Indian J Med Res 156, August 2022, pp 339-347
DOI: 10.4103/ijmr.ijmr_3130_21

Quick Response Code:

Assessment of zinc inadequacy among tribal adolescent population of central India - A cross-sectional study

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Received October 25, 2021

***Background & objectives*: Zinc is a crucial micronutrient in adolescence, required for promoting growth and sexual maturation. Adolescents of some tribes may be at high risk of zinc deficiency due to dietary inadequacy and poor bioavailability of zinc from plant-based diets. This study aimed to evaluate the risk of zinc deficiency by estimating prevalence of inadequate zinc intake, prevalence of low serum zinc and stunting among tribal adolescents.**

***Methods*: A cross-sectional community-based survey was conducted among adolescents (10-19 yr) in three purposively selected districts where *Bhil*, *Korku* and *Gond* tribes were in majority. Structured data collection instrument comprising information about sociodemographic characteristics and dietary recall data was used. Anthropometric assessment was conducted by standardized weighing scales and anthropometry tapes, and blood sample was collected from antecubital vein into trace element-free vacutainers. Serum zinc was estimated using an atomic absorption spectrophotometer.**

***Results*: A total of 2310 households were approached for participation in the study, of which 2224 households having 5151 adolescents participated. Out of these enlisted adolescents, 4673 responded to dietary recall (90.7% response rate). Anthropometry of 2437 participants was carried out, and serum zinc was analyzed in 844 adolescents. The overall prevalence of dietary zinc inadequacy was 42.6 per cent [95% confidence interval (CI) 41.2 to 44.1] with reference to the estimated average requirement suggested by International Zinc Nutrition Consultative Group (IZiNCG) and 64.8 per cent (95% CI 63.4 to 66.2) with Indian Council of Medical Research-recommended requirements. Stunting was observed in 29 per cent (95% CI 27.2 to 30.8) participants. According to IZiNCG cut-offs, low serum zinc was detected in 57.5 per cent (95% CI 54.1 to 60.8) of adolescents, whereas it was 34.4 per cent (95% CI: 31.2-37.5) according to the national level cut-off.**

***Interpretation & conclusions*: Risk of dietary zinc inadequacy and low serum zinc concentration amongst adolescents of the *Gond*, *Bhil* and *Korku* tribes is a public health concern.**

Key words Estimated average requirement - Indian tribal adolescents - serum zinc - zinc inadequacy

Zinc is regarded as a growth nutrient and functions as an essential co-factor in over 300 enzymes in human beings. Detrimental effects on growth and functioning are seen even in mild zinc deficiency let alone moderate and severe deficiency[1]. Symptoms of zinc deficiency are non-specific unlike deficiency symptoms or signs of other micronutrients. Long-term deficiency of zinc results in growth impairment and faltering, which could be indicated by stunting and thinness. Joint statement of the World Health Organization (WHO), the United Nations Children's Fund, the International Atomic Energy Agency and the International Zinc Nutrition Consultative Group (IZiNCG) recommended three indicators for assessing population-level zinc deficiency[2]. These include proportion of population with dietary intake of zinc below estimated average requirement (EAR), prevalence of stunting among children below five years of age and prevalence of low serum zinc levels[2].

Various studies have highlighted the extent of zinc deficiency among children under five, and the role of zinc supplementation with a dose of 10 mg/day or more especially in low birth weight infants to reduce under-five mortality[3]. Adolescence is another crucial stage in growth and development, and therefore, zinc deficiency could be detrimental in such stage of life. During adolescence, higher prevalence of inadequate zinc intake[4] and low serum zinc (ranging from 30 to 98%) have been reported[5,6]. Studies among tribal adolescent and non-adolescent populations have also reported higher prevalence[7,8]. Tribal health is identified as one of the priority areas for health interventions in National Health Policy 2017. Malnutrition indicators (underweight, stunting and wasting) have consistently been reported higher than national averages in tribal areas. Madhya Pradesh (MP) is a State with the highest number of tribal population (21%) in India and *Bhil*, *Gond* and *Korku* are predominant tribes in some of the districts of this State. Zinc status or intakes in adolescents in these tribes are unknown. Therefore, this study was planned to estimate the magnitude of zinc inadequacy among adolescent boys and girls of three major tribes (*Gond*, *Bhil* and *Korku*) of MP.

Material & Methods

Design and settings: A community based cross-sectional survey was conducted in three purposively selected districts where *Bhil* (Jhabua), *Korku* (Betul) and *Gond* (Mandla) tribes are in majority constituting >90 per cent of district's population after procuring approval from the Institutional Human Ethics Committee of All India Institute of Medical Sciences, Bhopal. Data collection for this survey was carried out during December 2015 to April 2016 in Mandla (*Gond* tribe), May to August 2016 in Betul (*Korku* tribe) and October 2017 to February 2018 in Jhabua (*Bhil* tribe).

Participants: This study was conducted among tribal adolescent boys and girls between 10 and 19 years of age. All adolescents residing in the selected households were eligible for participation in the study. Adolescents who were admitted in hospital or were fasting for any reason or had not consumed meals at home on the previous day were excluded.

Sampling and sample size: The required sample size was calculated separately for each specific objective of the study by using formula:

$$n = [(Z^2_{1-\alpha/2} \times p \times [1-p])/(d^2)] \times \text{design effect.}$$

We estimated sample size for this investigation assuming 15 per cent relative error for 95 per cent confidence interval (CI) around expected prevalence of 31.2, 46.6 and 53 per cent, respectively, for inadequate dietary intake, undernutrition (body mass index for age ≤−2SD) and low serum zinc[7,9]. Then, it was multiplied by design effect of 2 and non-response rate of 10 per cent. Final sample sizes required to estimate the prevalence of inadequate dietary intake of zinc, undernutrition and low serum zinc were 828, 430 and 328, respectively.

Multistage cluster random sampling was used. In the first stage, districts were purposively selected based on percentage of tribal population; then, in the second stage, two blocks with highest tribal population were selected, and in third stage, clusters (villages) were randomly selected. Each cluster was divided in to four geographic segments considering streets and dwelling pattern; one segment and first household within each segment were then randomly selected. Following such selection, subsequent neighbouring 33 households were approached for participation. Dietary intake assessment was conducted for all the participants, and they were invited for anthropometry and blood sampling the next morning.

Data collection:

<u>Data collection instrument</u>: A structured data collection instrument comprising information about sociodemographic characteristics, dietary recall, food frequency, anthropometric and morbidity related data was developed. This was pilot tested in tribal villages of Raisen district and was suitably modified.

After ascertaining eligibility of household, participant information sheet (PIS) in Hindi was handed over to participants and written informed consent from parent and assent from adolescents were obtained. In case of illiterate parent, consent was obtained in the presence of a literate witness from the same village.

Dietary intake assessment: Qualified field workers were recruited and trained for 24 h dietary recall method[10]. Field workers collected data about foods cooked during the last 24 h and raw contents utilized in the preparation of food. Then, the household member, who cooked the food, was asked to bring the same amount of raw material and it was weighted. SECA-852 Diet Portion Scale (Seca GmbH & Co. KG., Hamburg, Germany) and cups of various sizes were used for weighing raw materials. Volume of cooked food was estimated by using standardized containers of different sizes. Details about left over food and its utilization, food taken from outside the home and mid-day meal were also asked.

Anthropometry assessment: Anthropometric assessment was carried out by SECA Measuring Station (SECA 786; accuracy±500 g/±0.8%) and Columns Scale (SECA 224; graduation 1 mm) and tapes.

Blood sample collection, transportation and serum zinc estimation: Three millilitre of blood was collected from antecubital vein into trace element-free collection tubes (BD Vacutainer 368380). Standard techniques for collection and avoiding contamination were adopted, and biomedical waste management practice was followed by using collection containers (BD Recykleen Sharps Collector Container, USA) and coloured waste collection bags. Overnight fasting blood samples were collected on the subsequent day of dietary recall, and status of overnight fasting was noted at the time of sample collection. These samples were centrifuged at the field within one hour at 2236 g on a table top centrifuge to obtain serum, which were transported in a portable carrier box with ice packs maintaining cold chain (2-8°C). Serum was stored at −80°C deep freezer until serum zinc analysis was performed. Serum zinc was estimated using atomic absorption spectrophotometer (AAS model Varian, Varian Medical Systems Inc., CA, USA). Standard curve for serum zinc using commercial zinc atomic absorption standard solution was used. Each serum sample was diluted in 0.1N HCl in the ratio 1:10 and centrifuged to remove any debris. The supernatant was subject to zinc estimation by AAS using Hallow cathode lamp (PerkinElmer Inc., Waltham, MA, USA) 213.9 nm wavelength for detecting Zinc. In case of aberrant sample values, mainly due to blocking of the flame due to protein debris, the sample tests were repeated. Serum zinc analysis was performed at two locations: Indian Institute of Soil Sciences, Bhopal (for samples of Betul and Jhabua district), and Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (for Mandla).

Statistical analysis: Analysis was performed by using Microsoft Excel, R version 4.0.3 and SAS® software (OnDemand for Academics, SAS Institute Inc., Cary, NC, USA).

Prevalence of inadequate zinc intake: Nutrient composition tables from Indian Council of Medical Research (ICMR)-National Institute of Nutrition (NIN) book 'Nutritive Value of Indian Foods' with updated values as per the Indian Food Composition Table-2017 were used for calculating nutrient intakes[11]. We used the method proposed by Luo *et al*[12] to adjust for intraindividual variation for single-day recall data. External variance ratios for zinc intake were used from database by French *et al*[13,14]. Adjusted usual intakes were estimated by using TRAN1 and DISTRIB macros[12,13]. The prevalence of inadequate dietary zinc was calculated by estimating probability of having intake below EAR for given age and gender groups through DISTRIB macro in SAS. Age- and gender-wise EAR values as recommended by NIN in its 2020 recommendations[15] and IZiNCG[16] were used as nutrient requirements. TRAN1 and DISTRIB macros generate multiple simulations for usual intake for an individual based on reported dietary intake for an individual and external variance ratio. Monte Carlo simulation of the random effect methods is used to estimate the distribution of usual intake. It then computes probability of inadequate intake compared to EAR by averaging the individual probabilities.

Anthropometric assessment: Data obtained from anthropometry were analyzed by using WHO Anthro-PLUS software. Proportion of stunting (height for age Z score <−2SD) was calculated.

Prevalence of low serum zinc: It was estimated by calculating number of adolescents having serum zinc level less than the cut-off values for morning non-fasting (66 µg/dl for females and 70 µg/dl for males) and for the fasting samples (70 µg/dl for females and 74 µg/dl for males)[17].

Zinc inadequacy is considered a problem of public health concern when a country has 25 per cent of its population at risk of inadequate zinc intake based on data from quantitative dietary assessments, 20 per cent stunting among preschool children, and ≥20 per cent prevalence of low serum zinc concentration[18].

Results

A total of 2310 households were approached for participation in this study, of which 2224 households having 5151 adolescents participated. Of these enlisted adolescents, 4673 responded to dietary recall (90.7% response rate). Anthropometry of 2437 participants was conducted, and serum zinc was analyzed in 844 adolescents. Table I presents age- and sex-wise distribution of study participants among the three study tribes.

Dietary recall data of 180 participants were unreliable and excluded from further analysis. Single-day dietary zinc intakes were adjusted for intraindividual variation, and Figure 1 depicts probability density for observed intake and adjusted usual zinc intake (mg/day). Adjustments were done by using National Cancer Institute (NCI) one-day method[14] and external variance component, *i.e.* within to between individual variance was assumed as 1.3[13]. Change in prevalence with respect to different variance component assumption was assessed; the results are shown in Supplementary Figure 1. Prevalence estimates did not change substantially for range of variance ratios from 0.5 to 2.1. Table II shows the prevalence of inadequacy of dietary intake and its 95 per cent CI estimated with reference to EAR values recommended by ICMR and IZiNCG. Phytate to zinc molar ratio was more than 18 in our dataset (Supplementary Fig. 2), and therefore, IZiNCG EAR values for unrefined diet were used. The prevalence of dietary zinc inadequacy (ICMR EAR values) was found to be 61.1, 61.2 and 72.2 per cent, among *Bhil*, *Gond* and *Korku* tribes, respectively, while by IZiNCG EAR values prevalence was 39.5, 36.1 and 51.3 per cent, respectively (Table II).

Serum zinc analysis was performed on 844 samples, of which 741 were samples collected after overnight fasting and 103 were non-fasting samples; Figure 2 presents the distribution of serum zinc stratified by tribe and gender. The median serum zinc concentration for *Gond* and *Bhil* was less than the suggested lower cut off (for boys - 70 µg/dl and girls - 66 µg/dl), while for *Korku*, it was marginally higher than the suggested lower cut-off. Recently, modifications in serum zinc cut-offs based on the national level data for India have been suggested[19]. Table II shows the results by using IZiNCG recommended cut-off as well as modified Indian cut-off values. Prevalence of low serum zinc was highest among *Bhil* (70.2%) followed by *Gond* (59.9%) and *Korku* (36.1%) tribes based on IZiNCG cut-offs. However, this prevalence was markedly lower when estimated using modified cut-offs as suggested by Pullakhandam *et al*[19] and was found to be 35.7, 38.7 and 26.9 per cent, respectively, in *Bhil*, *Gond* and *Korku* tribes. Distribution of serum zinc in fasting and non-fasting samples is depicted in Supplementary Figure 3. Fasting serum zinc values [median (interquartile range) 63.5 µg/dl (51.8, 79.0)] were slightly higher than in non-fasting state [61.4 µg/dl (48.5, 78.1)].

The prevalence of any stunting among *Bhil*, *Gond* and *Korku* was 32.9, 24.0 and 29.0 per cent, respectively (Table II).

Prevalence of dietary zinc inadequacy, stunting and low serum zinc stratified by sociodemographic variables: The outcomes of this study were stratified by age group, sex, family type, type of roof of household (indicator of socio-economic condition), wealth index tertiles, drinking water sources and sanitation practices for exploring any pattern or trend. Evidently higher probability of dietary zinc inadequacy was observed in older adolescents (16-19 yr) and boys. Risk of inadequate dietary zinc was similar across types of families, wealth tertiles, sources of drinking water or sanitation practices. In addition, more dietary inadequacy was observed among *Korku* tribe than *Gond* and then *Bhil* tribe. However, considering huge prevalence of dietary zinc inadequacy (*i.e*. overall >40%) where every other adolescent was having inadequacy, trend among these factors needs to be interpreted with caution. The prevalence of lower serum zinc was higher in younger adolescents (10-12 yr) and boys. No specific trend was observed with respect to other factors (Table II).

Discussion

The present study reports that the tribal adolescents in MP are at high risk of inadequate dietary intake of zinc and have high prevalence of low serum zinc. Physical indicators such as burden of stunting were also found to be higher in the tribal adolescents.

A large scale National Nutrition Monitoring Bureau (NNMB) study[4] reported that dietary zinc inadequacy

Table I. Age and sex-wise distribution of the study population in three tribes

Characteristic	*Gond* (Mandla)			*Bhil* (Jhabua)			*Korku* (Betul)		
	Overall (n=1100), n (%)	Boys (n=515), n (%)	Girls (n=585), n (%)	Overall (n=2081), n (%)	Boys (n=1012), n (%)	Girls (n=1069), n (%)	Overall (n=1492), n (%)	Boys (n=672), n (%)	Girls (n=820), n (%)
Age (yr), mean±SD	14.6±2.7	14.4±2.7	14.7±2.7	14.1±2.8	14.0±2.8	14.2±2.8	14.5±2.8	14.4±2.7	14.6±2.9
Family type									
Nuclear	713 (73.3)	343 (77.3)	370 (69.9)	676 (32.8)	363 (36.4)	313 (29.4)	724 (49.4)	372 (56.2)	352 (43.7)
Joint	260 (26.7)	101 (22.7)	159 (30.1)	1383 (67.2)	633 (63.6)	750 (70.6)	743 (50.6)	290 (43.8)	453 (56.3)
WI tertiles									
First	330 (34.0)	145 (32.8)	185 (35.0)	644 (31.3)	315 (31.7)	329 (31.0)	476 (32.4)	223 (33.7)	253 (31.4)
Second	326 (33.6)	167 (37.8)	159 (30.1)	686 (33.4)	324 (32.6)	362 (34.1)	468 (31.9)	209 (31.6)	259 (32.2)
Third	315 (32.4)	130 (29.4)	185 (35.0)	725 (35.3)	355 (35.7)	370 (34.9)	523 (35.7)	230 (34.7)	293 (36.4)
Household (roof)									
Kachha	932 (95.8)	430 (96.8)	502 (94.9)	1617 (78.5)	791 (79.4)	826 (77.7)	1446 (98.6)	651 (98.3)	795 (98.8)
Pucca/Semi-Pucca	41 (4.2)	14 (3.2)	27 (5.1)	442 (21.5)	205 (20.6)	237 (22.3)	21 (1.4)	11 (1.7)	10 (1.2)
D/W source									
Improved	791 (71.9)	359 (69.7)	432 (73.8)	1714 (82.4)	833 (82.3)	881 (82.4)	1154 (77.3)	506 (75.3)	648 (79.0)
Sanitation									
Improved	133 (13.7)	51 (11.5)	82 (15.5)	1298 (63.0)	636 (63.9)	662 (62.3)	150 (10.2)	75 (11.3)	75 (9.3)

Data on family type, roof, drinking water and sanitation for 127, 22 and 25 participants and for wealth index, data of 129, 26 and 25 participants were missing among *Gond*, *Bhil* and *Korku* tribes, respectively. D/W, drinking water; SD, standard deviation; WI, wealth index

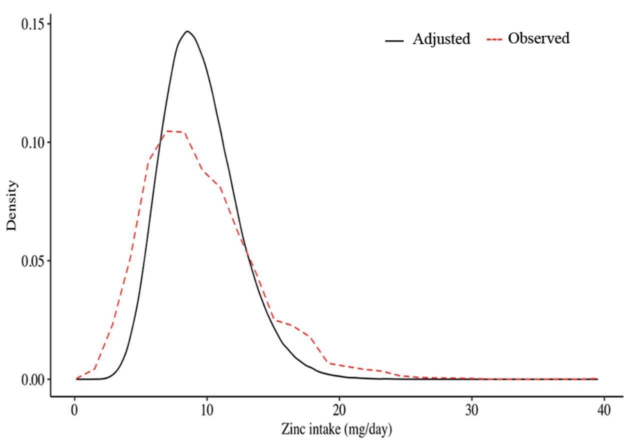

Fig. 1. Distribution of observed dietary zinc intake and adjusted (as per NCI one-day method) usual zinc intake.

in adolescents of 10 Indian States wherein per cent adequacy (intake relative to recommended dietary allowance *i.e*. RDA) was only 10 and seven per cent for normal and thin adolescents, respectively, indicating around 90 and three per cent inadequacy of intake. Similar findings from Rajasthan[20] and Maharashtra[21] were also reported (30-70%). In this study, higher probability of dietary zinc inadequacy was observed in adolescents boys, which could be attributed to their higher requirement of zinc for growth. The NNMB Study also reported higher age group, higher household wealth status, access to improved water, better maternal work status and living in better type of houses as predictors of good nutritional status[4].

Most commonly used indicator is nutrient adequacy which is expressed as per cent of intake as compared to RDA, and some studies have reported mean percent of adequacy, using cut-off like less than 50 per cent RDA. We have estimated probability of inadequacy with reference to EAR suggested by the ICMR[15] and IZiNCG[16]. Marked difference in estimates of zinc inadequacy is observed if these two references are used since EAR recommended by the ICMR-NIN is higher.

Recent secondary data analysis of the National Sample Survey Organization's food consumption data reported a prevalence of 24.6 per cent for inadequate intake of zinc nationally. This is relatively lower than the prevalence of inadequate zinc intake estimated in our study among tribal adolescent and the difference could be due to study population characteristics and/or different methods[22] used for estimation.

Table II. Prevalence of inadequate zinc intake, stunting, low serum zinc stratified by socio-demographic and environmental variables

Variables	Diet (n)	Mean usual zinc intake	Prevalence (95% CI) inadequate zinc intake		Anthropometric (n)	Prevalence (95% CI) of stunting	Serum zinc (n)	Prevalence (95% CI) of low serum zinc	
			EAR-ICMR[a]	EAR-IZiNCG[b]				IZiNCG cut-off[c]	New cut-off based on CNNS data[d]
Overall	4493	9.5	64.8 (63.4-66.2)	42.6 (41.2-44.1)	2437	29 (27.2-30.8)	844	57.5 (54.1-60.8)	34.4 (31.2-37.5)
Tribe (district)									
Bhil (Jhabua)	2055	9.6	61.1 (59-63.2)	39.5 (37.4-41.6)	964	32.9 (29.9-35.8)	325	70.2 (65.2-75.1)	35.7 (30.5-40.9)
Gond (Mandla)	971	10.1	61.2 (58.2-64.3)	36.1 (33.1-39.2)	745	24 (21-27.1)	292	59.9 (54.3-65.5)	38.7 (33.1-44.3)
Korku (Betul)	1467	8.9	72.2 (70-74.5)	51.3 (48.7-53.8)	728	29 (25.7-32.3)	227	36.1 (29.9-42.3)	26.9 (21.1-32.6)
Age group									
10-12	1377	8.5	30.6 (28.1-33)	29.7 (27.3-32.1)	872	23.5 (20.7-26.3)	179	62.6 (55.5-69.6)	36.3 (29.3-43.3)
13-15	1447	9.4	77.6 (75.5-79.8)	48.5 (45.9-51)	805	38.1 (34.8-41.5)	296	59.5 (53.9-65)	36.8 (31.4-42.3)
16-19	1669	10.3	81.9 (80-83.7)	48.2 (45.8-50.6)	760	25.7 (22.6-28.7)	340	53.2 (48-58.5)	30.9 (26-35.8)
Girls	2395	9.2	63 (61.1-64.9)	36.9 (34.9-38.8)	1064	30.9 (28.2-33.7)	390	62.1 (57.3-66.8)	35.1 (30.4-39.8)
Boys	2098	9.7	66.8 (64.8-68.8)	49.2 (47.1-51.3)	1373	27.5 (25.2-29.9)	425	53.4 (48.7-58.1)	33.4 (29-37.9)
Family type									
Joint	2386	9.4	64.9 (63-66.8)	42.5 (40.5-44.5)	1114	32.5 (29.8-35.2)	364	57.7 (52.6-62.7)	34.1 (29.2-38.9)
Nuclear	2107	9.5	64.6 (62.6-66.6)	42.8 (40.7-44.9)	1214	26.4 (23.9-28.8)	469	56.7 (52.3-61.2)	33.9 (29.6-38.2)
WI tertiles									
First	1450	9.4	63.8 (61.3-66.3)	42.3 (39.7-44.8)	711	27.4 (24.2-30.7)	250	54.4 (48.3-60.5)	33.6 (27.8-39.4)
Second	1480	9.4	63.4 (61-65.9)	42.1 (39.6-44.6)	777	29.9 (26.7-33.1)	281	57.7 (51.9-63.4)	35.2 (29.7-40.8)
Third	1563	9.5	67 (64.6-69.3)	43.5 (41.1-46)	837	30.1 (27-33.2)	301	58.8 (53.3-64.3)	32.9 (27.6-38.2)
Household (roof)									
Kachha	3989	9.4	64.9 (63.4-66.4)	42.9 (41.4-44.5)	2082	28.9 (27-30.9)	744	56.2 (52.6-59.7)	33.5 (30.1-36.8)
Pucca or Semi-Pucca	504	9.7	63.8 (59.6-68)	40.2 (35.9-44.5)	246	32.5 (26.7-38.3)	89	65.2 (55.3-75)	38.2 (28.2-48.2)
D/W source									
Improved	3655	9.4	64.3 (62.7-65.8)	42.5 (40.9-44.1)	1955	29.9 (27.9-31.9)	688	56.4 (52.7-60.1)	32.6 (29.1-36)
Not improved	838	9.5	67 (63.8-70.2)	43.1 (39.8-46.5)	482	25.3 (21.4-29.2)	156	62.2 (54.6-69.8)	42.3 (34.6-50)
Sanitation									
Improved	1581	9.6	62.9 (60.6-65.3)	40.6 (38.2-43)	761	31.7 (28.4-35)	267	59.6 (53.7-65.4)	31.1 (25.6-36.6)
Not improved	2912	9.4	65.8 (64-67.5)	43.7 (41.9-45.5)	1567	28.1 (25.9-30.4)	566	56 (51.9-60.1)	35.3 (31.4-39.3)

[a]Zinc EAR (mg/day)-ICMR 10-12 yr-8.5; 13-15 yr boys-14.3; 13-15 yr girls-12.8; 16-18 yr boys-17.6; 16-18 yr girls-14.2, [b]Zinc-EAR (IZiNCG) (mg/day) unrefined diets-9-13 yr-7; 14-18 yr boys-11; 14-18 yr girls-9, [c]Low serum zinc cut offs (µg/dl)-IZiNCG-Adolescent boys: Fasting-74 non-fasting-70; Adolescent girls fasting-70, non-fasting-66, [d]Modified low serum zinc cut offs (µg/dl) as per Pullakhandam *et al*., (2022)-Adolescent boys: Fasting-56 non-fasting-55; Adolescent girls fasting-54, non-fasting-53. D/W, drinking water; CI, confidence interval; WI, wealth index; EAR, estimated average requirement; ICMR, Indian Council of Medical Research; IZiNCG, International Zinc Nutrition Consultative Group

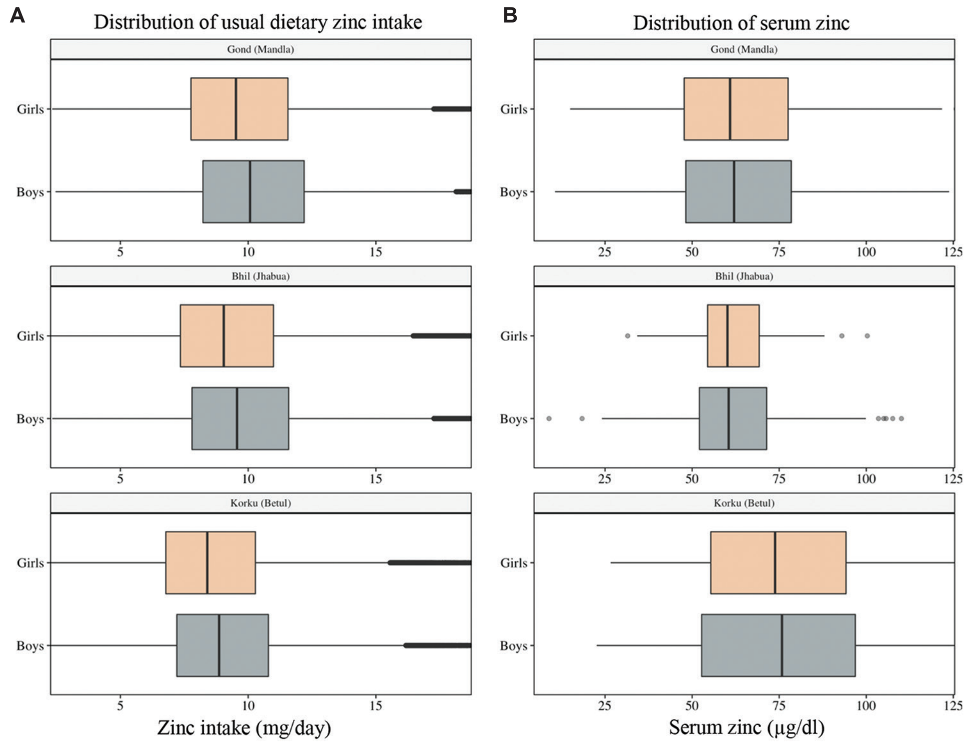

Fig. 2. Distribution of (A) usual zinc intake, and (B) serum zinc stratified by gender and tribe.

High prevalence of low serum zinc levels was reported by studies conducted among children, adolescents and pregnant women in various parts of India. Prevalence of low serum zinc levels ranges from 41 to 73 per cent in these reports. Among tribal adolescents of Jharkhand[7], the prevalence was found to be 53 per cent, and the prevalence was found to be 49.4 per cent in adolescents of Delhi[23]. Low serum zinc in schoolchildren (6-15 yr) has been reported to be more than 83 per cent in our neighbouring country Nepal[24], and in Cameroon[25], the prevalence of low serum zinc is reported to be 82 per cent in women (15-49 yr). Comprehensive National Nutrition Survey (CNNS) 2016-18 provides estimates of serum zinc deficiency in adolescents[6]. As per the survey report, nearly 32 per cent of the adolescents in India and nearly 20 per cent adolescents in MP were found to have low serum zinc. However, recently modified cut-offs for low serum zinc were suggested for LMICs because IZiNCG cut-offs were derived from NHANES datasets and were four decade older[19]. According to the revised estimates, the prevalence of low serum zinc among adolescents was found to be 5.6 per cent for India and 2.2 per cent for MP[19]. In our study, even after applying modified cut-offs, every third adolescent was classified as having low serum zinc. While CNNS is representative of entire adolescent population, our study included only tribal adolescents. Serum zinc concentration is not regarded as very reliable biomarker of one's zinc status. It is reported that even when a broad range of zinc intakes below and above the requirements are consumed, an individual's serum zinc concentration is retained within a relatively small range of values due to efficient homoeostatic mechanisms. Inflammation, fasting, pregnancy, oral contraceptive use and diurnal rhythm are some factors that affect zinc levels in the blood. Inflammation lowers zinc concentration while fasting raises it. In addition, biological samples, such as sample collection tubes, are easily contaminated with zinc from the environment, while haemolysis of blood samples increases plasma zinc concentrations[26]. Although the standard protocols were followed for collection and analysis in the present study, there are inherent issues with respect to use of serum zinc as biomarker of zinc deficiency as described above.

Intestinal morphology and functions are altered in conditions such as enteropathy. As it is associated with environmental factors, it is also known as environmental enteropathy (EE). This condition is almost universal in developing countries including India. EE leads to malabsorption of nutrients including zinc causing zinc deficiency[27]. Because the present investigation was conducted in tribal adolescents, whose macro-environments included rudimentary household structures (89% of the roofs were made of natural or rudimentary material), and lacked improved drinking water and sanitation, there was high likelihood of EE. This might be one of the reasons for high zinc deficiency observed in this study population.

We observed that all tribes mainly consumed cereal-based diet with higher phytate:zinc molar ratios. It has been reported that when Phy:Zn ratios are more than 18, then absorbable zinc content in men and women remain only at 18 and 25 per cent of dietary intake of zinc[28]. Burden of inadequate zinc intake, stunting and low serum zinc was considerably large even almost at all levels of sociodemographic and macro-environmental stratifiers.

Risk of zinc deficiency in any population is considered to be of public health concern when the prevalence of dietary intake of zinc below EAR reaches more than 25 per cent, the prevalence of stunting amongst children below five years of age goes beyond 20 per cent and the prevalence of low serum zinc levels is estimated to be more than 20 per cent[18]. All three tribes we covered under the current investigation had higher prevalence of these indicators and therefore were of public health concern. We also report considerable variation in prevalence of inadequate intake as well as low serum zinc as per recommendations of the international agencies *vis-a-vis* national guidlines.

The 24 h diet recall method has some errors which may be random or systematic[29]. Systematic errors may be due to inability to recall the number of times food was consumed, correct amount of raw material used, collecting such information on non-usual (festive or fasting) day with respect to diet, language comprehension, variety of foods used in different localities, *etc*. In order to reduce such biases, we identified qualified (MSc Nutrition or Home Science) investigators and trained them on study protocols. Our findings were based on one-day recall, which may not give a precise estimate and not be representative of usual intake of zinc for an individual, and may lead to overestimation of prevalence of the group level. However, with the use of NCI one day method to estimate usual intake, marked overestimation is not expected. In this study estimation of C-reactive protein was not considered to account for inflammation where serum zinc concentration may transiently decrease. This poses a risk of overestimation of prevalence of low serum zinc; however, this may not amount to a substantial magnitude.

Zinc deficiency is implicated as a risk factor for several chronic diseases including chronic depression, low birth weight, attention-deficit hyperactivity disorder, and pre-eclampsia[30]. In this study, we evaluated stunting as one of the clinical outcomes; however, there was no linear relationship with serum and dietary zinc levels. Meta-analysis of zinc supplementation and its impact on growth found limited improvement in growth in under five children[31]. However, it is known that micronutrient deficits frequently coexist, thus supplementing with one nutrient that limits growth is unlikely to be effective if other elements required for growth are deficient[32]. Thus, for tackling nutritional inadequacy which is of multifactorial origin and its chronic consequences, we need to act by formulating multipronged intervention strategies based on local evidence.

Overall, this large-scale study has generated evidence of high prevalence of inadequate dietary zinc intake, high levels of stunting as well as low serum zinc among tribal adolescents in MP. The diet of studied tribals of MP is not varied, and hence, measures to increase dietary diversity for increasing intake of micronutrients through public distribution system must be considered. As plant-based cereals were the main source of zinc in our study population, measures to increase bioavailability should be explored. Furthermore, zinc estimation should be included along with haemoglobin estimation in national level surveys. Such evaluation of serum zinc at population level will help in generating evidence regarding burden of serum zinc deficiency.

***Acknowledgment*:** Authors acknowledge the community health workers and officials from public health department for their cooperation during data collection. Facilitation by staff of Indian Institute of Soil Sciences (Sandeep Sharma and Ajay Tripathi) and Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (Drs H. Rai, G. Tagore and G.D. Sharma) is also acknowledged. Authors also acknowledge the assistance provided by the staff (Jyoti Malviya, Ritu Sharma, Pushplata Yadav, Deepika Lonkar, Indresh Kumar, Shivam Kumar and Lalita) employed in this project.

***Financial support & sponsorship*:** The study was funded by Indian Council of Medical Research, New Delhi, as extramural ad-hoc research project (IRIS-2013-0749).

***Conflicts of Interest*:** None.

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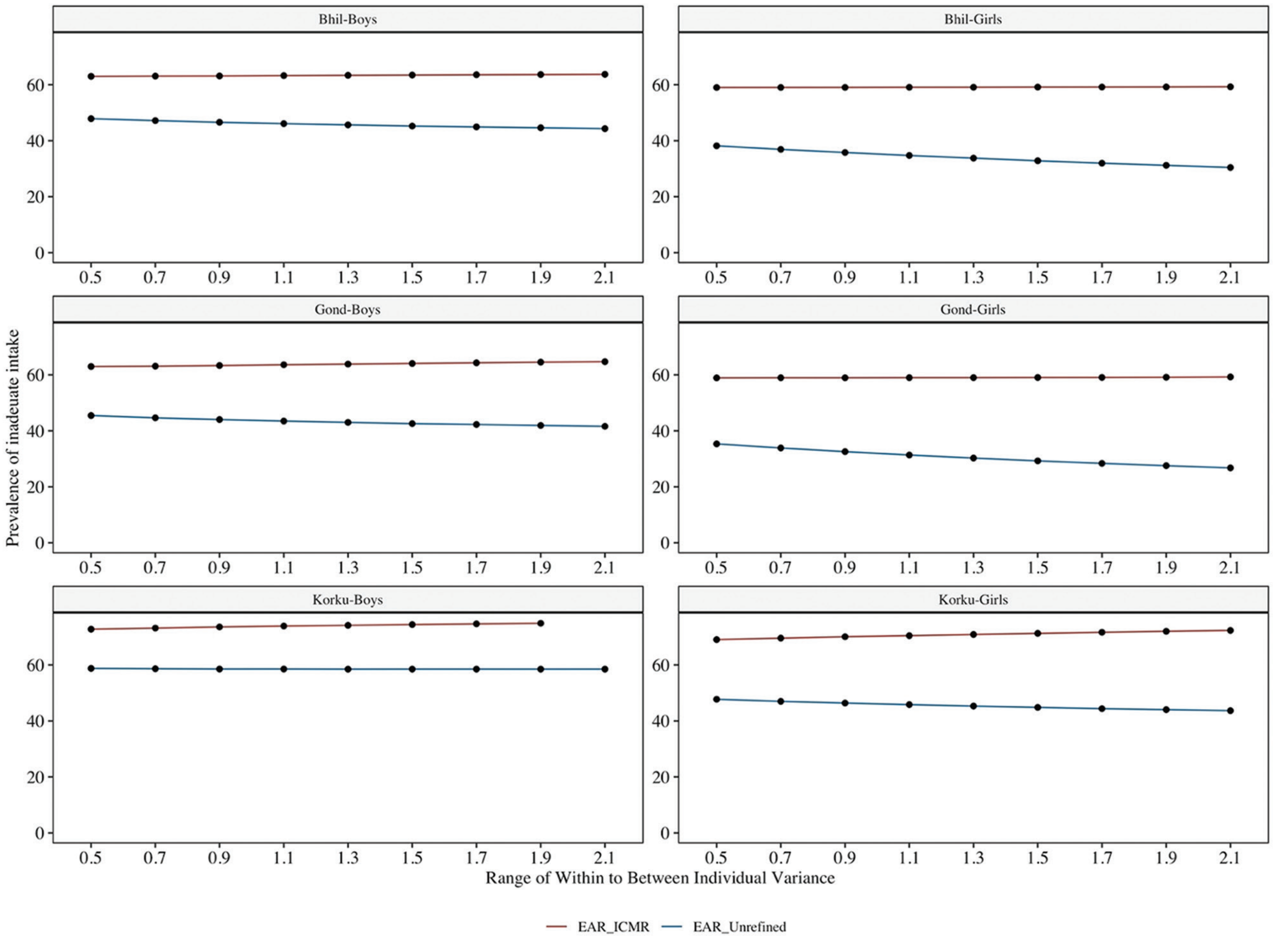
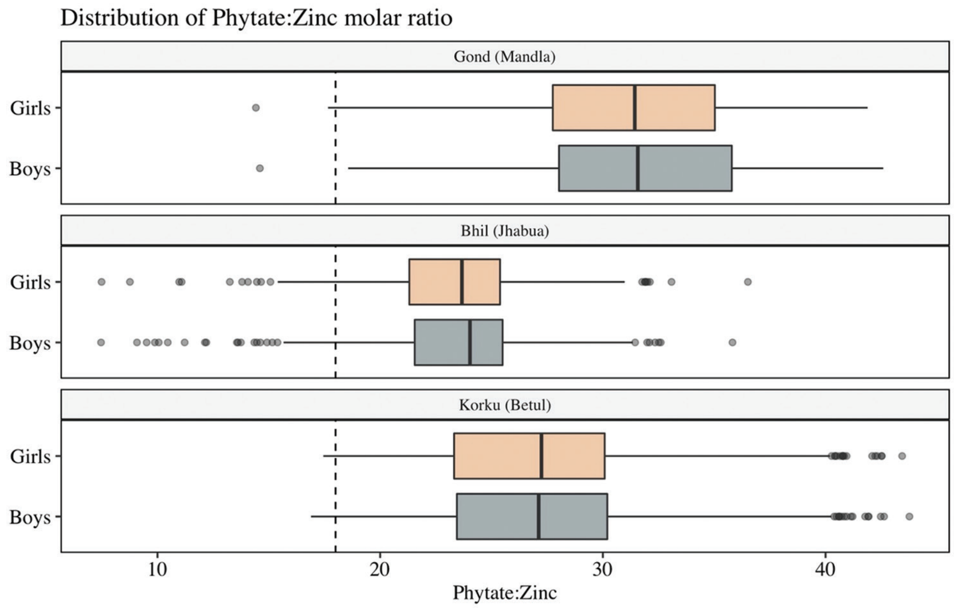
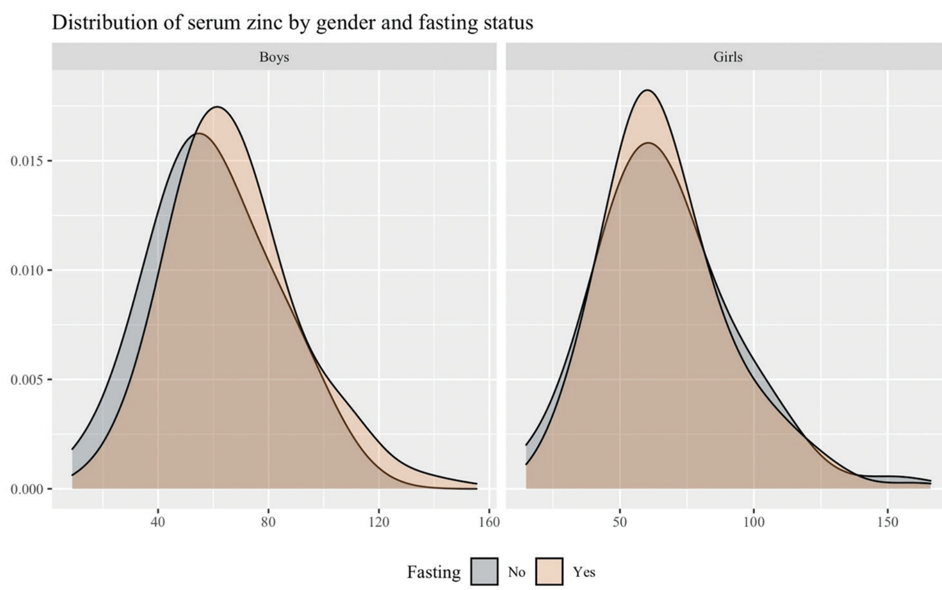


Supplementary Fig. 1. Prevalence of inadequate intake of zinc across range of external variance ratios stratified by gender and tribe.



Supplementary Fig. 2. Distribution of Phytate:zinc molar ratio stratified by gender and tribe.



Supplementary Fig. 3. Distribution of serum zinc stratified by gender and fasting status.