin B12 supplementation with IFA found no significant effect on Hb levels.[5,18] Conversely, studies incorporating injectable vitamin B12 with IFA reported significantly higher Hb levels compared to IFA alone.[19,20]

Although injectable forms of vitamin B12 are effective, they are associated with drawbacks such as injection site pain and the high cost of therapy, owing to the need for clinic visits and healthcare professional assistance for administration.[21] This highlights the need for a vitamin B12 formulation that is reliably absorbed and can be conveniently self-administered.

The nasal route is an appealing option for drug delivery due to its ease of access, extensive surface area, rich vascular supply, and permeable endothelial membrane, allowing it to bypass first-pass metabolism and potentially achieve greater systemic bioavailability compared to oral administration. Intranasal formulations have several advantages over intramuscular delivery such as: they are pain-free, can be self-administered, enhance patient compliance, eliminate the need for healthcare professional assistance, avoid injection-related complications, and lower treatment costs. Considering these advantages, Troikaa Pharmaceuticals Limited has developed an innovative intranasal methylcobalamin spray (500 mcg/0.1 mL) (NASO B12) to provide a superior alternative to existing formulations.[2]

In previous studies, supplementation of vitamin B12 with NASO B12 through the nasal route in individuals with vitamin B12 deficiency has been found to safe and effective.[22-24] It would be interesting to study the effect of this novel formulation along with IFA in treatment of anemia.

The present study was undertaken to evaluate the efficacy of NASO B12 supplementation along with iron-folate tablets on the treatment of anemia as compared to IFA + oral vitamin B12 or IFA alone in WRA. By targeting WRA, the study aimed to determine whether intranasal B12 supplementation could offer a more effective and patient-friendly approach to correcting anemia and improving hematological outcomes in this vulnerable population. This study fills a critical gap in understanding

the comparative advantages of nasal versus oral routes of B12 delivery in the context of anemia treatment.

METHODS

This study was a prospective, open-label, randomized, comparative clinical trial conducted in anemic women (Hb <11 gm/dl) of the reproductive stage. This multicenter study was conducted at three sites in Lucknow, Uttar Pradesh, India: Udyan health care Pvt. Ltd, Harsha clinic and diabetes centre, and Chandra diabetes and obesity clinic, from April 2023 to May 2024. The study was carried out in compliance with good clinical practice guidelines and the principles of the declaration of Helsinki. The study was approved by the institutional human ethics committee Udyaan center (registration no. ECR/1300/Inst/UP/2019) and was then registered with the clinical trials registry of India (CTRI)-CTRI/2023/04/052112. All participants were provided with details about the study and were included in the study when they gave their written informed consent to participate in the study.

Non-pregnant, non-lactating female patients of reproductive age (18-40 years), diagnosed with anemia (Hb<11 g/dL) and willing to comply with study requirements were included in the study. They were not considered in the study if they were suffering from severe anemia (Hb<7 gm/dl) or had hypersensitivity to study medication or were suspected to have illnesses, where iron supplementation is contraindicated (i.e. history of repeated blood transfusion, known cases of hemolytic anemia, chronic diarrhea, stomach ulcer, heart surgery/prosthetic valves, cancer, tuberculosis; those who are on medications like tetracycline or anticoagulants; and women with excessive bleeding problems).

The study enrolled 75 anemic WRA, who were randomly assigned into three groups in a 1:1:1 ratio to receive one of the following treatments-

NASO B12 group: NASO B12 (Methylcobalamin nasal spray 250 µg/spray; 500 mcg/day administered as two sprays, one in each nostril) of Troikaa pharmaceuticals Ltd. plus standard of care IFA (Sugar coated Ferrous sulfate equivalent to elemental Iron 60 mg + folic acid IP 500 mcg).

Oral B12 group: Oral B12 tablets (500 mcg/day, as methylcobalamin) plus standard of care IFA (Sugar coated Ferrous sulfate equivalent to elemental Iron 60 mg + folic Acid IP 500 mcg).

IFA group (Control): Standard of care IFA (Sugar coated ferrous sulfate equivalent to elemental iron 60 mg + folic acid IP 500 mcg).

Patients in NASO B12 and oral B12 group received the study drugs on alternate days during the first week, on days 1, 3, 5, 7, 9, 11, and 13 (treatment phase). Following this, the study drugs were administered once weekly on days 21, 28, 35, and 42 (maintenance phase). IFA supplementation was administered daily in all three treatment groups and continued for a total of 84 days.

The blood samples were collected from each patient at the screening visit (day 0) (i.e. baseline), and at day 7, day 14, day 42 and day 84 (end of study) for measurement of complete blood count including reticulocyte count, serum vitamin B12 levels, serum ferritin and serum folic acid.

Safety assessments involved monitoring vital signs, conducting physical examinations, and documenting any adverse events, whether local or systemic.

Study outcomes

The primary objective of the study was to evaluate the difference in Hb levels between the treatment arms at various time points. Secondary objectives included the comparison of the differences in vitamin B12 levels, other hematological parameters (such as reticulocyte count, RBC count), serum ferritin, serum folic acid, hematocrit and the occurrence of any local or the systemic adverse events.

Statistical analysis

Descriptive statistics such as the mean, the standard deviations were calculated for continuous data. A single-factor analysis of variance (ANOVA) was used to compare the differences observed in the study. A $p<0.05$ was considered as a statistically significant difference.

RESULTS

A total of 75 anemic women were enrolled and completed the study. The mean age of the study participants was 28±6.7 years. The baseline clinical characteristics of the participants are shown in Table 1.

Efficacy

Serum Hb levels

Upon comparison of the increase in Hb levels, from baseline it was observed that Hb increase was highest in the NASO B12 group at all-time points in comparison to oral B12 and IFA groups (Table 2 and Figure 1). Hb rapidly increased in the NASO B12 group after the days 7, 14, 42, and 84 from baseline 9.2 to 10.03, 10.6, 11.3 and 12.08 g/dL respectively.

With NASO B12 treatment significantly higher rise in Hb was observed at day 7 and day 14 as compared to IFA group, whereas with oral B12 treatment this rise was non-significant, depicting that NASO B12 acts faster as compared to oral B12 and IFA in Hb rise and the changes were evident as early as day 7.

Serum vitamin B12 levels

Upon analyzing the results for vitamin B12 levels, NASO B12 group consistently demonstrated a marked increase in mean levels and changes from baseline at each study visit (Table 3 and Figure 2). From as early as day 7, NASO B12 group showed a significant and sustained increase, achieving levels above 400 pg/mL (580.40±419.12 pg/mL), far exceeding the modest improvement seen in oral B12 group (314.24±158.96 pg/mL) and the slight decline observed in IFA group (286.40±155.36 pg/mL). This trend persisted throughout the study, with B12 levels in the NASO B12 group continuing to rise and were maintained above 400 pg/ml, even after treatment was stopped on day 42.

These results suggest that NASO B12 is the most effective option for correcting vitamin B12 deficiencies and maintaining adequate B12 levels in anemic patients.

RBC count

The results demonstrate that NASO B12 group exhibited superior efficacy at all the visits in improving RBC count as compared to Oral B12 group and IFA group. From day 7 onwards, NASO B12 group showed consistent increases in RBC count from baseline, with significant improvements observed at day 14, day 42, and day 84 as compared to IFA group (Table 4 and Figure 3). Whereas an increase in RBC count in oral B12 group was non-significant at all visits in comparison to IFA group.

Reticulocyte count

The results from Table 5 demonstrate that change in reticulocyte count after the administration of the study treatments. In NASO B12 group and oral B12 group there was quick rise in reticulocyte count in comparison to IFA. Thereafter a decrease in reticulocyte count depicting normalization of hematopoiesis was noted in all the groups.

Hematocrit

A significant rise in mean hematocrit levels from baseline was observed for all the treatments from day 14 onwards. The highest levels of hematocrit were found in NASO B12 group followed by oral B12 group and IFA group (Table 6). While the mean change in hematocrit from baseline was insignificant in the group supplemented with IFA alone, the addition of B12 supplementation resulted in significant improvements.

In the oral B12 group, the mean change from baseline in hematocrit showed inconsistency, with significant changes observed at day 14 and day 84 but not at day 42. In contrast, the NASO B12 group demonstrated consistent and significant changes in hematocrit from baseline at all three time points-day 14, day 42, and day 84-emphasizing its superior and sustained efficacy.

Ferritin and folic acid levels

Since IFA supplementation was provided to all three groups, an increase in ferritin levels was observed in each group. Similarly, as all three groups received folic acid supplementation, a significant increase from baseline was observed in all three groups (Table 7).

Safety

A total of 17 adverse events (AEs) were reported in the trial. Of these, 10 AEs occurred in the NASO B12 group, 3 in oral B12 group, and 4 in IFA group. All reported AEs were mild to moderate in severity and resolved without sequelae. No serious adverse events observed in study.

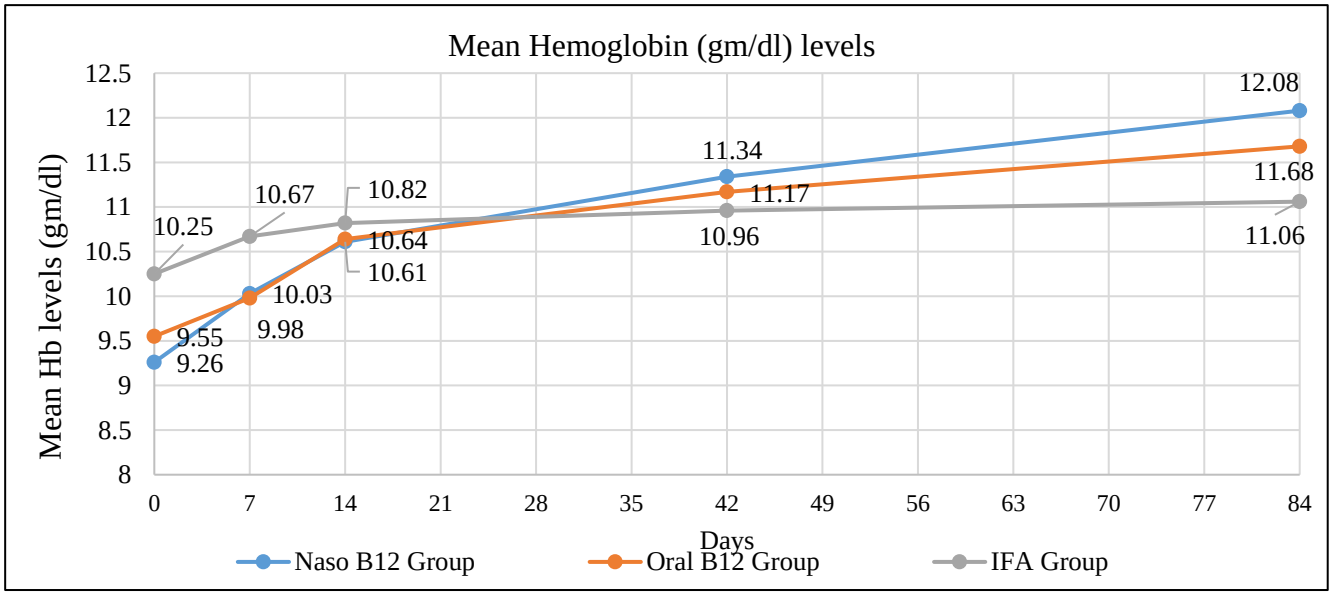

Figure 1: Change in mean serum Hb levels in the study groups at different time points.

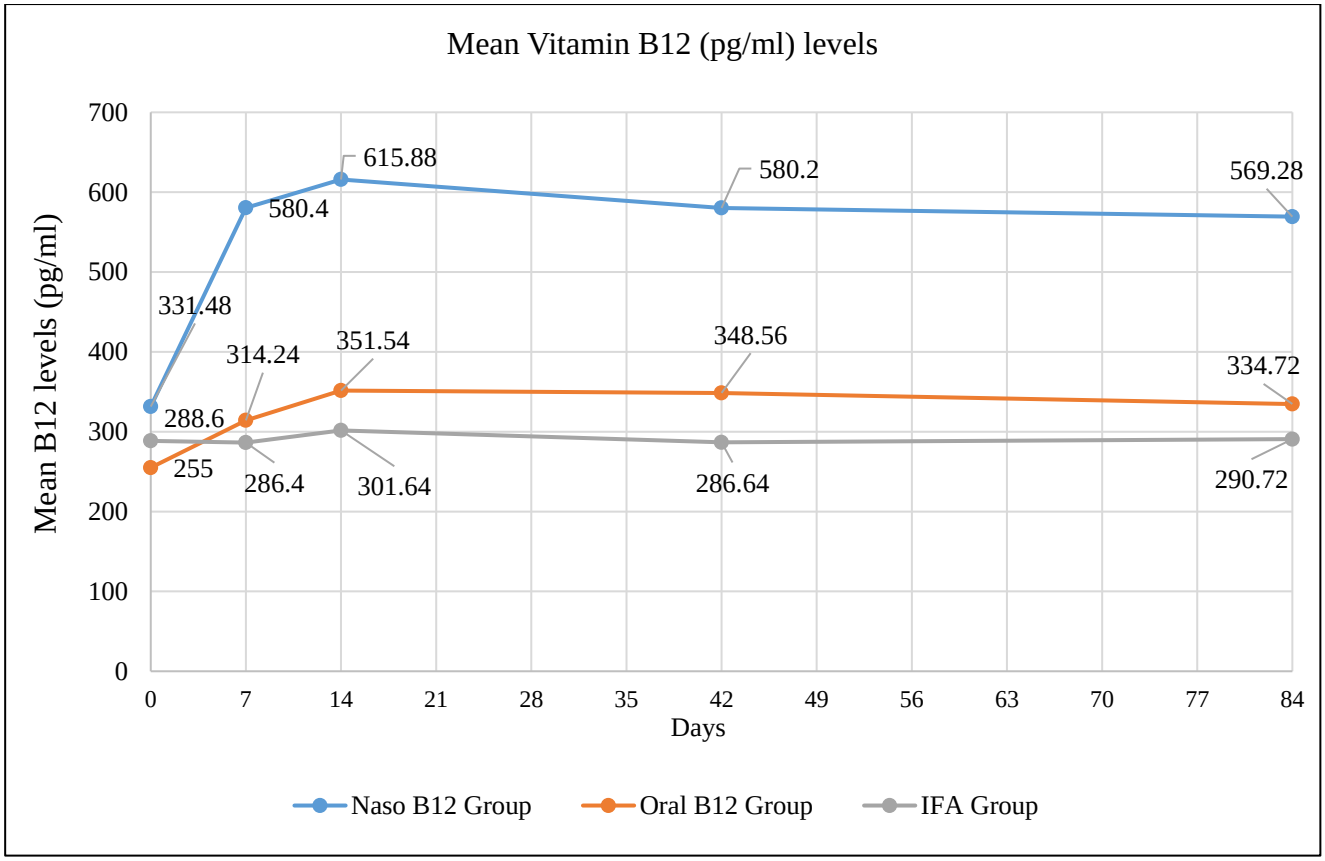

Figure 2: Change in mean serum vitamin b12 levels in the study groups at different time points.

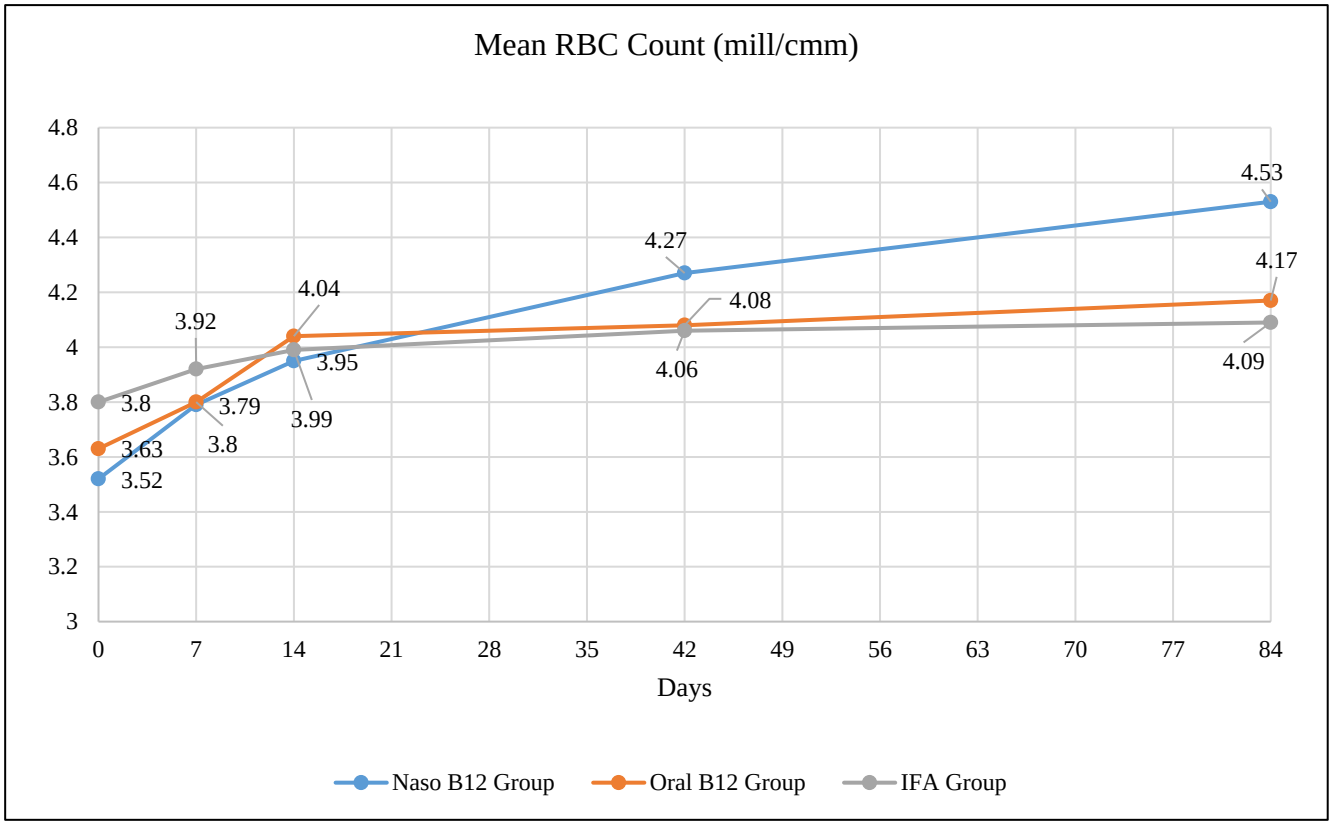

Figure 3: Change in RBC count in the study groups at different time points.

Table 1: Baseline clinical characteristics (mean±SD) of participants enrolled in the study.

Characteristics	NASO B12 group, (n=25)	Oral B12 group, (n=25)	IFA group, (n=25)
Age (in years)	27.04±6.06	29.16±7.13	28.72±7.20
Hb (gm/dl)	9.25±1.47	9.55±1.12	10.24±0.65
Serum vitamin B12 (pg/ml)	331.48±267.92	255±181.83	288.6±168.74
RBC count (mill/cmm)	3.51±0.63	3.63±0.5432	3.79±0.43
Reticulocyte count (%)	2.37±1.07	2.02±1.31	1.51±0.78
Hematocrit (%)	30.54±4.33	30.7±3.96	33.26±2.97
Serum ferritin (ng/ml)	51.08±98.001	27.79±42.31	19.51±14.92
Serum folic acid (ng/ml)	9.14±4.42	8.92±5.10	8±3.53

Table 2: Serum mean Hb levels (g/dl) in the study groups at different time points.

Study visits	NASO B12 group		Oral B12 group		IFA group	
	Mean level	Mean change from baseline	Mean level	Mean change from baseline	Mean level	Mean change from baseline
Baseline	9.26±1.47	-	9.55±1.13	-	10.25±0.65	-
Day 7	10.03±1.20$^{\#}$	0.78±0.57$^{\#}$	9.98±1.45$^{\#}$	0.43±0.67	10.67±0.84	0.42±0.56
Day 14	10.61±1.20*	1.35±1.11$^{\#}$	10.64±1.20*	1.08±1.06	10.82±0.78*	0.58±0.65
Day 42	11.34±0.90*	2.09±1.59$^{\#}$	11.17±0.98*	1.62±1.22$^{\#}$	10.96±0.89*	0.71±0.91
Day 84	12.08±0.96*$^{\#}$	2.82±1.84$^{\#}$	11.68±1.25*	2.12±1.66$^{\#}$	11.06±0.99*	0.8±0.96

*P<0.05 compared to baseline; $^{\#}$P<0.05 compared to IFA.

Table 3: Serum vitamin B12 levels (pg/ml) in the study groups at different time points.

Study visits	NASO B12 group		Oral B12 group		IFA group	
	Mean level	Mean change from baseline	Mean level	Mean change from baseline	Mean level	Mean change from baseline
Baseline	331.48±267.92	-	255.00±181.84	-	288.60±168.74	-
Day 7	580.40±419.12*$^{\#\S}$	248.92±366.23$^{\#\S}$	314.24±158.96	59.24±154.78	286.40±155.36	-2.20±74.00
Day 14	615.88±285.96*$^{\#\S}$	284.40±241.39$^{\#\S}$	351.54±187.06	105.67±180.52§	301.64±172.10	13.04±64.86
Day 42	580.20±257.02*$^{\#\S}$	248.72±271.05$^{\#\S}$	348.56±153.53	93.56±225.24	286.64±172.30	-1.96±92.40
Day 84	569.28±339.87*$^{\#\S}$	237.80±355.58$^{\#\S}$	334.72±168.54	79.72±195.38	290.72±190.80	2.12±138.87

*P<0.05 compared to baseline; $^{\#}$P<0.05 compared to Oral B12; §P<0.05 compared to IFA.

Table 4: RBC count (mill/cmm) in the study groups at different time points.

Study visits	NASO B12 group		Oral B12 group		IFA group	
	Mean level	Mean change from baseline	Mean level	Mean change from baseline	Mean level	Mean change from baseline
Baseline	3.52±0.63	-	3.63±0.55	-	3.80±0.43	-
Day 7	3.79±0.56	0.28±0.41	3.80±0.65	0.16±0.47	3.92±0.51	0.12±0.40
Day 14	3.95±0.50*	0.44±0.55§	4.04±0.58*	0.39±0.49	3.99±0.47	0.20±0.41
Day 42	4.27±0.54*	0.75±0.61§	4.08±0.45*	0.45±0.50	4.06±0.39*	0.26±0.39
Day 84	4.53±0.59*$^{\#\S}$	1.02±0.60$^{\#\S}$	4.17±0.47*	0.54±0.65	4.09±0.39*	0.30±0.57

*P<0.05 compared to baseline; $^{\#}$P<0.05 compared to Oral B12; §P<0.05 compared to IFA.

Table 5: Reticulocyte count (%) in the study groups at different time points.

Study visits	NASO B12 group		Oral B12 group		IFA group	
	Mean level	Mean change from baseline	Mean level	Mean change from baseline	Mean level	Mean change from baseline
Baseline	2.38±1.08	-	2.02±1.32	-	1.51±0.78	-
Day 7	3.02±2.07$^{\delta}$	0.64±2.01	2.68±1.62	0.66±1.59	1.72±0.81	0.21±0.67
Day 14	2.33±1.42	-0.05±1.54	1.83±0.89	-0.14±1.18	2.04±1.04	0.53±1.18
Day 42	1.52±0.78*$^{\#}$	-0.86±1.31$^{\delta}$	1.93±1.30	-0.09±1.30	1.78±1.15	0.27±1.39
Day 84	1.31±0.58*$^{\delta}$	-1.06±1.07$^{\delta}$	1.57±0.82	-0.45±1.55	1.72±0.79	0.20±1.11

*P<0.05 compared to baseline; $^{\#}$P<0.05 compared to Oral B12; §P<0.05 compared to IFA

Table 6: Hematocrit (%) in the study groups at different time points.

Study visits	NASO B12 group		Oral B12 group		IFA group	
	Mean level	Mean change from baseline	Mean level	Mean change from baseline	Mean level	Mean change from baseline
Baseline	30.54±4.17	-	30.70±5.17	-	33.26±2.97	-
Day 7	32.99±4.17	2.45±2.84	32.21±5.17	1.51±2.90	34.70±3.40	1.44±2.32
Day 14	34.60±3.83*	4.06±4.18$^{\delta}$	34.69±4.42*	3.86±3.31$^{\delta}$	35.28±3.24*	2.02±2.75
Day 42	36.86±3.93*	6.32±4.77$^{\delta}$	35.18±3.57*	4.48±3.45	35.94±3.10*	2.68±3.30
Day 84	39.12±4.67*$^{\delta}$	8.58±5.89$^{\delta}$	37.45±4.97*$^{\delta}$	6.75±4.73$^{\delta}$	36.66±3.57*	3.40±3.98

*P<0.05 compared to baseline; $^{\delta}$P<0.05 compared to IFA

Table 7: Mean serum ferritin level (ng/ml) and folic acid levels (ng/ml) in the study groups at different time points.

Study visits	NASO B12 group		Oral B12 group		IFA group	
	Ferritin level (ng/ml)$^{\#}$	Folic acid levels (ng/ml)	Ferritin level (ng/ml)	Folic acid levels (ng/ml)	Ferritin level (ng/ml)	Folic acid levels (ng/ml)
Baseline	32.81±36.20	9.15±4.43	27.79±42.32	8.92±5.11	19.52±33.90	8.00±3.53
Day 7	34.42±29.72	13.09±4.76*	26.54±19.86	11.35±4.88	28.25±33.90	11.07±4.05*
Day 14	34.48±23.15	14.66±4.24*	36.88±21.29	13.32±4.20*	29.97±28.65	11.95±5.04*
Day 42	35.02±22.81	12.73±4.31*	34.69±19.82	13.13±5.12*	29.79±20.49*	11.14±4.29*
Day 84	38.03±28.33	13.13±5.22*	34.94±22.70	13.13±5.17*	29.34±17.17*	11.20±4.56*

$^{\#}$Analysis of ferritin levels for the NASO B12 group was conducted with 24 participants due to the exclusion of one outlier with an abnormally high ferritin level at baseline to ensure accurate results; *P<0.05 compared to baseline.

DISCUSSION

This study underscores the pivotal role of vitamin B12 supplementation in addressing anemia among women, as demonstrated by a significant improvement in Hb levels. In the NASO B12 group, Hb levels increased remarkably from 9.26 g/dL to 12.08 g/dL by the end of the study, highlighting the efficacy of this intervention. Notably, the Hb level improvements were significantly higher and faster in the NASO B12 group compared to the IFA group, with a significant rise observable as early as day 7 of the treatment phase. It was also observed that the rise of Hb in the NASO B12 group was higher at all the visits as compared to oral B12 group. This finding emphasizes the necessity of supplementing NASO B12 alongside IFA to achieve favourable hematological outcomes in anemic women with the reproductive age group.

Our study results align with findings from studies by Sood et al and Chandelia et al where in injectable vitamin B12 was administered along with IFA supplementation and these studies reported an increase in Hb of 0.83 g/dL to 4.1 g/dL, suggesting that NASO B12 is as effective as injectable B12 and can be supplemented in place of injectable B12 which are painful and have a high cost of therapy, owing to the need for clinic visits and healthcare professional assistance for administration.[19,20,23]

The above observations can be described based on the levels of vitamin B12 achieved after the treatment. According to the American academy of family physicians, serum vitamin B12 levels are categorized as normal (≥400 pg/ml), low-normal (150-399 pg/ml), and low (<150 pg/ml).[25] Also, as per published studies, serum vitamin B12 levels of 400 pg/ml or more are crucial for optimal Hb formation and red blood cell parameters, as well as for neurological health and prevention of cognitive decline and dynapenia.[26-28]

In our study, the mean baseline levels for all the treatment groups had low-normal vitamin B12 levels. After treatment, only the NASO B12 group achieved normal B12 levels (≥400 pg/ml), with this increase observed as early as day 7. Notably, vitamin B12 levels in the NASO B12 group rose to 580.2 pg/ml by day 42 and were sustained above 400 pg/ml through day 84, even after discontinuing the intervention. In contrast, participants in the oral B12 group consistently remained within the low-normal range throughout the study, showing no significant improvement in serum B12 levels from baseline at any time point.

At the end of the study period, an average increase of 237.8 pg/ml was achieved with NASO B12, compared to a modest increase of 79.72 pg/ml in the oral B12 group. The rise in B12 levels after oral treatment are consistent with previous studies by Bansal et al and Gupta et al which reported increases of 63.2 pg/ml and 109 pg/ml, respectively, in adolescent girls receiving oral B12 supplementation.[5,6] Whereas, the achievement of higher vitamin B12 levels with NASO B12 aligns with studies by Seth et al which demonstrated similar outcomes in B12-deficient patients, and by Farookh et al involving diabetic patients on metformin therapy.[23,24] These findings underscore the rapid, consistent, and efficient ability of NASO B12 to elevate serum vitamin B12 levels-a

therapeutic outcome that oral B12 fails to replicate. The enhanced efficacy of NASO B12 can be attributed to its unique route of administration. Unlike oral B12, which relies on gastrointestinal absorption, NASO B12 is absorbed directly through the nasal epithelium, bypassing gastrointestinal barriers. This mechanism likely explains the rapid and significant increase in serum B12 levels observed with NASO B12.

Our study also evaluated RBC count as a secondary outcome, a parameter not addressed in the studies by Bansal et al and Gupta et al.[5,18] Our findings revealed a significant increase in RBC count across all three groups by the end of the study. However, the increase was markedly greater in the NASO B12 group compared to both the oral B12 and IFA groups. Notably, the rise in RBC count in the oral B12 group was not significantly different from that in the IFA group.

The evaluation of mean reticulocyte count revealed that, in the NASO B12 group, an initial increase in reticulocyte count was observed during the treatment phase, followed by a decreasing trend during the maintenance phase and at the study's conclusion. Reticulocytes represent the final immature stage of red blood cells (RBCs), spending approximately two days in the bone marrow and one day in the bloodstream before maturing into RBCs.[29] The observed early rise in reticulocyte count likely reflects active erythropoiesis triggered by the treatment. As therapy progressed, more reticulocytes matured into functional RBCs, resulting in a lower serum reticulocyte count by the end of the study. This pattern underscores the dynamic nature of erythropoiesis and the effectiveness of NASO B12 in promoting RBC production.

Ferritin, a marker of iron storage, reflects the body's iron reserves that are mobilized during erythropoiesis to support Hb synthesis and the production of new RBCs.[30,31] In our study, all three groups demonstrated an increase in mean serum ferritin levels, attributed to IFA supplementation provided across the groups.

In addition to other outcomes, our study evaluated hematocrit and serum folate levels. Hematocrit levels showed a significant increase from baseline across all three groups. Notably, the NASO B12 group demonstrated higher hematocrit rise than oral B12 group and IFA groups at all the visits due to raised RBC count. As anticipated, serum folate levels increased significantly in all three groups, highlighting the efficacy of the supplementation strategies in addressing folate requirements.

The results of our study hold significant clinical implications for WRA, a group particularly vulnerable to anemia and its associated complications. Anemia in WRA is a common and pressing concern, often leading to fatigue, reduced work capacity, and in severe cases, complications during pregnancy.[2]

Overall, our study demonstrates that NASO B12 supplementation, combined with IFA, provides a superior and consistent improvement in hematological parameters compared to oral B12 and IFA. NASO B12 not only achieved rapid normalization of serum vitamin B12 levels but also showed significant benefits in enhancing Hb, hematocrit, and RBC counts, alongside promoting effective erythropoiesis. These findings highlight the potential of NASO B12 as a more effective therapeutic option for managing anemia and improving overall hematological health, especially in WRA.

Our study included 75 anemic WRA so larger, population-based studies can be conducted to confirm these effects. Given the rise of hematologic parameters with NASO B12, future clinical studies can be designed including children, women, especially pregnant, lactating, the elderly population, and patients with neurologic conditions.

CONCLUSION

In conclusion, NASO B12, a nasal formulation of methylcobalamin, in addition to IFA demonstrated higher and faster efficacy in improving hematological parameters compared to oral B12+IFA and IFA alone. Its rapid absorption, enhanced bioavailability, and ability to stimulate effective erythropoiesis make NASO B12 a promising therapeutic option for addressing vitamin B12 deficiency and anemia, particularly in WRA.

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