

Hari Shankar\*, Neeta Kumar, Rajat Sandhir, Mrigendra Pal Singh, Suneeta Mittal<sup>a</sup>,  
Tulsi Adhikari, Mohd. Tarique, Parmeet Kaur, M.S. Radhika, Arun Kumar and D.N. Rao\*

# Association of dietary intake below recommendations and micronutrient deficiencies during pregnancy and low birthweight

<https://doi.org/10.1515/jpm-2019-0053>

Received February 16, 2019; accepted June 3, 2019

## Abstract

**Background:** Pregnancy is associated with biochemical changes leading to increased nutritional demands for the developing fetus that result in altered micronutrient status. The Indian dietary pattern is highly diversified and the data about dietary intake patterns, blood micronutrient profiles and their relation to low birthweight (LBW) is scarce.

**Methods:** Healthy pregnant women (HPW) were enrolled and followed-up to their assess dietary intake of nutrients, micronutrient profiles and birthweight using a dietary

recall method, serum analysis and infant weight measurements, respectively.

**Results:** At enrolment, more than 90% of HPW had a dietary intake below the recommended dietary allowance (RDA). A significant change in the dietary intake pattern of energy, protein, fat, vitamin A and vitamin C ( $P < 0.001$ ) was seen except for iron (Fe) [chi-squared ( $\chi^2$ ) = 3.16,  $P = 0.177$ ]. Zinc (Zn) deficiency, magnesium deficiency (MgDef) and anemia ranged between 54–67%, 18–43% and 33–93% which was aggravated at each follow-up visit ( $P \leq 0.05$ ). MgDef was significantly associated with LBW [odds ratio (OR): 4.21;  $P = 0.01$ ] and the risk exacerbate with the persistence of deficiency along with gestation (OR: 7.34;  $P = 0.04$ ). Pre-delivery (OR: 0.57;  $P = 0.04$ ) and postpartum (OR: 0.37;  $P = 0.05$ ) anemia, and a vitamin A-deficient diet (OR: 3.78;  $P = 0.04$ ) were significantly associated with LBW. LBW risk was much higher in women consuming a vitamin A-deficient diet throughout gestation compared to vitamin A-sufficient dietary intake (OR: 10.00;  $P = 0.05$ ).

**Conclusion:** The studied population had a dietary intake well below the RDA. MgDef, anemia and a vitamin A-deficient diet were found to be associated with an increased likelihood of LBW. Nutrient enrichment strategies should be used to combat prevalent micronutrient deficiencies and LBW.

**Keywords:** anemia; dietary intake; micronutrients; nutrition; pregnancy; recommended dietary allowance.

<sup>a</sup>Current affiliation: Fortis Memorial Research Institute, Gurgaon, Haryana 122001, India.

\*Corresponding authors: Dr. Hari Shankar, Epidemiology and Clinical Research Division, ICMR – National Institute of Malaria Research, Sector-8, Dwarka, New Delhi 110077, India; Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India; and Department of Biochemistry, Panjab University, Chandigarh 160014, India, Tel.: +91-9891011776, Fax: +91-11-25307111/77, E-mail: hshankar6127@gmail.com; and Dr. D.N. Rao, Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India, Tel.: +91-9868592706, Fax: +91-11-26588641/63, E-mail: dnrao311@rediffmail.com

**Neeta Kumar:** Department of Reproductive Biology and Maternal Health, Child Health, Indian Council of Medical Research, Ansari Nagar, New Delhi 110029, India

**Rajat Sandhir:** Department of Biochemistry, Panjab University, Chandigarh 160014, India

**Mrigendra Pal Singh:** National Institute of Malaria Research Field Unit Jabalpur, Madhya Pradesh, India

**Suneeta Mittal and Arun Kumar:** Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

**Tulsi Adhikari:** National Institute of Medical Statistics (ICMR), New Delhi, India

**Mohd. Tarique:** Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

**Parmeet Kaur:** Department of Dietetics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

**M.S. Radhika:** Clinical Division, National Institute of Nutrition (ICMR), Hyderabad, India

## Introduction

Maternal nutrition during pregnancy and even in the pre-conception period greatly influences the well-being of the mother, the development of the fetus at conception and at later stages of growth in children. Healthy diet and nutrition showed some positive associations with infant birthweight and a dietary pattern study about whole food consumption rather than specific nutrients intake may provide fruitful information [1]. The typical Indian diet contains cereals and millets which form the bulk of the

diet. The low Fe bioavailability of the Indian diet is due to a high phytate and low ascorbic acid content, which contributes to an increased risk of deficiency of micronutrients [2].

India is a country of varied ethnicity and religions, where food consumption patterns are greatly varied regionally, culturally and as regards family income. The production of agricultural food products in the defined region also influences the dietary pattern of the Indian population. Furthermore, nutrition during pregnancy is also affected by pregnancy-related complications such as nausea, vomiting, etc., which in turn results in highly diverse nutrition intake patterns. All these may result in single or multiple micronutrient deficiencies in the antenatal and/or postnatal period that have devastating effects on social and economic development of communities [3]. Micronutrient deficiencies such as iron (Fe) deficiency, vitamin A and iodine deficiency are the major nutritional deficiencies in India [4]; however, evidence of zinc (Zn) and magnesium deficiency (MgDef) prevalent in Indian pregnant women cannot be ignored [5]. The deleterious effects of such micronutrients alone or in combination with other etiological factors may result in complications in pregnancy and low birthweight (LBW) infants [1]. In order to avert such complications, addressing the magnitude of micronutrient deficiencies, assessing the nutritive value to food intake and its dietary pattern, food-based dietary diversification and food fortification are of prime importance [6]. Therefore, the present study aimed to assess the dietary intake pattern and serum micronutrient profile of pregnant women during gestation and their association with LBW.

## Materials and methods

### Subjects and study design

Pregnant 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, New Delhi during the period of January, 2011–December, 2013. Both oral and written consent was obtained from each participant. All the enrolled participants were dewormed by giving a single dose of albendazole 400 mg to be consumed at night on the day of enrolment. The enrolled participants were asked to fast overnight and on the next morning a venous blood sample (4 mL) was drawn from the antecubital vein in a supine position followed by refreshments. Likewise, a second sample (3 months after the first sampling) and a third sample (6 weeks' postpartum) were obtained in a similar manner. The inclusion criteria included: primigravida status of age 19–30 years; having a hemoglobin range 8.0–13.0 g/dL; a body mass index (BMI) in the range from 18 to 22 and belonging

to a middle socio-economic status according to Kuppuswami's scale for socio-economic status [7]. Briefly, the socio-economic status was described by considering the education score on the scale of 1–7 (illiterate as 1 and honors as 7), occupation score on the scale of 1–6 (1 = unemployed and 6 = semi-profession) and 10 as profession, family income per month 1–4 (1 ≤ Rs. 979 and 4 = Rs. 4894–7322), 6 = Rs. 7323–9787, 10 = Rs. 9788–19,574 and 12 = ≥ Rs. 19,575. A score between 11 and 25 was considered as being in the middle socio-economic bracket and the subject was eligible for recruitment. Exclusion criteria included a 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 Fe preparation in past 3 months. Low birthweight is defined as the weight of an infant at birth less than 2500 g [8].

### Ethical considerations

Ethical approval (IEC/NP-339/2010) for the study was obtained from the Institutional Ethical Committee of AIIMS, New Delhi, India. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

### Assessment of dietary intake

The assessment of diet during pregnancy was done using the 3 days 24-h dietary recall (24-HDR) method. This method was administered at each visit of the pregnant women. Dietary records were filled in by an experienced research officer of the Department of Obstetrics and Gynaecology and nutrient intakes per day in terms of raw quantity was calculated by the chief dietician. The detailed information about the meal patterns, time of consumption, ingredients used in the preparation of food, quantity of ingredients used, food intake, etc. were recorded for the preceding 3 days. A pre-determined preferred method of measurement for each food item or ingredients, i.e. using calibrated household utensils of varying volumes was used to assess the final volume of dishes and quantities consumed by the pregnant women. The nutrient composition of food items was calculated using standard food conversion tables for the ingredients to calculate the amount of Fe, calories, protein, fat, vitamin A and vitamin C from the diet [9]. The recommended dietary allowance (RDA) values for pregnant Indian women were considered according to the Indian Council of Medical Research were used to assess the adequacy of the nutrient intake [10]. According to National Iron Folic Acid (IFA) Supplementation Program to supplement daily IFA, all the enrolled pregnant women were prescribed nutritional supplement in the form of IFA tablets for 100 days during pregnancy and 100 days in the postpartum period.

For pregnant Indian women, the RDA as described by the National Institute of Nutrition (NIN), 2010 was considered in the study and the values below the RDA was described as (energy: 2580 kcal/day; protein: 82.2 g/day; fat: 30 g/day; Fe: 35 mg/day; vitamin A: 800 µg/day; vitamin C: 60 mg/day).

## Sample collection

Four milliliters of venous blood was taken from each participant and the 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  $-80^{\circ}\text{C}$  till further analysis.

## Analytical estimation

The serum was analyzed for Fe, copper (Cu), Zn and magnesium (Mg) 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 the application module: digestion time (5 min), sample volume (1 mL), and [4 mL Suprapur nitric acid ( $\text{HNO}_3$ ) (65%) and 1 mL hydrogen peroxide (20%) (Merck Chemicals, Pune, India)].

The digested serum samples were subjected for the analysis of Fe (259.940 nm), Cu (324.754 nm), Zn (213.856 nm) and Mg (279.553 nm) to ICP-AES. An ICP grade multi-element standard solution containing 1000 mg/L of 23 elements in 1 mol/L  $\text{HNO}_3$  (Product no. 1. 11355.0100, Batch no. HC081563, Merck Chemicals, Germany) was used as a reference standard. Pooled serum samples were used as biological material for the purpose of internal quality control.

Instrument parameters operating condition was power (1200 watt), 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). A wavelength was selected from a pre-defined 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 stepwise with 5% Suprapur  $\text{HNO}_3$  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.

The deficiency of micronutrients was considered when its serum levels were recorded below the reported values (Fe: 44.00/30.00/72.00  $\mu\text{g/dL}$  @ 2<sup>nd</sup>/3<sup>rd</sup> trimester/6 weeks post-partum; Cu: 63.69  $\mu\text{g/dL}$ ; Zn: 90.19  $\mu\text{g/dL}$  and Mg: 1500  $\mu\text{g/dL}$ ). The hemoglobin levels were recorded from hospital records and apart from three visits, pre-delivery hemoglobin levels were also taken into the analysis.

## Statistical analysis

Data were analyzed using STATA® software (Version 14.1) and the results were expressed as mean  $\pm$  standard deviation (SD). A chi square ( $\chi^2$ ) test was applied to test for any significant change in the pattern of dietary intake and serum micronutrients levels of pregnant women who were consuming a diet below the RDA or revealed any micronutrient deficiency. A table ( $3 \times 2$ ) was generated in which Pearson's  $\chi^2$  value with degree of freedom, total number of observations and level of significance was computed.

A logistic regression model was applied to analyze the likelihood of LBW in relation to the nutritional deficit diet and micronutrient

deficiencies. The single composite variable was created from the variable studied at three time-points (i.e. baseline, after 3 months and 6 weeks post-partum) to check the propensity of deficient micronutrient levels and deficient dietary intake during pregnancy and postpartum period and its association with LBW. Differences were considered to be statistically significant at an error probability of less than 0.05 ( $P < 0.05$ ).

## Results

Table 1 shows the characteristics of the study participants and delivery notes. The mean age of the pregnant women was 23.1 years. The weight and BMI of the pregnant women progressively increased till delivery. The delivery note showed a mean Apgar score at 1 and 5 min were 8.3 and 9.0; the head, chest and crown rum circumferences were 33, 32.2 and 48.8 cm, respectively. The mean infant weight recorded was 2813.3 g with a prevalence of 22.1% LBW and 10.8% preterm infants (Table 1). The nutrient dietary intake pattern of pregnant women according to the RDA is shown in Table 2. At the time of enrolment (second trimester), more than 90% of the pregnant women were consuming a diet below the RDA for energy, protein and vitamin A. Dietary intake below the RDA for fat and vitamin C was seen only in 10.5% and 34.2% of the pregnant women.

**Table 1:** Characteristics of the study participants and delivery notes.

Variables	Mean $\pm$ SD
Age, years	23.1 $\pm$ 2.7
Gestation age, days	269 $\pm$ 26.9
Weight at enrolment, kg	52.7 $\pm$ 12.2
Weight at third trimester, kg	58.2 $\pm$ 24.2
Weight at 6 weeks postpartum, kg	56.2 $\pm$ 10.9
BMI at enrolment, $\text{kg/m}^2$	21.6 $\pm$ 3.1
BMI at third trimester, $\text{kg/m}^2$	23.4 $\pm$ 4.7
BMI at 6 weeks postpartum, $\text{kg/m}^2$	23.0 $\pm$ 3.6
Apgar score	
1 min	8.3 $\pm$ 0.9
5 min	9.0 $\pm$ 0.3
Head circumference, cm	33.0 $\pm$ 7.6
Chest circumference, cm	32.2 $\pm$ 8.0
Crown rum length, cm	48.8 $\pm$ 8.1
Infant weight, g	2813.3 $\pm$ 515.6
	<b>Percentage</b>
Preterm infants	10.8%
Low birthweight	22.1%
Live birth	99.4%
Congenital anomaly	1.6%
Infant admission to NICU	6.7%

BMI, body mass index; NICU, neonatal intensive care unit.

**Table 2:** Trend of dietary nutrient intake during pregnancy and postpartum and categorization of pregnant women according to the recommended dietary allowance (RDA) in India.

Variables <sup>a,b</sup>	Mean $\pm$ SD	Below RDA <sup>c</sup>	Chi <sup>2</sup> test $\chi^2$
		n/d (%)	$\chi^2$ , P-value
Energy_1	1923.89 $\pm$ 518.36	342/380 (90.00)	77.72, 0.0001 <sup>d</sup>
Energy_2	2271.82 $\pm$ 528.10	224/306 (73.20)	
Energy_3	2456.66 $\pm$ 555.17	153/254 (60.24)	
Protein_1	59.58 $\pm$ 17.42	342/380 (90.00)	88.32, 0.0001 <sup>d</sup>
Protein_2	70.67 $\pm$ 17.96	233/306 (76.14)	
Protein_3	80.52 $\pm$ 22.06	147/254 (57.87)	
Fat_1	54.57 $\pm$ 21.67	40/380 (10.53)	30.47, 0.0001 <sup>d</sup>
Fat_2	69.65 $\pm$ 29.04	7/306 (2.29)	
Fat_3	84.02 $\pm$ 31.65	5/254 (1.97)	
Iron_1	18.26 $\pm$ 7.92	375/380 (98.68)	3.16, 0.177
Iron_2	19.52 $\pm$ 5.95	299/306 (97.71)	
Iron_3	19.75 $\pm$ 6.70	245/254 (96.46)	
Vitamin A_1	367.63 $\pm$ 566.49	347/379 (91.56)	14.83, 0.001 <sup>e</sup>
Vitamin A_2	493.14 $\pm$ 485.05	261/300 (87.00)	
Vitamin A_3	376.31 $\pm$ 172.88	111/112 (99.11)	
Vitamin C_1	105.43 $\pm$ 80.89	130/380 (34.21)	24.87, 0.0001 <sup>d</sup>
Vitamin C_2	104.34 $\pm$ 77.68	104/306 (33.99)	
Vitamin C_3	73.82 $\pm$ 59.09	132/254 (51.97)	

<sup>a</sup>Variables\_1/2/3: second trimester/third trimester/6 weeks postpartum. <sup>b</sup>Unit of measurement for energy, kcal/day; protein, g/day; fat, g/day; iron, mg/day; vitamin A,  $\mu$ g/day; vitamin C, mg/day. <sup>c</sup>Energy < RDA, energy intake < 2580 kcal/day; protein < RDA, protein intake < 82.2 g/day; Fat < RDA, fat intake < 30 g/day; iron < RDA, iron intake < 35 mg/day; vitamin A < RDA, vitamin A intake < 800  $\mu$ g/day; vitamin C < RDA, vitamin C intake < 60 mg/day. <sup>d</sup>P  $\leq$  0.05 considered statistically significant. <sup>e</sup>Fisher's exact test. SD, standard deviation; n, number of observations; n/d, numerator/denominator; %, percentage;  $\chi^2$ , chi square value; P-value, level of significance.

Dietary Fe intake below the RDA was seen in >96% of the pregnant women throughout the study period. The percentage of pregnant women consuming nutrients below the RDA was found to be reduced with the progression of pregnancy and postpartum period except for vitamin A intake, where nearly 100% women were consuming a vitamin A-deficient diet at postpartum. The results showed alterations in the dietary intake pattern which was evident from the significant changes in the dietary intake of energy ( $\chi^2=77.72$ ; P=0.0001), protein ( $\chi^2=88.32$ ; P=0.0001), fat ( $\chi^2=30.47$ ; P=0.0001), vitamin A ( $\chi^2=14.83$ ; P=0.001) and vitamin C ( $\chi^2=24.87$ ; P=0.0001) except for dietary Fe intake ( $\chi^2=3.16$ ; P=0.177) which was not altered throughout the pregnancy and postpartum period (Table 2).

Serum micronutrient levels revealed the prevalence of Zn and Mg deficiency in ~55% and 18% pregnant women at enrolment (second trimester), which aggravated to 67% and 33% after 3 months (third trimester) and 54% and

43% at postpartum, respectively. The results revealed that there was a significant change in the frequency distribution of serum Zn ( $\chi^2=7.33$ ; P=0.026) and Mg ( $\chi^2=22.93$ ; P=0.0001) deficient pregnant women during pregnancy and postpartum period. At enrolment (second trimester), prevalence of anemia (hemoglobin deficiency) was observed in 33% of the pregnant women, which was increased to 56% after 3 months (third trimester), followed by 51% at pre-delivery and ~93% during the postpartum period. The change in the status of anemia in pregnant women was found to be significant at different time-points during pregnancy and postpartum ( $\chi^2=270.57$ ; P=0.0001). Although the pregnant women in this study were found to be Fe and Cu sufficient the serum levels of Fe ( $\chi^2=58.63$ ; P=0.0001) fluctuated significantly during pregnancy and the postpartum period (Table 3).

Table 4 shows that the pregnant women having Mg deficiency at second trimester had a >4 times higher chance of delivering an LBW baby (OR: 4.21, CI: 1.38–12.87; P=0.01);

**Table 3:** Serum levels of micronutrients, their deficiency and change in the pattern during pregnancy and postpartum.

Variables <sup>a,b</sup>	Mean $\pm$ SD	Deficient <sup>c</sup>	Chi <sup>2</sup> test
		n/d (%)	$\chi^2$ , P-value
S. Iron_1	140.25 $\pm$ 83.91	2/220 (0.91)	58.63, 0.0001 <sup>d</sup>
S. Iron_2	150.42 $\pm$ 115.53	0/162 (0.00)	
S. Iron_3	124.26 $\pm$ 63.25	16/91 (17.58)	
S. Cu_1	181.40 $\pm$ 43.81	1/220 (0.45)	1.15, 1.000
S. Cu_2	203.10 $\pm$ 49.02	0/162 (0.00)	
S. Cu_3	141.95 $\pm$ 40.03	0/91 (0.00)	
S. Zn_1	92.74 $\pm$ 46.65	119/218 (54.59)	7.33, 0.026 <sup>e</sup>
S. Zn_2	100.85 $\pm$ 96.08	109/162 (67.28)	
S. Zn_3	91.52 $\pm$ 21.65	49/91 (53.85)	
S. Mg_1	1846.33 $\pm$ 648.57	40/220 (18.18)	22.93, 0.0001 <sup>e</sup>
S. Mg_2	1857.61 $\pm$ 885.49	53/161 (32.92)	
S. Mg_3	1718.09 $\pm$ 818.79	39/90 (43.33)	
Hb_1	11.07 $\pm$ 1.22	228/690 (33.04)	270.57, 0.0001 <sup>e</sup>
Hb_2	10.79 $\pm$ 1.11	323/579 (55.79)	
Hb_pre-del.	10.94 $\pm$ 1.22	159/309 (51.46)	
Hb_3	10.19 $\pm$ 0.94	231/248 (93.15)	

<sup>a</sup>Variables\_1/2/Pre-del./3, second trimester/third trimester/pre-delivery/6 weeks postpartum. <sup>b</sup>Unit of measurement for serum iron (S. Iron),  $\mu$ g/dL; serum copper (S. Cu),  $\mu$ g/dL; serum zinc (S. Zn),  $\mu$ g/dL; serum magnesium (S. Mg),  $\mu$ g/dL; hemoglobin (Hb), g/dL. <sup>c</sup>S. Iron deficiency\_1/2/3, serum iron levels < 44.00/30.00/72.00  $\mu$ g/dL; S. Cu deficiency, serum copper levels < 63.69  $\mu$ g/dL; S. Zn deficiency, serum zinc levels < 90.19  $\mu$ g/dL; S. Mg deficiency, serum magnesium levels < 1500  $\mu$ g/dL; Anemia\_1/2/pre-del./3, Hb levels < 10.5 g/dL/11.0 g/dL/11.0 g/dL/12.0 g/dL. <sup>d</sup>Fisher's exact test. <sup>e</sup>P  $\leq$  0.05 considered statistically significant. SD, standard deviation; n, number of observations; n/d, numerator/denominator; %, percentage;  $\chi^2$ , chi square value; P-value, probability.



**Table 4:** Likelihood of low birthweight infant associated with micronutrient deficiency and dietary intake below the recommended dietary allowance (RDA) during pregnancy and postpartum period.

Nutrient deficiency <sup>a,b</sup>	n	Odds ratio	95% CI	P-value
Zn deficiency_1	137	0.91	0.40–2.09	0.83
Zn deficiency_2	113	1.26	0.42–3.79	0.68
Zn deficiency_3	56	1.00	0.29–3.39	1.00
Mg deficiency_1	137	4.21	1.38–12.87	0.01 <sup>c</sup>
Mg deficiency_2	113	3.74	1.38–10.15	0.01 <sup>c</sup>
Mg deficiency_3	55	2.33	0.65–8.33	0.19
Anemia_1	298	0.88	0.47–1.64	0.68
Anemia_2	288	0.58	0.33–1.02	0.06
Anemia_pre-del.	308	0.57	0.33–0.99	0.04 <sup>c</sup>
Anemia_3	247	0.37	0.13–1.02	0.05 <sup>c</sup>
Energy < RDA_1	156	0.56	0.17–1.81	0.33
Energy < RDA_2	160	0.85	0.39–1.87	0.68
Energy < RDA_3	164	0.57	0.29–1.14	0.11
Protein < RDA_1	156	0.81	0.24–2.80	0.74
Protein < RDA_2	160	1.27	0.53–3.07	0.59
Protein < RDA_3	164	0.76	0.38–1.51	0.43
Vitamin A < RDA_1	155	0.70	0.28–1.78	0.46
Vitamin A < RDA_2	156	3.78	1.08–13.20	0.04 <sup>c</sup>
Vitamin A < RDA_3 <sup>d</sup>	–	–	–	–
Vitamin C < RDA_1	156	0.43	0.19–0.99	0.05 <sup>b</sup>
Vitamin C < RDA_2	160	1.48	0.71–3.08	0.30
Vitamin C < RDA_3	164	0.87	0.44–1.73	0.69

<sup>a</sup>Variables\_1/2/pre-del./3, second trimester/third trimester/pre-delivery/6 weeks postpartum. <sup>b</sup>Zinc deficiency (Zn deficiency), serum Zn levels < 90.19 µg/dL; magnesium deficiency (Mg deficiency), serum Mg levels < 1500 µg/dL; Anemia\_1/2/pre-del./3, Hb levels < 10.5 g/dL/11.0 g/dL/11.0 g/dL/12.0 g/dL; energy < RDA, energy intake < 2580 kcal/day; protein < RDA, protein intake < 82.2 g/day; vitamin A < RDA, vitamin A intake < 800 µg/day; vitamin C < RDA, vitamin C intake < 60 mg/day. <sup>c</sup>P ≤ 0.05 considered statistically significant. <sup>d</sup>Omitted due to collinearity effect. CI, confidence interval; n, number of observations; P-value, probability.

however, third trimester Mg deficiency was found to be associated with relatively less likelihood of delivering an LBW baby (OR: 3.7, CI: 1.38–10.15; P = 0.01) compared with second trimester Mg deficiency. Anemia, characterized by low hemoglobin levels, was also found to be significantly associated with LBW. Pre-delivery and postpartum hemoglobin levels (OR: 0.37, CI: 0.13–1.02; P = 0.05) were significantly associated with LBW; out of these, hemoglobin deficiency prior to delivery had more chances of delivery of an LBW baby (OR: 0.57, CI: 0.33–0.99; P = 0.04). Further, vitamin A-deficient dietary intake during the third trimester of pregnancy was also significantly associated with LBW (OR: 3.78, CI: 1.08–13.20; P = 0.04). Pregnant women whose dietary intake of vitamin A during the third trimester was below the RDA had a ~4 times higher risk of delivering an LBW fetus (Table 4).

Pregnant women who were Mg deficient at any one time-point of the study showed less chances of LBW (OR: 1.09, CI: 0.33–3.58; P = 0.89). In contrast, the chances of delivering an LBW fetus was found to be proportionately and significantly increased in Mg-deficient pregnant women when the deficiency persisted at any two visits (OR: 4.08, CI: 1.13–14.75; P = 0.03) or all three visits (OR: 7.34, CI: 1.15–46.98; P = 0.04). Similarly, Zn deficiency at any one time-point was associated with lesser chances of delivering an LBW baby (OR: 0.63, CI: 0.23–1.77; P = 0.38) compared to those who were Zn deficient at all the three visits (OR: 2.20, CI: 0.47–10.30; P = 0.32), although the association was non-significant. Further, anemia was also found to be significantly associated with the risk of LBW, but the likelihood was non-significantly higher when anemia persisted at any two time-points of the studied periods (OR: 0.46, CI: 0.21–1.03; P = 0.06). The association between anemia at any one time-point and LBW was found to be significant (OR: 0.43, CI: 0.20–0.96; P = 0.04), followed by the likelihood of LBW when anemia was observed at all the studied time-points (OR: 0.31, CI: 0.11–0.87; P = 0.03) (Table 5).

Similarly, the increased propensity of deficient dietary intake during gestation resulted in an increased risk of LBW, although the risk was statistically non-significant except for vitamin A intake. The likelihood of LBW delivery was 4.46 times higher in pregnant women who were taking dietary vitamin A below the RDA at any time-point of study (OR: 4.46, CI: 0.56–35.40; P = 0.16). The risk of LBW delivery was 10-fold more when deficient vitamin A intake was observed at all the time-points of the study (OR: 10.00, CI: 1.00–102.87; P = 0.05) (Table 5). Discussion Nutritious dietary intake during pregnancy plays a vital role in the development and growth trajectory of the fetus. The requirements of energy and other dietary nutrients increased with the progression of pregnancy to fulfil the enhanced maternal metabolic demands, expansion of plasma volume, red cell mass and the delivery of nutrients to the fetus [11]. These enhanced requirements may result in micronutrient deficiencies, which remain a major public health concern mostly in developing countries [12]. In this study, a significant percentage of pregnant women showed dietary intake of energy below the RDA and dietary fat intake above the RDA, which corroborates with the interpretation of earlier published meta-analysis [13]. Dietary intake of Fe ~43%–47% lower than the RDA was observed in the pregnant women enrolled in the present study. An Indian study has also reported the intake of dietary Fe less than 50% of the RDA [2] which supports the current estimates of dietary Fe intake in our study. The dietary Fe intake below the RDA throughout the gestation

**Table 5:** Likelihood of low birthweight with respect to the propensity of micronutrient deficiencies and less nutritive diet during pregnancy and postpartum period.

Nutrient deficiency <sup>a</sup>	n	Odds ratio	95% CI	P-value
Mg deficiency				
At any time-point	150	1.09	0.33–3.58	0.89
At any two visits	150	4.08	1.13–14.75	0.03 <sup>b</sup>
At all three visits	150	7.34	1.15–46.98	0.04 <sup>b</sup>
Zn deficiency				
At any time-point	150	0.63	0.23–1.77	0.38
At any two visits	150	0.48	0.15–1.50	0.21
At all three visits	150	2.20	0.47–10.30	0.32
Anemia				
At any time-point	316	0.43	0.20–0.96	0.04 <sup>b</sup>
At any two visits	316	0.46	0.21–1.03	0.06
At all three visits	316	0.31	0.11–0.87	0.03 <sup>a</sup>
Energy intake < RDA				
At any time-point	245	0.48	0.21–1.13	0.09
At any two visits	245	0.71	0.30–1.71	0.45
At all three visits	245	0.64	0.23–1.79	0.39
Protein intake < RDA				
At any time-point	245	0.43	0.18–1.04	0.06
At any two visits	245	0.79	0.33–1.92	0.61
At all three visits	245	0.88	0.32–2.42	0.81
Iron intake < RDA				
At any time-point	245	1.70	0.19–15.01	0.63
At any two visits	245	1.89	0.21–16.80	0.57
At all three visits	245	2.56	0.29–22.44	0.40
Vitamin A < RDA				
At any time-point	218	4.46	0.56–35.40	0.16
At any two visits	218	5.88	0.73–47.62	0.10
At all three visits	218	10.00	1.00–102.87	0.05 <sup>a</sup>
Vitamin C < RDA				
At any time-point	245	0.97	0.51–1.85	0.93
At any two visits	245	0.95	0.40–2.26	0.91
At all three visits	245	0.99	0.19–5.18	0.99

<sup>a</sup>Magnesium deficiency (Mg deficiency), serum Mg levels < 1500 µg/dL; zinc deficiency (Zn deficiency), serum Zn levels < 90.19 µg/dL; anemia\_1/2/Pre-del./3, Hb levels < 10.5 g/dL/11.0 g/dL/11.0 g/dL/12.0 g/dL; energy intake < RDA, energy intake < 2580 kcal/day; protein intake < RDA, protein intake < 82.2 g/day; iron intake < RDA, iron intake < 35 mg/day; vitamin A intake < RDA, vitamin A intake < 800 µg/day; vitamin C intake < RDA, vitamin C intake < 60 mg/day. <sup>b</sup>P ≤ 0.05 considered statistically significant. CI, confidence interval; n, number of observations; P-value, probability.

and postpartum period signifies the importance of exogenous Fe supplement to meet the increased Fe demands of the developing fetus. The prevalent micronutrient deficiencies in India, such as Fe and vitamin A deficiency [14], might be the cause of dietary inadequacy of such micronutrients during pregnancy, which later accumulates in childhood and adult life.

There are sufficient reports on nutritional recommendations and dietary intake pattern during pregnancy

and lactation [15, 16]. Some of them explained the maternal adaptations, macro- and micronutrient requirements during pregnancy; others have summarized the advocacy to promote a balanced nutritious diet that is usually lacking in regular pregnancy. The current study not only assessed the dietary intake pattern of pregnant women and the prevalence of micronutrient deficiencies, but also monitored the fluctuations in their dietary nutrient intake and micronutrient levels during pregnancy/postpartum period, and interpolated them into the risk of an LBW infant. Although the dietary intake and level of Fe, Zn and vitamin A in the blood have been assessed in Burkina Faso, they challenged the bio-fortification and food-based intervention strategies as no association whatsoever was observed between the dietary intake and biological status of studied micronutrients [17]. We observed the Zn and Mg deficiency in pregnant women whereas serum Fe and Cu levels were well maintained despite having the dietary Fe intake below the RDA, thereby coinciding with the results of Martin-Prevel [17]. The optimum levels of the observed serum Fe might be the compensatory effect of oral Fe supplement routinely consumed by pregnant women to sustain their increasing Fe demands during pregnancy. In the studied cohort, the prevalence of Mg deficiency nearly doubled during the third trimester and was further 2.4-fold higher at 6 weeks postpartum. This shows that more and more pregnant women were becoming deficient in serum Mg levels as pregnancy progressed. It was supported by the literature in which Mg levels were lower in mid and late gestation as compared to early pregnancy due to mineral requirements by growing fetus, hemodilution, renal clearance and partly due to estrogen activity [16]. While inspecting the mean serum Mg levels reported in our study, Mg levels at the end of the second trimester and initiation of the third trimester were comparable to the results of Pathak et al. [18], although they reported a higher prevalence of third trimester Mg deficiency. The reason for the varying prevalence is due to the difference in considering reference values of serum Mg for defining Mg deficiency (1.80 mg/dL in their case), unless a similar prevalence is expected from the earlier study as well. Anemia during pregnancy is frequently observed and widely studied. In our study also, the prevalence of anemia increased with the progression of pregnancy and peaked at the postpartum period.

A low protein diet during the third trimester is non-significantly associated with an increased likelihood of LBW (OR: 1.27, CI: 0.53–3.07; P = 0.59) as suggested by the experimental model of food restriction and low protein diets that resulted in LBW and intrauterine growth retardation via the mechanism of placental insufficiency [1].

LBW is a proxy marker to depict the maternal undernutrition status; therefore, we studied maternal nutrition using a 24-h dietary recall, assessed the micronutrient deficiencies, if any, and its impact on infant birthweight. Though it is very difficult to predict the optimal maternal dietary intake for optimal birthweight, still an attempt was made to examine the maternal dietary intake and their serum micronutrient status on the risk of delivering an LBW infant. In the present study, Mg deficiency during the second (OR: 4.21, CI: 1.38–12.87;  $P=0.01$ ) and third trimesters (OR: 3.7, CI: 1.38–10.15;  $P=0.01$ ) was significantly associated with a high risk of LBW which is in support of previous research that reported the association of Mg deficiency with the incidence of LBW and small-for-gestational-age infants [19]. The likelihood of LBW increased dramatically when Mg deficiency persisted at all the studied intervals (OR: 7.34, CI: 1.15–46.98;  $P=0.04$ ) as compared to any one (OR: 1.09, CI: 0.33–3.58;  $P=0.89$ ) or two time-points (OR: 4.08, CI: 1.13–14.75;  $P=0.03$ ) of the study. There is no such information available about the propensity of Mg deficiency in relation to LBW; therefore, the magnitude of the problem is under-estimated.

The prevalence of anemia at all the studied intervals was significantly associated with the increased likelihood of LBW (OR: 0.31, CI: 0.1–0.87;  $P=0.03$ ); besides, even when anemia persisted at any time-point during pregnancy or postpartum, it also increased the risk in a significant manner (OR: 0.43, CI: 0.20–0.96;  $P=0.04$ ). A similar observation was reported from a prospective cohort study carried out in Maharashtra where maternal anemia during early-mid second trimester was associated with a significantly but lower risk of LBW (unadjusted OR: 0.34, 95% CI: 0.13–0.92;  $P=0.03$ ) [20].

Dietary intake of vitamin A is critical for fetal development because of its involvement in growth, vision, protein synthesis and cell differentiation. A significant association of an inadequate vitamin A diet with LBW (OR: 3.78, CI: 1.08–13.20;  $P=0.04$ ) was similar to the preliminary observations of Tu et al. [21] in which they observed that ~40% women had inadequate intake of Fe, Zn, vitamin A, folate and vitamin B, and about 10% women have had LBW babies [21]. On the other hand, vitamin A dietary intake was estimated to be ~38%–54% lower than the RDA for pregnant women. Therefore, an effective strategy and design should be operationalized for the screening and treatment of vitamin A deficiency during pregnancy [22]. While assessing the impact of inadequate vitamin A intake on LBW, the log likelihood increased proportionately to the persistence of deficient dietary intake (OR: 10.00, CI: 0.97–102.87;  $P=0.05$ ). Despite the non-appearance of clinical manifestation

of vitamin A deficiency in the present population, its persistence predicts a likelihood of LBW and the data revealed that vitamin A-deficient diet is an early predictor of LBW.

The prevalence of Zn deficiency in the present study (67.3% at the third trimester) is well in accordance with the report of Pathak et al. [23] who showed ~65% Zn deficiency during the third trimester of pregnancy with a mean serum Zn level of  $61.1 \pm 16.6$   $\mu\text{g/dL}$ . Mean Zn levels were, however, considerably lower than the present study; indeed, the mean Zn levels in our study corroborate with the other studies [24, 25]. Zn deficiency is associated with pregnancy-related complications, fetal growth retardation, congenital abnormalities and retarded neurobehavioral and immunological development [26]. We predicted the risk of delivering an LBW infant in relation to Zn deficiency during pregnancy, and found that Zn deficiency if persistent throughout the gestation/postpartum may increase the chances of LBW (OR: 2.20, CI: 0.47–10.30;  $P=0.32$ ) in a non-significant manner.

Due to the highly interrelated nature of dietary nutrients, the specific effect of a single nutrient on birthweight is not easy to assess; however, the current longitudinal study comprised age, primi and gravidity status-matched pregnant women, belonging to middle socio-economic status to minimize the caveats associated with nutrition studies. In addition, assessing the impact of some additional micronutrients such as folate, iodine and vitamin B12 on LBW could help in improving our understanding of the impact of such micronutrient deficiencies on LBW. Nutrition research also has this demerit as there is no comprehensive model available, except regression/correlation, that can clearly explain the cohesion between dietary intake, micronutrient status and LBW.

## Conclusion

The dietary pattern in pregnant women revealed very low intake of Fe, vitamin A along with a low caloric and proteinaceous diet. A high prevalence of Zn and Mg deficiencies with the compounding effect of anemia are strongly associated with LBW. The results of the study reflect the micronutrient deficiency profile of the studied pregnant women and the obtained results will be beneficial for the authorities to take appropriate steps in the direction of enhancing the nutritive value of food by fortification, creating awareness and implementing supplementation schemes in India.

**Acknowledgments:** The authors are highly thankful to Prof. Y.K. Gupta and Dr. Amita Srivastava for helping in carrying out serum micronutrient level estimations. The authors also wish to acknowledge the continuous support provided by Dr. Rohini Sehgal, Mrs. Leema Maithi and Mrs. Shobha Kandpal for the enrolment and follow-up of patients.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** This study was funded by the Indian Council of Medical Research, New Delhi, India (grant no. 5/7/165/06-RHN, Funder Id: <http://dx.doi.org/10.13039/501100001411>).

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

## References

- Grieger J, Clifton V. A review of the impact of dietary intakes in human pregnancy on infant birthweight. *Nutrients* 2014;7: 153–78.
- Nair KM, Iyengar V. Iron content, bioavailability & factors affecting iron status of Indians. *Indian J Med Res* 2009;130: 634–45.
- Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, et al. Maternal and child undernutrition: consequences for adult health and human capital. *Lancet* 2008;371:340–57.
- Laxmaiah A, Arlappa N, Balakrishna N, Mallikarjuna Rao K, Galreddy C, Kumar S, et al. Prevalence and determinants of micronutrient deficiencies among rural children of eight states in India. *Ann Nutr Metab* 2013;62:231–41.
- Kapil U, Pathak P, Singh P, Singh C. Zinc and magnesium nutriture amongst pregnant mothers of urban slum communities in Delhi: a pilot study. *Indian Pediatr* 2002;39:365–8.
- Hotz C, McClafferty B. From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status. *Food Nutr Bull* 2007; 28(2 Suppl):S271–9.
- Kumar N, Gupta N, Kishor J. Kuppaswamy's socioeconomic scale: updating income ranges for the year 2012. *Indian J Public Health* 2012;56:103–4.
- World Health Organization. ICD-10: International Statistical Classification of Diseases and Related Health Problems: tenth revision. 2nd ed. Geneva, Switzerland: World Health Organization. <http://www.who.int/iris/handle/10665/42980>.
- Gopalan C, Rama Sastri B, Balasubramanian S. Nutritive value of Indian foods. In: Narasinga Rao BS, Deosthale YG, Pant KC, editors. Revised & updated. New Delhi: National Institute of Nutrition-Indian Council of Medical Research Press; 2011.
- NIN. Nutrient requirements and recommended dietary allowances for Indians. Hyderabad, India: National Institute of Nutrition (ICMR); 2010. <http://www.icmr.nic.in/final/rda-2010.pdf>. Assessed 10 January 2018.
- Kaiser LL, Allen L, American Dietetic Association. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. *J Am Diet Assoc* 2002;102:1479–90.
- Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet* 2013;382:427–51.
- Blumfield ML, Hure AJ, Macdonald-Wicks L, Smith R, Collins CE. Systematic review and meta-analysis of energy and macronutrient intakes during pregnancy in developed countries. *Nutr Rev* 2012;70:322–36.
- Vijayaraghavan K. Control of micronutrient deficiencies in India: obstacles and strategies. *Nutr Rev* 2002;60(5 Pt 2):S73–6.
- Kominiarek MA, Rajan P. Nutrition recommendations in pregnancy and lactation. *Med Clin North Am* 2016;100:1199–215.
- Liu F-L, Zhang Y-M, Parés GV, Reidy KC, Zhao W-Z, Zhao A, et al. Nutrient intakes of pregnant women and their associated factors in eight cities of China: a cross-sectional study. *Chin Med J (Engl)* 2015;128:1778–86.
- Martin-Prevel Y, Allemand P, Nikiema L, Ayassou KA, Ouedraogo HG, Moursi M, et al. Biological status and dietary intakes of iron, zinc and vitamin A among women and preschool children in rural Burkina Faso. *PLoS One* 2016;11:e0146810.
- Pathak P, Kapoor SK, Kapil U, Dwivedi SN. Serum magnesium level among pregnant women in a rural community of Haryana State, India. *Eur J Clin Nutr* 2003;57:1504–6.
- Almonte RA, Heath DL, Whitehall J, Russell MJ, Patole S, Vink R. Gestational magnesium deficiency is deleterious to fetal outcome. *Neonatology* 1999;76:26–32.
- Ahankari AS, Myles PR, Dixit JV, Tata LJ, Fogarty AW. Risk factors for maternal anaemia and low birth weight in pregnant women living in rural India: a prospective cohort study. *Public Health* 2017;151:63–73.
- Tu N, King JC, Dirren H, Thu HN, Ngoc QP, Diep ANT. Effect of animal-source food supplement prior to and during pregnancy on birthweight and prematurity in rural Vietnam: a brief study description. *Food Nutr Bull* 2014;35(4 Suppl):S205–8.
- Singh P. Micronutrient deficiency in India. *J Indian Soc Agric Stat* 2007;61:128–31.
- Pathak P, Kapil U, Dwivedi SN, Singh R. Serum zinc levels amongst pregnant women in a rural block of Haryana state, India. *Asia Pac J Clin Nutr* 2008;17:276–9.
- Jain S, Sharma P, Kulshreshtha S, Mohan G, Singh S. The role of calcium, magnesium, and zinc in pre-eclampsia. *Biol Trace Elem Res* 2010;133:162–70.
- Jeswani RM, Vani SN. A study of serum zinc levels in cord blood of neonates and their mothers. *Indian J Pediatr* 1991;58:683–6.
- Black RE. Micronutrients in pregnancy. *Br J Nutr* 2001;85 (Suppl 2):S193–7.