Micronutrients Drift During Daily and Weekly Iron Supplementation in Non-anaemic and Anaemic Pregnancy

Hari Shankar^{1,3} · Neeta Kumar² · Rajat Sandhir³ · Suneeta Mittal^{1,5} ·
Tulsi Adhikari⁴ · Arun Kumar¹ · D. N. Rao^{1,6}

Received: 8 November 2016 / Accepted: 4 January 2017 / Published online: 17 January 2017
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Abstract

Aims Pregnancy is a phenomenon associated with dynamic changes in physical, mental and biochemical status of body and demands increased nutritional intake for developing foetus. The level of various micronutrients which act as co-factors for antioxidant enzymes or it-self as antioxidants gets altered with the progression of pregnancy. The present longitudinal study summarized the trend of selected micronutrients level in anaemic (AP) and non-anaemic primigravida (NAP) supplemented with daily and weekly oral iron folic acid (IFA) tablet during pregnancy and postpartum.

Methods A total of 200 primigravida {N = 100; NAP (Hb > 11 g/dl) and N = 100 AP (Hb = 8–11 g/dl) assigned daily (N = 50) and weekly (N = 50) supplementation} were recruited and overnight fasting blood samples were withdrawn at 13–16 weeks, after 3 months and 6 weeks postpartum. The serum iron, copper, zinc,

magnesium and manganese were estimated by inductively coupled plasma-atomic emission spectrophotometer.

Results Serum manganese ($p < 0.05$) at baseline and magnesium ($p < 0.01$) at postpartum was significantly different between NAP and AP supplemented with daily IFA tablets. The trend of copper found to be increased during pregnancy and later declined at postpartum in both the groups. Daily supplementation resulted in significantly high iron ($p < 0.05$) in NAP during third trimester.

Conclusions Hypozincemia and hypomagnesemia was observed in anaemic pregnancy supplemented with weekly and daily IFA respectively. Clear evidence of altered micronutrients levels during healthy and anaemic pregnancy was seen. The reference values may be drawn from this study for the nutritional assessment during pregnancy for healthy pregnancy outcomes.

Trial Registration Clinical Trial Registry-India, <http://ctri.nic.in>, CTRI/2014/10/005135.

Keywords Anaemia · ICP-AES · Iron folic acid supplementation · Pregnancy · Trace elements · Micronutrients

✉ D. N. Rao
dnrao311@rediffmail.com

¹ Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

² Indian Council of Medical Research, Ansari Nagar, New Delhi 110029, India

³ Panjab University, Chandigarh 160014, India

⁴ National Institute of Medical Statistics (ICMR), New Delhi, India

⁵ Present Address: Fortis Memorial Research Institute, Gurgaon, Haryana 122001, India

⁶ GITAM Institute of Medical Sciences and Research, GITAM University, Rushikonda, Visakhapatnam 530045, India

Introduction

Pregnancy is a period of physiological changes in women's body which take place in response to meet the increasing metabolic demands of growing foetus [1]. The deficiency of micronutrients often co-exists and the deficiency of copper, zinc and manganese can lead to poor pregnancy outcomes like preeclampsia [2] and foetal growth restriction [3]. Thus, an intricate balance between various

essential micronutrients is vital for growth and development of foetus and well-being of the mothers.

The deficiency of micronutrients can disturb the growth, cognition, reproductive performance and also may lead to mortality and morbidity in new born [4]. Although, the multiple micronutrients deficiency related to intake of poor nutritious diet during pregnancy have been studied; but iron deficiency is most extensively investigated [5]. Thus, it is pre-requisite to monitor the level of micronutrients which are involved in maintaining healthy pregnancy. In addition, iron deficiency anaemia, one of the major nutritional deficiencies during pregnancy in developed as well as developing countries [6] is associated with low birth weight, prenatal mortality and increased rates of premature birth [5]. Further, zinc and copper deficiency has been associated with the complications of pregnancy and delivery [7], childbirth and foetal growth retardation [8]. Although, manganese is one of the least studied micronutrients, scanty and inconclusive data is available on its association with pregnancy complications [9], an association of manganese deficiency with foetal growth restriction [10]; and reduced umbilical cord manganese level in neonates born to preeclampsia mothers has been observed [11]. Randomized controlled trials are the best approach for studying the micronutrient supplementation on pregnancy outcome; while most of the trials are largely been done for individual micronutrient/trace elements or minerals. A programme of supplementing multiple micronutrients during pregnancy may be projected based upon the availability of adequate data on micronutrient deficiencies during pregnancy.

Materials and Methods

Subjects

A total of two hundred primigravid women between 13 and 16 weeks gestation were recruited on their first visit to the antenatal clinic at the Outpatient Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences (AIIMS), New Delhi. On the basis of Hb values, they were grouped into non-anaemic ($11.0 \leq \text{Hb} \leq 13.0$; $n = 100$) and anaemic group ($8.0 \leq \text{Hb} \leq 11.0$ g/dl; $n = 100$). The inclusion criteria included primigravid status of age 19–30 years having Hb range 8.0–13.0 g/dl; body mass index (BMI) ranged 18–22, belonging to middle socio-economic status according to Kuppuswami's scale for socio-economic status. Exclusion criteria included history of chronic illness or any metabolic disease like diabetes mellitus, malignancy and heart disease, infectious diseases like tuberculosis, HIV, endocrine disorders or intake of any

iron preparation in past three months. After enrolment, 2 ml of overnight fasting blood was drawn at baseline. Likewise, second sample (3 months after first sampling) and third sample (6 weeks postpartum) was obtained in a similar manner.

Randomization

After screening the eligibility and informed consent process, envelopes containing random number slips for 100 pregnant women in daily and 100 pregnant women in weekly arm was prepared at Indian Council of Medical Research and provided to the participating centre. Random numbers was generated in a ratio of 1/1 by means of a customized computer programme. According to the serial number of the enrolled subject, one sealed envelope containing slip with instructions for random allocation was opened for each subject. The enrolled participants were categorized into two groups as follows:

Group-1: pregnant non-anaemic ($\text{Hb} > 11$ g/dl) women ($N = 100$)

Group-2: pregnant anaemic ($\text{Hb} = 8\text{--}11$ g/dl) women ($N = 100$)

Informed consent was obtained from each subject before enrolled into the study.

Study Design

All the enrolled participants (anaemic and non-anaemic pregnant women) were dewormed by giving single dose of Albendazole 400 mg to be consumed at night on the day of enrolment. The enrolled participants were asked to fasting overnight and on the next morning venous blood sample was drawn from antecubital vein in supine position followed by refreshments. Likewise, second sample and third sample was obtained in similar manner.

According to random allocation slip instructions, participants were given either one tablet of FeSO_4 containing 100 mg elemental iron and 500 μg folic acid (Iron folic acid tablet/IFA tablet; Hindustan Antibiotics Ltd.—A govt. of India Enterprises, Pune 411 018, India) till 6 weeks postpartum daily or two IFA tablets weekly till 6 weeks postpartum. Thus, both the groups i.e. group 1 and 2 further subdivided into daily IFA supplementation group (DISG; $N = 50$) and weekly IFA supplementation group (WISG; $N = 50$).

Sample Collection

2 ml venous blood was taken from each participant and serum was separated by centrifuging the blood in clot

activator tubes at 3000 rpm for 15 min. The different aliquots were prepared and stored in -80°C till further analysis.

Analytical Estimation

The serum was analysed for iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg) and manganese (Mn) by means of inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) (Model JY 2000@2, HORIBA Jobin-Yvon, France). Serum samples were digested using MW800 Microwave digestion system (Aurora instruments Ltd., Vancouver, Canada). The digestion procedure was optimized as in application module: digestion time (5 min), sample volume (1 ml), and [4 ml suprapur nitric acid (HNO_3) (65%) and 1 mL hydrogen peroxide (20%) (Merck Chemicals, India)].

The digested serum samples were subjected for the analysis of iron (Fe 259.940 nm), copper (Cu 324.754 nm), zinc (Zn 213.856 nm), magnesium (Mg 279.553 nm) and manganese (Mn 257.610 nm) to ICP-AES. ICP grade multi-element standard solution containing 1000 mg/L of 23 elements in 1 mol/L HNO_3 (Product no. 1. 11355. 0100, Batch no. HC081563, Merck Chemicals, Germany) was used as reference standard. Pooled serum samples were used as biological material for the purpose of internal quality control.

Instrument parameters operating condition was power (1200 W), nebulizer flow rate (0.78 L/min), pump speed 15(rates/min), nebulizer pressure 2.39(bar) dual detector and sweep/reading of 3, reading/replicate of 3, dwell time (5 s), and integration time (10 s). Wavelength was selected from a predefined set for each trace element using the ICP software version 5.2. The blank solution was run for background correction. For calibration curves, the standard solution was diluted step-wise with 5% suprapur HNO_3 in the concentration range of 5–100 ppb. The linearity of the calibration curves was considered to be good (correlation coefficient, $r \geq 0.993$). The limit of detection (LOD), as described in the manufacturer's instrument manual was used for calculations.

Statistical Analysis

Data was analysed using SPSS for windows (Version 20.0) and the results were expressed as mean $\pm$ SE. Mean values were compared between the two groups using the student's unpaired t test and within the same group using paired t test. Correlation study was performed by calculating Pearson coefficient of correlation. Differences were considered to be statistically significant at an error probability of less than 0.05 ($p < 0.05$).

Results

Daily IFA Supplementation Groups

Table 1 depicts non-significant difference between non-anaemic and anaemic pregnancy in all the studied trace elements except Mn that showed statistically significant difference at baseline (61.71 ± 5.55 vs. 95.47 ± 9.30 ; 95% CI -54.50 to -13.02 ; $p < 0.01$) and postpartum (76.14 ± 7.56 vs. 113.9 ± 12.05 ; 95% CI -65.06 to -10.48 ; $p < 0.01$). The mineral Mg level was also significantly high in NAP as compared to AP (18.70 ± 0.98 vs. 13.04 ; 95% CI 261.3 – 870.3 ; $p < 0.001$). Low level of serum Mg (hypomagnesemia) less than 15 mg/l was observed in AP at postpartum. The serum level of Fe, Cu and Zn were non-significant between the two groups. However, during the progression of pregnancy i.e. from second to third trimester the serum level of Fe, Cu and Mn increased significantly ($p < 0.001$ and $p < 0.05$) in NAP. The increased level of Cu and Mn remained significant ($p < 0.01$ and $p < 0.05$) till postpartum when compared to second visit. The Serum level of Zn and Mg did not showed much change when compared during studied intervals in NAP (Table 1). The serum level of Fe found positively correlated with serum Cu at postpartum ($p < 0.0001$), serum Zn at second visit and postpartum ($p < 0.05$), serum Mg throughout the pregnancy and postpartum ($p < 0.0001$) and negatively correlated with serum Mn insignificant in NAP (Table 2).

In AP; however, only serum Cu increased significantly ($p < 0.001$) at second visit as compared to baseline and decreased significantly ($p < 0.0001$) at postpartum when compared to second visit. Rest all the studied parameters viz. Fe, Zn, Mn and Mg were found non-significant at studied intervals except Mn where serum level declined significantly ($p < 0.05$) at postpartum (Table 1). Hypozincemia at baseline was observed in AP women supplemented with daily IFA tablets. The positive correlation between serum Fe and Zn was found statistically significant ($p < 0.0001$) at second visit in AP supplemented with daily IFA tablets. Further, serum Fe was also positively correlated with serum Mg levels ($p < 0.05$) at all studied intervals (Table 2).

Weekly IFA Supplementation Group

There was non-significant difference observed in all the studied micronutrients when compared between NAP and AP (Table 1). Zn deficiency defined by serum Zn concentration $<13.8 \mu\text{mol/l}$ ($90.19 \mu\text{g/dl}$) was observed at

Table 1 Micronutrient status during pregnancy and postpartum

Micronutrients		Non-anaemic primigravida		Anaemic primigravida	
		DISG	WISG	DISG	WISG
S. Fe (µg/dl)	2nd trimester	125.6 ± 7.52	131.1 ± 11.05	118.2 ± 9.05	120.7 ± 8.67
	3rd trimester	162.5 ± 11.64*	124.6 ± 10.21	145.7 ± 11.19	143.2 ± 14.43
	6 week PP	134.0 ± 12.87	126.6 ± 12.70	112.2 ± 11.89	119.2 ± 15.37
S. Cu (µg/dl)	2nd trimester	181.0 ± 5.31	182.2 ± 6.75	185.5 ± 6.11	184.7 ± 6.29
	3rd trimester	200.3 ± 7.23 [†]	211.3 ± 8.20 [†]	195.4 ± 6.76 ^α	203.7 ± 9.78
	6 week PP	147.3 ± 8.79 [†]	143.1 ± 6.43 ^α	131.7 ± 9.39 ^α	142.9 ± 10.06 ^α
S. Zn (µg/dl)	2nd trimester	91.25 ± 3.39	91.12 ± 3.27	88.85 ± 2.57	101.0 ± 12.91
	3rd trimester	90.06 ± 5.31	89.80 ± 5.72	95.79 ± 6.67	101.8 ± 13.99
	6 week PP	96.32 ± 4.51	89.82 ± 3.70	91.49 ± 5.85	87.81 ± 4.68
S. Mg (mg/l)	2nd trimester	17.96 ± 0.41	17.99 ± 0.38	17.33 ± 0.57	18.78 ± 0.96
	3rd trimester	18.78 ± 0.91	18.99 ± 1.43	17.83 ± 1.36	18.42 ± 1.70
	6 week PP	18.70 ± 0.98 ^a	19.04 ± 2.23	13.04 ± 1.14	16.36 ± 1.48
S. Mn (µg/l)	2nd trimester	61.71 ± 5.55	63.78 ± 5.12	95.47 ± 9.30 ^a	76.27 ± 7.36
	3rd trimester	79.71 ± 6.54 [†]	74.20 ± 4.77	89.37 ± 9.14	71.83 ± 4.81
	6 week PP	76.14 ± 7.56 [†]	68.51 ± 5.15 [†]	113.9 ± 12.05 ^{a,*}	70.62 ± 4.26

Data expressed as mean ± SE; Statistical significance

DISG daily iron folic acid supplementation group, PP postpartum, S. Cu serum copper, S. Fe serum iron, S. Mg serum magnesium, S. Mn serum manganese, S. Zn serum zinc, WISG weekly iron folic acid supplementation group

* $p \leq 0.05$ between the groups; ^a $p \leq 0.05$ between non-anaemic and anaemic group in respective supplementation; [†] $p \leq 0.01$ within the group; ^α $p \leq 0.001$ within the group

second trimester and postpartum in NAP supplemented with weekly IFA tablets. Serum level of Cu was significantly increased in NAP during third trimester of pregnancy ($p < 0.01$). However, serum Cu found significantly declined at postpartum ($p < 0.001$) along with serum Mn ($p < 0.01$) in NAP (Table 1). The correlation study revealed statistical significant positive correlation between serum Fe and serum Cu, Zn and Mg at baseline ($p < 0.05$) and second visit ($p < 0.0001$) in NAP supplemented with weekly IFA tablets (Table 2).

In AP, the level of most of the micronutrients did not changed significantly during pregnancy; albeit only, serum level of Cu was found significantly declined at postpartum ($p < 0.001$) when compared to the second visit (Table 1). Apart from this, hypozincemia was observed in AP at postpartum. In AP supplemented with weekly IFA, serum Fe found positively correlated with serum Mg at baseline and postpartum ($p < 0.05$); whereas, negatively correlated with serum Mn at all intervals but this correlation found statistically significant only at postpartum ($p < 0.05$).

Daily Versus Weekly IFA Supplementation

The effect of daily and weekly IFA tablet supplementation on serum level of Fe, Cu, Zn, Mg, Mn was evaluated in NAP and AP women. The results revealed insignificant

difference in levels of all the micronutrients except Fe at second visit ($p < 0.05$) in NAP women. Apart from this, serum Mn level was also found significantly different at postpartum ($p < 0.05$) in AP women (Table 1).

Discussion

The present study comprised primigravida with similar age group, BMI ranged 18–22 kg/m², belonging to middle socio-economic status non-anaemic and anaemic pregnant women. The levels of Fe, Cu and Mn in serum increased progressively in NAP; whereas profound increase was seen only in serum Cu levels in case of AP women supplemented with daily IFA tablets. These differences in micronutrients levels between NAP and AP might be the result of poor dietary habits and less nutrients intake which could lead to altered micronutrient levels in women who had anaemia during pregnancy. The significant rise in Cu levels in anaemic pregnant women corroborate with the results obtained by Ghosh et al. [12] except that the increase in Cu level was less profound in non-anaemic pregnant women in their study; however, a significant rise was observed in NAP women supplemented with daily IFA tablets in present study. This increase in Cu level during pregnancy is due to increase in the carrier protein ceruloplasmin in response to stimulation by elevated estrogen

Table 2 Association of serum level of iron with other micronutrients during pregnancy and postpartum

	Non-anaemic daily IFA group			Non-anaemic weekly IFA group			Anaemic daily IFA group			Anaemic weekly IFA group		
	2nd trimester r (CI)	3rd trimester r (CI)	6 weeks PP r (CI)	2nd trimester r (CI)	3rd trimester r (CI)	6 weeks PP r (CI)	2nd trimester r (CI)	3rd trimester r (CI)	6 weeks PP r (CI)	2nd trimester r (CI)	3rd trimester r (CI)	6 weeks PP r (CI)
S. Cu (µg/ dl)	0.2727 (-0.003202 to 0.5100)	0.3000 (-0.00048 to 0.5508)	0.7051*** (0.4296 to 0.8605)	0.4199** (0.1660 to 0.6216)	0.5787*** (0.3258 to 0.7543)	0.1674 (-0.2122 to 0.5031)	0.2828 (-0.004784 to 0.5272)	0.1379 (-0.1734 to 0.4241)	0.2491 (-0.2466 to 0.6414)	0.04269 (-0.2445 to 0.3230)	0.2530 (-0.05929 to 0.5203)	0.2289 (-0.2516 to 0.6188)
S. Zn (µg/ dl)	0.1465 (-0.1346 to 0.4058)	0.3768* (0.08612 to 0.6083)	0.4088* (0.01622 to 0.6922)	0.3217* (0.05341 to 0.5467)	0.6282*** (0.3937 to 0.7860)	0.3356 (-0.03530 to 0.6253)	0.2591 (-0.03042 to 0.5085)	0.6153*** (0.3794 to 0.7761)	-0.04838 (-0.5040 to 0.4283)	0.02673 (-0.2594 to 0.3086)	0.1848 (-0.1304 to 0.4660)	-0.04125 (-0.4864 to 0.4209)
S. Mg (mg/ l)	0.4708*** (0.2243 to 0.6607)	0.5585*** (0.3066 to 0.7373)	0.7012*** (0.4233 to 0.8585)	0.3436* (0.07796 to 0.5637)	0.6668*** (0.4484 to 0.8101)	0.3567 (-0.01901 to 0.6441)	0.3983** (0.1254 to 0.6152)	0.7098*** (0.5175 to 0.8339)	0.4811* (0.01825 to 0.7742)	0.4397** (0.1777 to 0.6435)	0.3030 (-0.005236 to 0.5586)	0.5200* (0.08596 to 0.7881)
S. Mn (µg/ l)	-0.2732 (-0.5301 to 0.02968)	-0.05237 (-0.3791 to 0.2859)	-0.2113 (-0.5598 to 0.2007)	0.05039 (-0.2403 to 0.3328)	-0.08557 (-0.3945 to 0.2408)	-0.1275 (-0.5050 to 0.2910)	-0.2089 (-0.5245 to 0.1571)	-0.05237 (-0.3791 to 0.2859)	-0.05089 (-0.5189 to 0.4406)	-0.1339 (-0.4662 to 0.2315)	-0.1997 (-0.5082 to 0.1543)	-0.5763* (-0.8220 to -0.1496)

CI confidence interval (95%), IFA iron folic acid, PP postpartum, r correlation coefficient, S. Cu serum copper, S. Zn serum zinc, S. Mg serum magnesium, S. Mn serum manganese

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.0001$

levels [13]. Daily IFA tablets caused varied Mn and Mg levels in AP and NAP women. This shows that anaemia contributed in the altered level of micronutrients. Liu et al. [14] in a longitudinal study analysed blood Cu, Ca, Zn and Mg levels during different time interval of healthy pregnancy and found altered levels of these elements. They found no statistically significance between the Mg levels during the pregnancy which is in accordance with the results obtained in present study. Tholin et al. [15] studied blood Mn level during pregnancy in iron supplemented and non-supplemented women and found no significant difference between the groups. The median values of Mn in trimester-I and trimester-II were 154 (range 79–360) nmol/L and 190 (range 98–408) nmol/L respectively, which was lower than the values obtained in the present study. This difference might be due to the analytical procedure adopted, using less sensitive instrument or less intake of Mn rich diet by the subjects. Mena et al. [16] has reported higher blood Mn levels in manganese miners and anaemic subjects, which is in relation with the results obtained in present study where significantly high Mn levels were observed in AP women compared to NAP women.

Serum Fe levels both in daily or weekly IFA supplementation group, irrespective of anaemic status was found positively correlated significantly with Mg levels during pregnancy and postpartum. Statistically positive correlation between serum Fe and Cu was observed during pregnancy and postpartum in NAP supplemented with weekly and daily IFA tablets respectively. However, serum Fe and Zn was positively correlated at most of the studied intervals in NAP supplemented with daily or weekly IFA tablets; and in AP supplemented with daily IFA tablet only at second visit (Table 2). These correlations between the trace elements revealed the possible association between them and also confirm the interaction during the progression of pregnancy.

This is noteworthy that weekly IFA supplementation developed hypozincemia both in AP and NAP women. Low serum level of Zn in anaemic pregnant women has also been reported in previous studies [12]. Although, low baseline level of Zn in AP women was also observed; but it was recovered after supplementing them with daily IFA tablets. The serum Zn levels were marginally decreased during the pregnancy in present finding, which is in accordance with earlier studies where Zn level declined during pregnancy, but the difference was statistically non-significant [14, 17–19]. Since, fetal demands for various trace elements increase as gestation increases and plasma volume expands; this results in the decreased levels of trace elements. Interactions between different trace elements are well known; and high concentration of iron can interfere with zinc uptake and copper absorption. These elements are more likely to interact with each other when given as

supplement and the outcomes may also vary greatly [20]. Serum Mg levels were stable during pregnancy with the exception that daily IFA supplementation in anaemic pregnancy showed hypomagnesaemia at postpartum. Further, serum Mg was below the normal reference range (18.0–29.0 mg/l) at baseline, which was continued in AP supplemented with daily IFA tablets. Low levels of Mg reported to be associated with hemodilution, increased requirement of Mg demands by the growing fetus and also increased renal clearance [21–23].

The limitation of the study is that there are various factors like vitamin A, folate and riboflavin deficiency and iron deficiency which is most commonly associated with anaemia during pregnancy along with other confounders viz. genetic factors, nutritional deficiencies, repeated pregnancies, bioavailability of iron tablets, burden of infectious diseases and malaria during pregnancy [24]. In the present study, however a few of them viz. Vitamin A, vitamin B12 and folate levels were not studied; yet most of the confounders tried to be ruled out by (1) deworming the subjects with Albendazole tablet before enrolling into the study; thus, the subjects were assumed to be free from infections like hookworm infection etc. (2) maintaining the uniformity of iron supplementation by providing the iron folic acid tablets in the form of ferrous sulphate + folic acid (IFA) to each study participant. The participants were asked to adhere with the supplied IFA tablets as there are numerous combinations of iron tablets viz. ferrous sulphate, ferrous fumarate, ferrous ascorbate, ferrous citrate etc. are available in the market and each one is having varying bioavailability and compliance. The tablets were manufactured from Hindustan Antibiotics Ltd.—A govt. of India Enterprises, Pune, India to maintain the homogeneity about the type of iron supplement consumed by subjects (3) excluding the participants having pregnancy related complications such as repeated pregnancy, history of infections, symptoms of any co-morbidity and parity, which contributes for anaemia during pregnancy [25]. These would aid in minimizing the factors from the list of confounders responsible for anaemia during pregnancy and altered micronutrient levels.

The present study has several advantages over previous studies that examined micronutrient status during pregnancy. Some of them assessed micronutrients levels either in healthy pregnancy or in anaemic pregnancy [12, 26], some have reported differences in micronutrient levels between anaemic and non-anaemic pregnant women [25] and very few [14] have analysed the micronutrient levels during pregnancy and postpartum in longitudinal manner. However, all these published information can be gathered from the present finding as it is comprehensively designed parallel group randomised longitudinal study, comparing micronutrient status in anaemic and non-anaemic pregnant

women at different time intervals of pregnancy and postpartum; and also compared the effect of IFA supplementation in daily or weekly schedule between NAP and AP women. This is the first report on micronutrient profile of North Indian anaemic and non-anaemic primigravida pregnant women as no information is available according to our knowledge. Furthermore, apart from having a large sample size, a wide range of micronutrients was examined simultaneously in homogenous population, allowing estimation of the extent of maternal micronutrient states throughout the pregnancy as well as post-delivery. Various studies have documented the role of trace elements deficiency in developing anaemia during pregnancy [12, 25]. However; Bhutta et al. [27] reported that there is no effect of multiple micronutrient supplementation as compared to IFA supplementation on maternal anaemia. Considering the fact that enrolled participants were age matched, with middle socioeconomic status, similar BMI and supplemented IFA tablets of same pharmacological ingredients throughout the pregnancy and postpartum to maintain the homogeneity. Thus, the results of the present study also have the potential to provide reference values for assessing nutritional status.

Conclusions

The present study gives clear evidence that all the studied trace elements are altered visibly but statistically non-significantly except Mn and Fe during different stages of normal and anaemic pregnancy. This data may be treated as reference range of micronutrients in mild anaemic and non-anaemic pregnant women which will be helpful in assessing the health status of pregnant women with socioeconomic and racial background similar to those of our study participants and give treatments to them promptly.

Acknowledgements The authors are highly thankful to Indian Council of Medical Research, New Delhi, India for funding the study. The authors are also thankful to Prof. YK Gupta, Head of Pharmacology Department, AIIMS, New Delhi for providing instrumentation facility (ICP-AES) for performing trace elements analysis.

Funding This study was funded by Indian Council of Medical Research, New Delhi, India (Grant No. 5/7/165/06-RHN).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study were in accordance with the ethical standards of the Institutional Ethics Committee of All India Institute of Medical Sciences, New Delhi, India (Approval No.: IEC/NP-339/2010) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Awritten informed consent was obtained from all study participants included in the study.

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