 doi:10.20944/preprints202012.0667.v1

Prevalence and predictors of zinc deficiency among children and non-pregnant women in Nepal: analysis of Nepal micronutrients status survey 2016

[1*]Suresh Mehata, [2]Man Kumar Tamang, [3]Kedar Raj Parajuli, [4,5]Binod Rayamajhee, [6,7,8,9]Uday Narayan Yadav, [10] Ranju Mehta, [11]Dipendra Raman Singh

[1] Ministry of Health and Population, Government of Nepal, Kathmandu, Nepal
[2]Queensland Brain Institute, The University of Queensland, Australia
[3] Chief, Nutrition Section, Family Welfare Division, Department of Health Services, Ministry of Health and Population, Kathmandu, Nepal
[4]School of Optometry and Vision Science, Faculty of Science, Kensington NSW 2033, UNSW, Sydney, Australia
[5]Department of Infectious Diseases and Immunology, Kathmandu Research Institute for Biological Sciences (KRIBS), Lalitpur, Nepal
[6]Centre for Primary Health Care and Equity, University of New South Wales, Sydney, Australia
[7]School of public Health and Community Medicine, UNSW, Sydney, Australia
[8]Centre for research policy and implementation, Biratnagar, Nepal
[9]Department of Public Health, Torrens University, Sydney, Australia
[10]Little Buddha College of Health Sciences, Min Bhawan, Kathmandu, Nepal
[11]Director General, Department of Health Services, Ministry of Health and Population, Kathmandu, Nepal

*Corresponding author

Dr Suresh Mehata
Ministry of Health and Population
Ram Shah Path, Kathmandu, Nepal
Email: sureshmht@gmail.com

 doi:10.20944/preprints202012.0667.v1

Abstract

Zinc deficiency, a common malnutrition in children and women is a global public health problem. Burden of zinc deficiency is more in countries with low meat and high cereal food consumption like Nepal. Nationally representative data on zinc status in Nepal is lacking at present. This study analysed the data from the recent Nepal National Micronutrient status survey 2016(NNMSS-2016) to determine the prevalence of zinc deficiency and associated risk factors among children aged 6-59 months (n=1462) and non-pregnant women aged 15-49 years(n=1923) from three ecological zones, Hill, Terai, and Mountain of the country. Venous blood was collected from the participants to measure micronutrients such as zinc, markers of anaemia, vitamin A, and markers of inflammation. Stool was collected to assess the soil-transmitted helminths (STHs) and *Helicobacter pylori* infection. Socio-demographic, household and other relevant information were collected by a structured questionnaire. Logistic regression was used to examine the predictors of zinc deficiency among the participants. The overall zinc deficiency in children was found to be 22.9% while it was higher in non-pregnant women (24.7%). Predictors associated with zinc deficiency among enrolled children in the study were, living in rural areas (AOR=2.25, 95% CI, [1.13, 4.49]), occurrence of diarrhoea during the two weeks preceding the survey (AOR=1.57, 95% CI, [1.07, 2.30]), household wealth quintile (AOR= 0.48, 95% CI,[0.25, 0.92]) and vitamin A status (AOR=0.49, 95% CI,[0.28, 0.85]. Risk factors associated with zinc deficiency among the non-pregnant women were being underweight (AOR=1.60, 95% CI,[1.15, 2.23]), fever occurrence during two weeks preceding the survey (AOR=1.45, 95% CI,[1.06, 1.99]), *H. pylori* in the stool (AOR=1.32, 95% CI, [1.03, 1.70]), being rich (AOR=0.64, 95% CI,[0.42, 0.98]) and being in the risk of folate deficiency (AOR=0.60, 95% CI,[0.37, 0.96]). We conclude that community focussed intervention programs including health and nutrition counselling and livelihood opportunities focusing groups at high-risk may improve the zinc status in Nepal.

Keywords: Zinc deficiency, children, non-pregnant women, national micronutrient status survey, Nepal

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 December 2020 doi:10.20944/preprints202012.0667.v1

Introduction

Zinc deficiency is a common and long-standing public health problem of low and middle-income countries (LMICs). According to the WHO data, zinc deficiency is a leading cause of mortality and morbidity in LMICs (Organization, 2002). Zink deficiency is considered as a public health concern when the prevalence of low serum zinc concentration is >20% (Whitehead Jr et al., 2017). Nearly one-third of the South Asian population is at risk of inadequate zinc supply (Wessells & Brown, 2012) but zinc deficiency varies from 4% to 73% across the subregions. Mild to moderate level of zinc deficiency is more common throughout the world than severe zinc deficiency (Brief, 2007; Ezzati, Lopez, Rodgers, & Murray, 2004). Since the first documentation of zinc as a nutrient for human health in 1963, several studies have proven zinc as an essential micronutrient with a key role in myriad of biological functions which ranges from DNA synthesis to physical growth (Brown, Peerson, Rivera, & Allen, 2002; MacDonald, 2000; Prasad, 1991). Zinc is equally important forideal growth of the foetus and maternal tissue development (King, 2000). Zinc deficiency is mainly associated with insufficient intake and/or absorption from the foods. Additionally, the human body has no tissue reservoir for zinc, unlike iron and vitamin A so, adequate zinc supply through dietary is necessary to prevent zinc deficiency (Ackland, Michalczyk, & nutrition, 2006; Mackenzie, Iwasaki, Tsuji, & signaling, 2008). Children, especially from low-income countries such as Nepalwith poor diet and gastrointestinal infections are at higher risk of zinc insufficiency (Brown et al., 2004). Zinc deficiency can appear as a symptom of the disease which leads to detrimental effects in human health including immune anomalies as zinc involves in innate immunity, rough skin, dwarfism, poor appetite, and mental fatigue, among others (Livingstone, 2015; Report & Reducing risks, 2002). Zinc deficiency is also correlated with anaemia, cardiac diseases, and impairs neurogenesis at the early stage of development (Adamo et al., 2019; Atasoy & Bugdayci, 2018). Globally, zinc deficiency is highly associated with chronic and infectious diseases like cancer, and diabetes, and measles, HIV, tuberculosis, and pneumonia, respectively (Sigel, Sigel, & Sigel, 2013). Zinc supplementation can reduce the risk of low-birthweight infants' deaths, and it is also used as an adjuvant with rehydration treatment of diarrheal diseases (Sazawal et al., 2001; "Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials %J The American journal of clinical nutrition," 2000).

The Government of Nepal (GoN) has introduced zinc supplements to manage childhood diarrhoea in 2007 but, it demands regular evaluation and proper monitoring (Ghimire, Agho, Renzaho, Dibley, & Raynes-Greenow, 2018). GoN has listed Zinc tablet of 10 mg, 20 mg (scored tablets) in the national list of essential medicines to use in acute diarrheal cases as an adjunct to ORSs (Alam et al., 2017).

Micronutrient deficiencies is a hidden hunger among Nepalese community, particular women and children, and the inadequate zinc status of maternal women results in adverse consequences such as abortion, low-birthweight, and congenital malformation (Jiang, Christian, Khatry, Wu, & West Jr, 2005). In essence, the monotonous Nepali food menu consists of more cereal items but food like red meat is limited in the daily supply of kitchen of many households. Phytate rich nutrients of cereals inhibit zinc absorption, particularly when phytate:zinc molar ratio (P:Z) is >15 in the consumed diet (Ram K Chandyo et al., 2009; Lonnerdal, 2000). Poor dietary intake, inappropriate food supply, food insecurity, presence of more phytate and/or fiber in diets, and improper food preparation can cause zinc deficiency

(Bruno De Benoist et al., 2007). National data on zinc deficiency based on population studies and associated predictors are lacking in Nepal. To address this gap, this study aimed to assess the national level prevalence of and factors associated with the zinc deficiency among children aged 6-59 months and non-pregnant women aged 15-49 years in Nepal.

Materials and methods

Study participants

We used cross-sectional data from a nationally representative Nepal National Micronutrient Status Survey 2016 (NNMSS-2016). A detail methodology has been presented elsewhere(Ministry of Health and Population et al., 2018). In brief, the NNMSS-2016 study was conducted to provide up to date status on the basic health and associated demographic statistics. Complete details about the study population, study area, and sampling techniques are published in the NNMSS report. Stratified multistage cluster sampling without replacement approach was applied in the study. Three geographical regions (Mountain, Hill, and Terai) and five development zones (Eastern, Central, Western, Mid-western, and Far western) were included in the study considering 180 clusters (75 from the Terai and Hill each, and 30 clusters from the Mountain) and 15 strata by systemic sampling where clusters were used as the primary sampling units (PSUs) (Ford et al., 2020). A total of 24 households were selected from each cluster (n = 4,320) using a systematic random sampling where 4,309 (99.7%) households have completed the interviews. Geometric mean zinc and prevalence of zinc deficiency were determined among 1,462children aged 6-59 months and 1,923 non-pregnant women aged 15-49 years. Serum zinc was corrected for inflammation in children 6-59 months but not the women. Zinc deficiency was defined using the cut off as below (kIZiNCG, 2007):

For children 6-59 months: morning, non- fasting: <65 µg/dL or afternoon, non-fasting: <57 µg/dL. For non-pregnant women: morning, non-fasting: < 66 or afternoon, non-fasting<59 µg/dL depending on the time of day: morning (until noon), non-fasting: 66 µg/dL; afternoon, non-fasting: 59 µg/dL

Biological specimen collection and laboratory processes

Blood samples were collected to measure micronutrient status and inflammation markers. Trained phlebotomists and pathologists collected blood and stool samples from the participants at their houses. Blood samples were non-fasted samples as fasting was not possible in the survey design. Stool samples were collected by the survey staffs within 24 hrs of the interview. The collected stool samples were examined for *Helicobacter pylori* antigen detection using an enzyme-linked immunoassay (ELISA) test kit on a Mago clinical analyzer which provides both negative and positive controls in each analytical test. For the validity of each analytical test, positive and negative controls were used where the absorbance was at least 0.8 OD units and less than 0.09 OD units, respectively. Internal quality control of laboratory was strictly followed.

Serum zinc concentration was determined by atomic absorption spectrophotometry (AAS). A BioRad serum control with three levels of control materials was used for the AAS and samples were run in duplicate. Haemoglobin was measured by photometric method using Hemocue®

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 December 2020 doi:10.20944/preprints202012.0667.v1

Hb 301 analyser at the household on small blood samples(Whitehead et al., 2017). Following criteria was used for defining anaemia(WHO, 2011):Children 6-59 months: < 11.0 g/dL; Non-pregnant women 15-49 years: <12.0 g/dL.

The other marker of anaemia such as ferritin and soluble transferrin receptor (sTfR) was measured by ELISA. Vitamin A and retinol binding protein (RBP) were measured by HPLC and ELISA, respectively. RBC folate level was determined by a gold standard microbiological method. The markers of inflammation namely AGP and CRP were measured by ELISA. Body mass index (BMI) was calculated for the anthropometric measurement of children and women. The detailed methodology has been described in detail elsewhere (Ministry of Health and Population et al., 2018).
Data on socio-demographic characteristics, participation in national nutrition and other intervention, recent micronutrient supplementation (zinc, iron, folic acid, vitamin A, multiple micronutrient supplementation or powders), two week recall of fever, cough and diarrhoea and other relevant information were collected by using structured questionnaire.

Statistical analysis

All analyses were performed using Stata 15. The reported values were weighted by sample weights in order to obtain the national estimates. Logistic regression was used to assess unadjusted and adjusted odds ratio (AOR) considering the sampling design where ecological zones were used as strata and 'wards' as a cluster. Only significant association observed in bivariate logistic regression were included in the multivariate model. P<0.05 was considered to be statistically significant.

Ethics statement

The ethical approval to conduct the survey was approved by the Ethical Review Board (ERB) of Nepal Health Research Council (NHRC) (Reg. No.: 201/2015). All participants gave informed consent before they were included in the study.

Results:

This study reports the data on micronutrient deficiencies in children (6-59 months) and non-pregnant women (15-49 years) from a national micronutrient survey of Nepal 2016. A total of 1462 (N=1462) children and 1923 (N=1923) non-pregnant women were sampled for the survey representing all geographical regions and socio-demographic groups.

The prevalence of zinc deficiency among the children was 22.9% (N=335). Besides zinc deficiency, other nutritional problems such as anaemia, stunting and underweight were also significantly prevalent in these children [Table 1]. The final multivariate regression analysis showed few variables associated with zinc status in children. The children living in rural areas (AOR=2.25, 95% CI, [1.13, 4.49]) and occurrence of diarrhoea during the two weeks preceding the survey (AOR=1.57, 95% CI, [1.07, 2.30]) had increased risk of zinc deficiency. The variables which were associated with decreased odds of zinc deficiency were household wealth quintile (AOR= 0.48, 95% CI,[0.25, 0.92]) and vitamin A status (AOR=0.49, 95% CI,[0.28, 0.85]) [Table 2]. The location of residence (rural or urban) is a non-modifiable risk factor while diarrhoea possibly can be modifiable and manageable.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 December 2020 doi:10.20944/preprints202012.0667.v1

Table 1: Socio-demographic and health characteristics of children aged 6 to 59 months by status of zinc deficiency, Nepal National Micronutrient Status Survey, Nepal, 2016

Socio-demographic and health characteristics	Zinc deficiency[a] (N=335, 22.9% [95%CI 18.9, 25.1]		No Zinc deficiency (N=1127, 77.1% [95%CI 74.9, 81.1]		Total (N=1462)	
	n		n		n	
Socio-demographic characteristics						
Age, months	335	33.3 (31.4, 35.2)	1127	34.0 (32.7, 35.2)	1462	33.8 (32.7, 34.9)
Sex (%)						
Male	177	54.7 (49.1, 60.1)	573	54.9 (51.5, 58.2)	750	54.8 (51.7, 57.9)
Female	158	45.4 (40.0, 50.8)	554	45.1 (41.8, 48.5)	712	45.2 (42.1, 48.3)
Rurality (%)						
Rural	314	94.5 (88.6, 97.5)	959	85.8 (77.8, 91.2)	1273	87.7 (80.6, 92.4)
Urban	21	5.5 (2.5, 11.4)	168	14.2 (8.8, 22.2)	189	12.3 (7.7, 19.4)
Ecological zone (%)						
Mountain	68	10.6 (7.5, 14.9)	172	7.2 (5.3, 9.7)	240	7.9 (6.1, 10.3)
Hill	157	46.2 (38.1, 54.5)	464	42.2 (36.3, 48.3)	621	43.1 (37.3, 49.0)
Terai	110	43.2 (34.7, 52.1)	491	50.6 (44.5, 56.8)	601	49.0 (43.1, 55.0)
Household wealth quintile (%)						
Poorest	139	33.0 (25.0, 39.9)	271	18.2 (14.2, 23.1)	410	21.2 (16.9, 26.3)
Poorer	60	17.7 (12.8, 24.0)	246	20.6 (17.0, 24.7)	306	19.9 (16.5, 23.9)
Middle	50	17.2 (12.7, 22.9)	208	20.3 (16.3, 24.9)	258	19.6 (16.0, 23.8)
Richer	53	19.9 (14.3, 27.1)	208	19.7 (16.3, 23.5)	261	19.7 (16.6, 23.3)
Richest	33	13.2 (7.8, 21.7)	194	21.2 (15.8, 27.9)	227	19.5 (14.4, 25.8)
Ethnicity (%)						
Brahmin or Chettri	130	32.4 (25.5, 40.2)	389	30.6 (25.3, 36.4)	519	31.0 (26.0, 36.5)
Other Terai Castes	22	13.4 (7.3, 23.1)	84	13.9 (7.9, 23.3)	106	13.8 (7.9, 22.9)
Hill Dalit	55	10.6 (7.1, 15.5)	184	11.5 (8.6, 15.1)	239	11.3 (8.6, 14.6)
Terai Dalit	18	6.9 (3.3, 13.8)	59	7.2 (4.2, 12.0)	77	7.1 (4.2, 11.9)
Newar	6	2.0 (7.6, 5.0)	39	4.1 (2.4, 6.8)	45	3.6 (2.2, 5.9)
Hill Janajati	75	25.6 (18.5, 34.1)	257	23.1 (17.8, 29.5)	332	23.7 (18.2, 30.1)
Terai Janajati	24	7.4 (4.4, 12.2)	72	5.1 (3.4, 7.5)	96	5.6 (3.9, 8.0)
Muslims	4	1.6 (0.4, 5.8)	42	4.6 (2.5, 8.3)	46	4.0 (2.2, 7.2)
Others	1		1		2	
Haemoglobin[b]	335	11.77(11.61, 11.93)	1127	11.85 (11.74, 11.96)	1462	11.83 (11.73, 11.93)
Anemia[c] (%)		21.3 (16.7, 26.9)		18.7 (15.3, 22.7)		19.3 (16.2, 22.8)
Anthropometry (%)						
Stunting	144	37.6 (30.1, 45.8)	409	35.2 (30.7, 40.0)	553	35.7 (31.6, 40.1)
Wasting	32	12.1 (7.6, 18.8)	124	12.0 (9.8, 14.6)	156	12.0 (9.6, 15.0)
Underweight	112	31.8 (25.2, 39.2)	331	29.8 (25.8, 34.2)	443	30.3 (26.3, 34.5)
Two week morbidity recall (%)						
Fever	115	33.3 (26.9, 40.4)	413	36.6 (32.7, 40.7)	528	35.9 (32.5, 39.4)
Cough	105	33.6 (27.5, 40.2)	439	38.8 (34.8, 43.1)	544	37.7 (34.1, 41.4)
Diarrhoea	78	25.5 (19.6, 32.3)	202	18.3 (15.3, 21.6)	280	19.8 (17.1, 22.9)
Took zinc tablet in last 7 days (%)	3	0.87 (0.26, 2.93)	14	0.95 (0.51, 1.75)	17	0.93 (0.53, 1.62)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 December 2020 doi:10.20944/preprints202012.0667.v1

CRP	335	1.85 (1.28, 2.41)	1127	1.98 (1.70, 2.25)	1462	1.95 (1.69, 2.20)
AGP	335	0.84 (0.76, 0.92)	1127	0.87 (0.84, 0.91)	1462	0.87 (0.83, 0.90)
Malaria (%)	0		0		0	
Helicobacter pylori (%)	80	22.6 (16.9, 29.5)	208	18.7 (15.4, 22.6)	288	19.6 (16.4, 23.2)
Any soil transmitted helminths[d] (%)	52	16.1 (10.6, 23.7)	128	11.2 (9.0, 13.8)	180	12.3 (9.8, 15.3)
Micronutrient status						
Serum ferritin	335	24.60(22.15, 27.05)	1127	26.72 (24.73, 28.71)	1462	26.26 (24.53, 27.98)
Iron deficiency by ferritin[e] (%)	71	23.5 (18.4, 29.6)	236	22.2 (18.6, 26.2)	307	22.5 (19.5, 25.8)
Serum RBP	335	0.97 (0.94, 1.01)	1127	1.03 (1.01, 1.05)	1462	1.01 (1.00, 1.03)
Vitamin A deficiency[f] (%)	5	6.6 (2.7, 15.1)	11	3.2 (1.6, 6.3)	16	4.0 (2.4, 6.6)
RBC folate	335	711.28(663.06, 759.51)	1127	709.34 (678.69, 739.99)	1462	709.76 (680.93, 738.59)
Risk of folate deficiency[g] (%)	15	5.2 (2.8, 9.6)	59	5.9 (3.8, 9.1)	74	5.8 (3.8, 8.8)
Serum Zinc	335	45.88 (43.64, 48.12)	1127	100.49 (97.58, 103.41)	1462	88.55 (85.32, 91.79)

Note. Ns are unweighted. Values presented are mean(95%CI) or percent(95%CI). All estimates account for weighting and complex sampling design. Abbreviations: AGP, α-1 acid glycoprotein; CI, confidence interval; CRP, C-reactive protein; RBC, red blood cell; RBP, retinol binding protein.

[a]Zinc deficiency defined as serum zinc <65.0 µg/ dL for nonfasted, morning (i. e. before 12 pm) samples and < 57.0 µg/ dL for non-fasted, afternoon (i. e. after 12 p.m.) samples, inflammation adjusted (IZiNCG 2012).

[b]Haemoglobin adjusted for altitude and smoking (WHO, 2017a).

[c] Anaemia defined as altitude-and smoking-adjusted Hb <12.0 g/ dL (WHO, 2017a).

[d]Soil-transmitted helminths including hookworm, Trichuris trichura, and Ascaris lumbricodes.

[e]Iron deficiency defined as inflammation-adjusted serum ferritin <15.0 µg/ L (WHO, 2017a).

[f]Vitamin A deficiency was defined as RBP <0.64 µmol/ L.

[g]Folate cut off based on the risk of megaloblastic anaemia defined as RBC folate <305.0 nmol/ L (Institute of Medicine 1998).

Table 2: Predictors of zinc deficiency among children aged 6 to 59 months, Nepal National Micronutrient Status Survey, Nepal, 2016

Potential predictors	**Unadjusted odds ratio (95% CI)**	**Adjusted odds ratio (95% CI)**	**P**
Rurality (%)			
Urban	1	1	
Rural	2.87 (1.51, 5.46)	2.25 (1.13, 4.49)	0.021
Household wealth quintile (%)			
Poorest	1	1	
Poorer	0.49 (0.34, 0.71)	0.50 (0.34, 0.74)	0.001
Middle	0.48 (0.31, 0.74)	0.49 (0.30, 0.82)	0.006
Richer	0.58 (0.35, 0.93)	0.67 (0.40, 1.23)	0.132

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 25 December 2020 doi:10.20944/preprints202012.0667.v1

Richest	0.35 (0.21, 0.61)	0.48 (0.25, 0.92)	0.028
Ethnicity			
Brahmin or Chettri	3.00 (0.91, 9.86)	2.98 (0.77, 11.45)	0.111
Other Terai Castes	2.72 (0.71, 10.39)	2.84 (0.66, 12.13)	0.158
Hill Dalit	2.61 (0.79, 8.65)	2.27 (0.58, 8.92)	0.24
Terai Dalit	2.72 (0.75, 9.82)	2.68 (0.63, 11.40)	0.18
Newar	1.36 (0.30, 6.10)	1.78 (0.32, 10.03)	0.511
Hill Janajati	3.12 (0.98, 9.96)	2.76 (0.72, 10.49)	0.136
Terai Janajati	4.10 (1.14, 14.75)	4.06 (0.99, 16.68)	0.052
Muslims	1	1	
Others	10.10 (3.25, 31.36)	6.07 (1.60, 23.02)	0.008
Diarrhea in last two week			
No	1	1	
Yes	1.53 (1.05, 2.23)	1.57 (1.07, 2.30)	0.02
Ln RBP in µmol/L	0.45 (0.26, 0.79)	0.49 (0.28, 0.85)	0.011

Among the non-pregnant women, the mean zinc concentration was 46.94 µg/dL in the deficient group, and the overall prevalence of zinc deficiency was 24.7% (N=497). Besides, anaemia and obesity were also one of the significant health problems among women [Table 3].Being underweight (AOR=1.60, 95% CI,[1.15, 2.23]), fever occurrence during two weeks preceding the survey (AOR=1.45, 95% CI,[1.06, 1.99]) and *Helicobacter pylori* detection in the stool (AOR=1.32, 95% CI, [1.03, 1.70]) were associated with oddsof developing zinc deficiency. The protective factors were being rich (AOR=0.64, 95% CI,[0.42, 0.98]) and being in risk of folate deficiency (AOR=0.60, 95% CI,[0.37, 0.96]), which were associated with decreased odds of zinc deficiency [Table 4].

Table 3: Socio-demographic and health characteristics of non-pregnant women aged 15-49 years by status of zinc deficiency, Nepal National Micronutrient Status Survey, Nepal, 2016

Socio-demographic and health characteristics	**Zinc deficiency[a] (N=497, 24.7% [95%CI 21.7, 27.9]**		**No Zinc deficiency (N=1426, 75.4% [95%CI 72.1, 78.3]**		**Total (N=1923)**	
	n		**n**		**n**	
Socio-demographic characteristics						
Age group, %						
15-29 years	235	44.4 (39.2, 49.7)	738	52.0 (48.8, 55.2)	973	50.1 (47.5, 52.7)
30-49 years	262	55.6 (50.3, 60.8)	688	48.0 (44.8, 51.2)	950	49.9 (47.3, 52.5)
Lactating, %	143	25.9 (22.9, 29.1)	393	23.7 (19.3, 28.8)	536	25.4 (22.7, 28.3)
Gave birth in last 5 years, %	210	38.3 (32.4, 44.5)	533	36.2 (32.9, 39.6)	743	36.7 (33.7, 39.8)
Married/cohabitating, %	70	86.2 (81.6, 89.7)	220	84.8 (81.8, 87.4)	290	85.1 (82.7, 87.3)
Rurality (%)						

	Rural	422	87.5 (80.6, 92.1)	1235	86.2 (78.6, 91.5)	1657	86.5 (79.4, 91.5)
	Urban	75	12.5 (7.9, 19.4)	191	13.8 (8.5, 21.4)	266	13.5 (8.5, 20.6)
Ecological zone (%)							
	Mountain	82	7.6 (5.4, 10.8)	240	5.9 (4.6, 7.7)	322	6.4 (5.0, 8.0)
	Hill	207	43.9 (36.7, 51.3)	615	44.6 (39.2, 50.1)	822	44.4 (39.5, 49.4)
	Terai	208	48.5 (41.3, 55.7)	571	49.5 (44.1, 55.0)	779	49.3 (44.4, 54.2)
Household wealth quintile (%)							
	Poorest	130	19.9 (15.1, 25.8)	299	13.8 (11.0, 17.3)	429	15.3 (12.3, 19.0)
	Poorer	114	21.1 (16.4, 26.6)	300	18.5 (15.0, 22.6)	414	19.1 (15.9, 22.8)
	Middle	91	18.8 (14.6, 23.8)	282	20.5 (17.1, 24.4)	373	20.1 (17.0, 23.5)
	Richer	74	16.0 (12.1, 20.8)	276	21.0 (17.3, 25.4)	350	19.8 (16.6, 23.5)
	Richest	88	24.3 (16.7, 33.9)	269	26.2 (19.7, 33.9)	357	25.7 (19.5, 33.1)
Ethnicity (%)							
	Brahmin or Chettri	201	37.2 (30.0, 44.9)	575	37.8 (32.1, 43.8)	776	37.6 (32.2, 43.4)
	Other Terai Castes	23	6.2 (3.2, 11.8)	83	9.9 (5.5, 17.0)	106	9.0 (5.2, 14.9)
	Hill Dalit	70	10.3 (7.1, 14.6)	160	8.1 (6.1, 10.7)	230	8.6 (6.7, 11.1)
	Terai Dalit	22	6.8 (3.4, 13.1)	59	6.4 (3.6, 10.9)	81	6.5 (3.8, 10.8)
	Newar	12	3.7 (1.8, 7.4)	52	5.1 (3.0, 8.5)	64	4.8 (2.8, 7.8)
	Hill Janajati	111	23.4 (17.4, 30.8)	344	21.8 (17.4, 26.8)	455	22.2 (17.9, 27.1)
	Terai Janajati	54	11.5 (7.3, 17.5)	122	8.9 (6.0, 12.9)	176	9.5 (6.5, 13.7)
	Muslims	4	1.0 (0.4, 2.7)	29	2.1 (0.9, 4.4)	33	1.8 (0.9, 3.7)
	Others	0		2		2	
Level of education (%)							
	Never attended school	189	37.2 (32.1, 42.7)	464	29.9 (25.4, 34.9)	653	31.7 (27.7, 36.0)
	Primary	71	16.1 (12.2, 21.0)	237	16.1 (13.8, 18.6)	308	16.1 (14.2, 18.2)
	Some secondary	175	34.8 (30.0, 40.0)	527	38.2 (34.5, 42.2)	702	37.4 (34.0, 40.9)
	Higher	62	11.9 (8.8, 15.8)	198	15.8 (13.1, 18.8)	260	14.8 (12.5, 17.5)
Haemoglobin[b]		497	12.88 (12.70, 13.05)	1426	13.00 (12.89, 13.12)	1923	12.97 (12.86, 13.08)
	Anemia[c] (%)	98	18.0 (15.4, 21.0)	222	22.0 (17.1, 27.8)	320	19.0 (16.4, 22.0)
BMI (%)							
	Underweight	102	18.4 (14.4, 23.2)	195	13.9 (11.7, 16.3)	297	15.0 (12.9, 17.3)
	Normal weight	286	54.0 (47.2, 60.6)	926	63.6 (59.6, 67.5)	1212	61.3 (58.0, 64.4)
	Overweight/Obese	109	27.7 (20.6, 36.1)	305	22.5 (19.1, 26.3)	414	23.8 (20.6, 27.3)
Two week morbidity recall (%)							
	Fever	95	18.0 (14.2, 22.5)	207	12.9 (10.6, 15.6)	302	14.1 (11.9, 16.7)
	Cough	88	16.3 (12.8, 20.6)	231	14.7 (12.3, 17.3)	319	15.1 (13.0, 17.4)
	Diarrhoea	48	9.6 (6.9, 13.3)	136	9.5 (7.6, 11.9)	184	9.6 (7.8, 11.6)
Took zinc tablet in last 7 days (%)		2	0.2 (0.04, 0.8)	2	0.5 (0.1, 2.5)	4	0.3 (0.08, 0.8)

CRP	497	1.58 (1.20, 1.95)	1426	1.35 (1.13, 1.57)	1923	1.41 (1.23, 1.59)
AGP	497	0.62 (0.59, 0.64)	1426	0.60 (0.58, 0.61)	1923	0.60 (0.59, 0.62)
Malaria (%)	0		0		0	
Helicobacter pylori (%)	224	45.4 (39.2, 51.6)	571	38.3 (34.5, 42.4)	795	40.1 (36.2, 44.0)
Any soil transmitted helminths[d] (%)	73	16.2 (12.1, 21.3)	261	18.9 (15.5, 22.8)	334	18.2 (15.2, 21.6)
Micronutrient status						
Serum ferritin	497	39.38 (36.31, 42.45)	1426	38.82 (36.76, 40.88)	1923	38.96 (37.07, 40.85)
Iron deficiency by ferritin[e] (%)	85	18.4 (14.0, 23.8)	251	18.8 (16.2, 21.7)	336	18.7 (16.3, 21.4)
Serum RBP	497	1.42 (1.37, 1.46)	1426	1.45 (1.42, 1.47)	1923	1.44 (1.41, 1.47)
Vitamin A deficiency[f] (%)	3	1.8 (0.5, 6.5)	8	3.0 (1.4, 6.1)	11	2.7 (1.4, 5.1)
RBC folate	497	616.92 (574.14, 659.70)	1426	591.27 (561.34, 621.20)	1923	597.59 (568.94, 626.24)
Risk of folate deficiency[g] (%)	58	7.5 (5.0, 11.1)	174	11.7 (9.3, 14.6)	232	10.7 (8.7, 13.1)
Zinc	497	46.94 (45.13, 48.76)	1426	96.25 (93.53, 98.97)	1923	84.10 (81.35, 86.84)

Note. Ns are unweighted. Values presented are mean(95%CI) or percent(95%CI). All estimates account for weighting and complex sampling design. Abbreviations: AGP, α-1 acid glycoprotein; BMI, Body Mass Index; CI, confidence interval; CRP, C-reactive protein; RBC, red blood cell; RBP, retinol binding protein.
[a]Zinc deficiency defined as serum zinc <66.0 µg/ dL for nonfasted, morning (i. e. before 12 pm) samples and < 59.0 µg/ dL for non-fasted, afternoon (i. e. after 12 p.m.) samples (IZiNCG 2012).
[b]Haemoglobin adjusted for altitude and smoking (WHO, 2017a).
[c] Anaemia defined as altitude-and smoking-adjusted Hb <12.0 g/ dL (WHO, 2017a).
[d]Soil-transmitted helminths including hookworm, Trichuris trichura, and Ascaris lumbricodes.
[e]Iron deficiency defined as inflammation-adjusted serum ferritin <15.0 µg/ L (WHO, 2017a).
[f]Vitamin A deficiency was defined as RBP <0.64 µmol/ L.
[g]Folate cut off based on the risk of megaloblastic anaemia defined as RBC folate <305.0 nmol/ L (Institute of Medicine 1998).

Table 4: Predictors of zinc deficiency s among non-pregnant women aged 15-49 years, Nepal National Micronutrient Status Survey, Nepal, 2016

Potential predictors	**Unadjusted odds ratio (95% CI)**	**Adjusted odds ratio (95% CI)**	P
Age group			
15-29 years	1	1	
30-49 years	1.36 (1.05, 1.76)	1.22 (0.89, 1.66)	0.215
Household wealth quintile			
Poorest	1	1	
Poorer	0.79 (0.55, 1.14)	0.80 (0.55, 1.16)	0.232
Middle	0.64 (0.44, 0.93)	0.66 (0.45, 0.95)	0.026
Richer	0.53 (0.35, 0.79)	0.53 (0.34, 0.83)	0.006
Richest	0.64 (0.43, 0.96)	0.64 (0.42, 0.98)	0.041
Level of education			
Never attended school	1	1	
Primary	0.81 (0.52, 1.25)	0.81 (0.52, 1.24)	0.33

	Some secondary	0.73 (0.55, 0.97)	0.88 (0.62, 1.25)	0.475
	Higher	0.60 (0.41, 0.89)	0.82 (0.53, 1.27)	0.369
Body Mass Index				
	Underweight	1.56 (1.11, 2.19)	1.60 (1.15, 2.23)	0.005
	Normal weight	1	1	
	Overweight/Obese	1.45 (0.92, 2.29)	1.54 (0.97, 2.46)	0.069
Fever in two week recall				
	No	1	1	
	Yes	1.48 (1.08, 2.03)	1.45 (1.06, 1.99)	0.021
Helicobacter pylori				
	No	1	1	
	Yes	1.34 (1.05, 1.71)	1.32 (1.03, 1.70)	0.027
Risk of folate deficiency[a]				
	no	1	1	
	Yes	0.61 (0.38, 0.98)	0.60 (0.37, 0.96)	0.035

Note. Estimates are unadjusted odds ratios and adjusted odds ratios with 95% confidence intervals from logistic regression models, accounting for weighting and complex sampling design.
[a]Folate cut off based on the risk of megaloblastic anaemia defined as RBC folate <305.0 nmol/ L (Institute of Medicine 1998).

Discussion:

This paper details the findings from the Nepal National Micronutrient Status Survey 2016 (NNMSS2016) focusing on zinc deficiency in pre-school children aged 6-59 months and non-pregnant women (NPW) aged 15-49 years. This is the first nationally representative data on the zinc status of Nepal. Our results suggest that zinc deficiency in Nepalese children and NPW is a significant nutritional problem. A few cross-sectional studies conducted among school-age children, women of reproductive age, and pregnant women have also shown a high prevalence of zinc deficiency in Nepal (Ram K. Chandyo et al., 2009; Nepal et al., 2014; Tamang, Yadav, Acharya, & Lamsal, 2020). Zinc deficiency is not only a public health problem in developing countries like Nepal, but recent data from some developed countries such as Japan and New Zealand also report a higher burden of zinc deficiency (Daniels et al., 2018; Yasuda & Tsutsui, 2016). We didn't find nationally representative data from India on zinc deficiency, a neighbouring country of Nepal that share similar socio-cultural and food practices(Gonmei& Toteja, 2018). However, several studies from various regions of India report that prevalence of zinc deficiency were high (43.8%) in 6-60 months children and about 53% in non-pregnant women (Kapil & Jain, 2011; Menon et al., 2011).

The National Demographic and Health Surveys (NDHS) from the Government of Nepal provide the nationally representative data on micronutrient status in the Nepalese population(Ministry of Health and Population (MOHP) [Nepal], New ERA , & Inc., 2012; Ministry of Health Nepal, New ERA, & ICF, 2017) but these measures have not measured zinc status as one of the parameters in assessing infant and maternal nutrition. The prevalence of zinc deficiency in the current study was 22.7% in children and 24.7% in NPW. Zinc deficiency is considered a public health concern when the prevalence reaches 20% (de Benoist, Darnton-Hill, Davidsson, Fontaine, & Hotz, 2007).A community-based study in Bhaktapur district had

observed >2/3rdof the participating non-pregnant women (13-35years)were zinc deficient(Ram K. Chandyo et al., 2009).Since there are no clinical trials aiming role of zinc supplementation in pregnancy and infant outcomes in Nepal, some cross-sectional community-based studies(Ram K. Chandyo et al., 2009)have reported higher burden of zinc deficiency across the country. Based on our findings, zinc deficiency is a public health concern in Nepal and thus, we suggest longitudinal studies or interventional studies targeting the risk groups of different ecological zones of the country.

Studies have shown that inflammation influences nutritional markers including zinc concentration (Karakochuk et al., 2017), one study has reported 31% of Nepalese children of 6-8 years of age had high levels of α-acid glycoprotein (AGP) and C-reactive protein (CRP), prominent markers of inflammation(Schulze et al., 2014). Also, an unpublished data from a baseline survey in 2012 showed a high percentage of children (6-23 months of age) with sub-clinical inflammation(Ministry of Health and Population et al., 2018).Therefore, the micronutrient concentration of the included participants in this study has been adjusted based on the values of AGP and CRP, which will further minimise bias(Strand, Adhikari, Chandyo, Sharma, & Sommerfelt, 2004).

Our finding showed that children residing in rural areas had higher risk of zinc deficiency compared with those from urban areas. An Ethiopian study also reported higher odds of zinc deficiency in pregnant women residing in rural areas (Kumera et al., 2015). The location of residence (rural vs urban) is a non-modifiable predictor of zinc status. The increased risk of zinc deficiency in children residing in rural areas might reflect the limited consumption of food products from animal sources compared with those living in urban areas. Meat products are quite expensive, and poor people often cannot afford to buy them. Animal products such as meat and oysters are a good source of zinc(Ma & Betts, 2000) which are very limited and often hard to get in the kitchen of rustic communities in Nepal. Plant-based diets, the typical Nepalese staple foods contain a high amount of phytates which are potent inhibitors of zinc absorption in the intestines(Lönnerdal, 2000). A study from Africa showed significant association of serum zinc status with dietary diversity in pregnant women(Kumera et al., 2015). However, this study did not assess food consumption pattern in the participants, which could have further highlighted the contribution of dietary intakeof zinc.

The occurrence of diarrhoea in children during two weeks preceding the survey was associated with increased risk of zinc deficiency. A study conducted among 6-35 months Nepalese children has reported significant association of dysentery with zinc status (Strand et al., 2004). Diarrhoea or dysentery leads to loss of body fluids and zinc can be excreted in stool (Castillo-Duran, Vial, & Uauy, 1988). Studies have suggested zinc supplementation improves the gastrointestinal mucosal integrity and promotes immune system, thereby potentially reducing severity and duration of diarrhoea (Lazzerini & Wanzira, 2016). Based on our findings, nutritional awareness, improving the dietary pattern to include meat products, provision of health/medical services and livelihood programs along with nutritional counselling for the people living in rural areas might help to improve zinc status as well as overall food habit to improve nutritional status. The body mass index is a modifiable predictor of zinc status in the NPW. As our data show that underweight increases the odds of zinc deficiency, maintaining body weight, fitness and consumption of adequate nutrients might help prevent zinc deficiency in those groups of NPW.

The infection of *H. pylori* increased the likelihood of zinc deficiency as did the fever occurring during two weeks preceding the survey. *H. pylori* infection is more commonly discussed in the context of anaemia(Cardaropoli, Rolfo, & Todros, 2014; John, Baltodano, Mehta, Mark, & Murthy, 2018) and evidence linking association of *H.pylori* with zinc status is scarce. A study in dyspepsia patients showed an association of *H. pylori*-induced gastric inflammation with reduced zinc concentration in gastric tissues. *H. pylori* infection can induce increased reactive oxygen isotypes resulting in oxidative stress and zinc deficiency further exacerbates the inflammation (Sempértegui et al., 2007). There are evidences that *H. pylori* infection can transmit in the family through personal contact and proper hygiene maintenance may prevent its transmission and spread (Salih, 2009). Relationship between fever and zinc deficiency is less clear; however, a study found that dengue diagnosed in children with zinc deficiency had slightly longer duration of fever and hospital stay as compared to children with normal zinc level(Rerksuppaphol& Rerksuppaphol, 2019). Probably zinc level drop in blood during fever due to higher hepatic synthesis of zinc binding acute phase proteins including metallothioneins (Gammoh & Rink, 2017).

Our study observed that good economic status (rich vs poor) and risk of folate deficiency were protective factors for zinc deficiency in NPW. Economic status is a non-modifiable factor. However, through proper economic support and livelihood opportunities from the government, people with low economic condition can gradually increase their living standard. The growth of income will support them to buy nutritious food that may help to improve their overall nutrition uptake. Low folate levels as a protective factor for zinc deficiency in our study is quite intriguing as many studies suggest no significant interaction between folate and zinc at the context of intestinal zinc absorption (Butterworth et al., 1988; Hambidge, Hackshaw, & Wald, 1993; Tamura et al., 1992). Evidence of the interaction between folate and zinc is less clear (Hansen et al., 2001). Thus, future studies, preferably interventional in design, should investigate the role of folate in maintaining zinc status in NPW.

Low zinc levels in high proportion of 6-59 months children and NPW of 15-49 years in our study suggest that zinc deficiency is a public health concern in Nepal. Our study identified several modifiable predictors of zinc deficiency such as body mass index, diarrhoea and fever occurrence, and appropriate intervention as discussed above could improve the zinc as well as the overall nutritional status of children and women. Interventional studies with zinc supplements, particularly in vulnerable groups are warranted to verify the relationship between zinc status and its observed risk factors.

Strengths and limitation of the study

The NNMSS 2016 is a nationally representative sample addressing all ecological regions and socio-demographic groups, which is its biggest strength. As far as our knowledge, this study is the first study from Nepal using nationally representative data in order to assess the predictors of zinc deficiencies. This study has some limitations as well, perhaps the greatest limitation of our study is its cross-sectional design; therefore, we could not ascertain the causality between the predictors and zinc deficiency. We were also unable to determine the contribution of dietary zinc on serum zinc levels because of a lack of dietary information.

Conclusion

 doi:10.20944/preprints202012.0667.v1

Our study suggests that zinc deficiency is a significant public health concern among NPW and children in Nepal, especially among children and NPW from rural communities with poor economic status. Community focussed interventional programs, awareness, counselling and sustainable livelihood policies may help to improve zinc status as well as overall health of the target population.

Acknowledgements

The authors thankfully acknowledge the participants of the study and also to field enumerators who spent tough houses in sample collection and transport.

Author contributions

SM conceptualized the study, acquired and analysed the data and supervised the manuscript writing, MKT, BR, UNY and RM wrote and reviewed the manuscript, KRP designed the study and supervised manuscript writing, Dipendra Raman Singh conceptualized, designed the study and supervised manuscript writing.

Conflicts of interests

The authors declare that there are no conflicts of interests on this manuscript.

References:

Ackland, M. L., Michalczyk, A. J. G., & nutrition. (2006). Zinc deficiency and its inherited disorders-a review. *1*(1), 41-49.

Adamo, A. M., Liu, X., Mathieu, P., Nuttall, J. R., Supasai, S., & Oteiza, P. I. J. F. i. c. n. (2019). Early developmental marginal zinc deficiency affects neurogenesis decreasing neuronal number and altering neuronal specification in the adult rat brain. *13*, 62.

Alam, K., Poudel, A., Palaian, S., Koirala, B., Shankar, P. R. J. J. o. P. P., & Medicine, C. (2017). Role of Zinc in childhood diarrhea management: a case of Nepal. *3*(1), 34-36.

Atasoy, H. I., & Bugdayci, G. J. P. I. (2018). Zinc deficiency and its predictive capacity for anemia: Unique model in school children. *60*(8), 703-709.

Brief, I. Z. N. C. G. J. I. T. (2007). Quantifying the risk of zinc deficiency: Recommended indicators. (1).

Brown, K. H., Peerson, J. M., Rivera, J., & Allen, L. H. J. T. A. j. o. c. n. (2002). Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *75*(6), 1062-1071.

Brown, K. H., Rivera, J. A., Bhutta, Z., Gibson, R. S., King, J. C., Lönnerdal, B., . . . bulletin, n. (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document# 1. Assessment of the risk of zinc deficiency in populations and options for its control. *25*(1 Suppl 2), S99-S203.

Butterworth, C. E., Jr., Hatch, K., Cole, P., Sauberlich, H. E., Tamura, T., Cornwell, P. E., & Soong, S. J. (1988). Zinc concentration in plasma and erythrocytes of subjects receiving folic acid supplementation. *Am J Clin Nutr, 47*(3), 484-486. doi:10.1093/ajcn/47.3.484

Cardaropoli, S., Rolfo, A., & Todros, T. (2014). Helicobacter pylori and pregnancy-related disorders. *World journal of gastroenterology, 20*(3), 654-664. doi:10.3748/wjg.v20.i3.654

Castillo-Duran, C., Vial, P., & Uauy, R. (1988). Trace mineral balance during acute diarrhea in infants. *J Pediatr, 113*(3), 452-457. doi:10.1016/s0022-3476(88)80627-9

Chandyo, R. K., Strand, T. A., Mathisen, M., Ulak, M., Adhikari, R. K., Bolann, B. J., & Sommerfelt, H. (2009). Zinc Deficiency Is Common among Healthy Women of Reproductive Age in Bhaktapur, Nepal. *The Journal of Nutrition, 139*(3), 594-597. doi:10.3945/jn.108.102111

Chandyo, R. K., Strand, T. A., Mathisen, M., Ulak, M., Adhikari, R. K., Bolann, B. J., & Sommerfelt, H. J. T. J. o. n. (2009). Zinc deficiency is common among healthy women of reproductive age in Bhaktapur, Nepal. *139*(3), 594-597.

Daniels, L., Williams, S. M., Gibson, R. S., Taylor, R. W., Samman, S., & Heath, A.-L. M. (2018). Modifiable "Predictors" of Zinc Status in Toddlers. *Nutrients, 10*(3), 306. doi:10.3390/nu10030306

de Benoist, B., Darnton-Hill, I., Davidsson, L., Fontaine, O., & Hotz, C. (2007). Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. *Food Nutr Bull, 28*(3 Suppl), S480-484. doi:10.1177/15648265070283s306

De Benoist, B., Darnton-Hill, I., Davidsson, L., Fontaine, O., Hotz, C. J. F., & bulletin, n. (2007). Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. *28*(3_suppl3), S480-S484.

Ezzati, M., Lopez, A. D., Rodgers, A. A., & Murray, C. J. (2004). *Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors*: World Health Organization.

Ford, N. D., Bichha, R., Parajuli, K. R., Paudyal, N., Joshi, N., Whitehead Jr, R. D., . . . Adhikari, D. P. J. T. J. o. N. (2020). Age, Ethnicity, Glucose-6-Phosphate Dehydrogenase Deficiency, Micronutrient Powder Intake, and Biomarkers of Micronutrient Status, Infection, and Inflammation Are Associated with Anemia Among Children 6–59 Months in Nepal. *150*(4), 929-937.

Gammoh, N. Z., & Rink, L. (2017). Zinc in Infection and Inflammation. *Nutrients, 9*(6), 624. doi:10.3390/nu9060624

Ghimire, P. R., Agho, K. E., Renzaho, A. M., Dibley, M., & Raynes-Greenow, C. J. P. o. (2018). Association between health service use and diarrhoea management approach among caregivers of under-five children in Nepal. *13*(3), e0191988.

Gonmei, Z., & Toteja, G. (2018). Micronutrient status of Indian population. *Indian Journal of Medical Research, 148*(5), 511-521. doi:10.4103/ijmr.IJMR_1768_18

Hambidge, M., Hackshaw, A., & Wald, N. (1993). Neural tube defects and serum zinc. *Br J Obstet Gynaecol, 100*(8), 746-749. doi:10.1111/j.1471-0528.1993.tb14266.x

Hansen, M., Samman, S., Madsen, L. T., Jensen, M., Sørensen, S. S., & Sandström, B. (2001). Folic acid enrichment of bread does not appear to affect zinc absorption in young women. *The American Journal of Clinical Nutrition, 74*(1), 125-129. doi:10.1093/ajcn/74.1.125

Jiang, T., Christian, P., Khatry, S. K., Wu, L., & West Jr, K. P. J. T. J. o. n. (2005). Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. *135*(5), 1106-1112.

John, S., Baltodano, J. D., Mehta, N., Mark, K., & Murthy, U. (2018). Unexplained iron deficiency anemia: does Helicobacter pylori have a role to play? *Gastroenterology Report, 6*(3), 215-220. doi:10.1093/gastro/goy001

Kapil, U., & Jain, K. (2011). Magnitude of zinc deficiency amongst under five children in India. *Indian J Pediatr, 78*(9), 1069-1072. doi:10.1007/s12098-011-0379-z

Karakochuk, C. D., Barr, S. I., Boy, E., Bahizire, E., Tugirimana, P. L., Akilimali, P. Z., . . . Green, T. J. (2017). The effect of inflammation on serum zinc concentrations and the prevalence estimates of population-level zinc status among Congolese children aged 6–59 months. *European Journal of Clinical Nutrition, 71*(12), 1467-1470. doi:10.1038/ejcn.2017.127

King, J. C. J. T. A. j. o. c. n. (2000). Determinants of maternal zinc status during pregnancy. *71*(5), 1334S-1343S.

kIZiNCG. (2007). *Assessing population zinc status with serum zinc concentration. Technical Brief No.2, 2007*. Retrieved from

Kumera, G., Awoke, T., Melese, T., Eshetie, S., Mekuria, G., Mekonnen, F., . . . Gedle, D. (2015). Prevalence of zinc deficiency and its association with dietary, serum albumin and intestinal parasitic infection among pregnant women attending antenatal care at the University of Gondar Hospital, Gondar, Northwest Ethiopia. *BMC Nutrition, 1*(1), 31. doi:10.1186/s40795-015-0026-6

Lazzerini, M., & Wanzira, H. (2016). Oral zinc for treating diarrhoea in children. *Cochrane Database of Systematic Reviews*(12). doi:10.1002/14651858.CD005436.pub5

Livingstone, C. J. N. i. C. P. (2015). Zinc: physiology, deficiency, and parenteral nutrition. *30*(3), 371-382.

Lönnerdal, B. (2000). Dietary Factors Influencing Zinc Absorption. *The Journal of Nutrition, 130*(5), 1378S-1383S. doi:10.1093/jn/130.5.1378S

Lonnerdal, B. J. T. J. o. n. (2000). Dietary factors influencing zinc absorption. *130*(5), 1378S-1383S.

Ma, J., & Betts, N. M. (2000). Zinc and Copper Intakes and Their Major Food Sources for Older Adults in the 1994–96 Continuing Survey of Food Intakes by Individuals (CSFII). *The Journal of Nutrition, 130*(11), 2838-2843. doi:10.1093/jn/130.11.2838

MacDonald, R. S. J. T. J. o. n. (2000). The role of zinc in growth and cell proliferation. *130*(5), 1500S-1508S.

Mackenzie, E. L., Iwasaki, K., Tsuji, Y. J. A., & signaling, r. (2008). Intracellular iron transport and storage: from molecular mechanisms to health implications. *10*(6), 997-1030.

Menon, K. C., Skeaff, S. A., Thomson, C. D., Gray, A. R., Ferguson, E. L., Zodpey, S., . . . Pandav, C. S. (2011). Concurrent micronutrient deficiencies are prevalent in nonpregnant rural and tribal women from central India. *Nutrition, 27*(4), 496-502. doi:10.1016/j.nut.2010.02.012

Ministry of Health and Population (MOHP) [Nepal], New ERA , & Inc., I. I. (2012). *Nepal Demographic and Health Survey 2011*. Retrieved from Kathmandu, Nepal:

Ministry of Health and Population, N., New ERA, UNICEF, EU, USAID, & CDC. (2018). *Nepal National Micronutrient Status Survey, 2016*. Retrieved from Kathmandu, Nepal:

Ministry of Health Nepal, New ERA, & ICF. (2017). *Nepal Demographic and Health Survey 2016*. Retrieved from Kathmandu, Nepal:

Nepal, A. K., Gelal, B., Mehta, K., Lamsal, M., Pokharel, P. K., & Baral, N. (2014). Plasma zinc levels, anthropometric and socio-demographic characteristics of school children in eastern Nepal. *BMC research notes, 7*, 18-18. doi:10.1186/1756-0500-7-18

Organization, W. H. (2002). *The world health report 2002: reducing risks, promoting healthy life*: World Health Organization.

Prasad, A. S. J. T. A. j. o. c. n. (1991). Discovery of human zinc deficiency and studies in an experimental human model. *53*(2), 403-412.

Report, W. H. O. J. T. W. H., & Reducing risks, p. h. l. (2002). Quantifying selected major risks to health.

Rerksuppaphol, L., & Rerksuppaphol, S. (2019). Zinc deficiency in children with Dengue viral infection. *Pediatric reports, 11*(1), 7386-7386. doi:10.4081/pr.2019.7386

Salih, B. A. (2009). Helicobacter pylori infection in developing countries: the burden for how long? *Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association, 15*(3), 201-207. doi:10.4103/1319-3767.54743

Sazawal, S., Black, R. E., Menon, V. P., Dinghra, P., Caulfield, L. E., Dhingra, U., & Bagati, A. J. P. (2001). Zinc supplementation in infants born small for gestational age reduces mortality: a prospective, randomized, controlled trial. *108*(6), 1280-1286.

Schulze, K. J., Christian, P., Wu, L. S.-F., Arguello, M., Cui, H., Nanayakkara-Bind, A., . . . West, K. P., Jr. (2014). Micronutrient Deficiencies Are Common in 6- to 8-Year-Old Children of Rural Nepal, with Prevalence Estimates Modestly Affected by Inflammation. *The Journal of Nutrition, 144*(6), 979-987. doi:10.3945/jn.114.192336

Sempértegui, F., Díaz, M., Mejía, R., Rodríguez-Mora, O. G., Rentería, E., Guarderas, C., . . . Reeves, P. G. (2007). Low concentrations of zinc in gastric mucosa are associated with increased severity of Helicobacter pylori-induced inflammation. *Helicobacter, 12*(1), 43-48. doi:10.1111/j.1523-5378.2007.00476.x

Sigel, A., Sigel, H., & Sigel, R. K. (2013). *Interrelations between essential metal ions and human diseases* (Vol. 13): Springer.

Strand, T. A., Adhikari, R. K., Chandyo, R. K., Sharma, P. R., & Sommerfelt, H. (2004). Predictors of plasma zinc concentrations in children with acute diarrhea. *The American Journal of Clinical Nutrition, 79*(3), 451-456. doi:10.1093/ajcn/79.3.451

Tamang, M. K., Yadav, U. N., Acharya, A., & Lamsal, M. (2020). Assessment of Zinc Status Among Pregnant Women Attending a Tertiary Level Hospital of Sunsari District, Nepal. *J Nepal Health Res Counc, 17*(4), 468-473. doi:10.33314/jnhrc.v17i4.2065

Tamura, T., Goldenberg, R. L., Freeberg, L. E., Cliver, S. P., Cutter, G. R., & Hoffman, H. J. (1992). Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. *Am J Clin Nutr, 56*(2), 365-370. doi:10.1093/ajcn/56.2.365

Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials %J The American journal of clinical nutrition. (2000). *72*(6), 1516-1522.

Wessells, K. R., & Brown, K. H. J. P. o. (2012). Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *7*(11), e50568.

Whitehead Jr, R. D., Zhang, M., Sternberg, M. R., Schleicher, R. L., Drammeh, B., Mapango, C., & Pfeiffer, C. M. J. C. b. (2017). Effects of preanalytical factors on hemoglobin measurement: a comparison of two HemoCue® point-of-care analyzers. *50*(9), 513-520.

Whitehead, R. D., Zhang, M., Sternberg, M. R., Schleicher, R. L., Drammeh, B., Mapango, C., & Pfeiffer, C. M. (2017). Effects of preanalytical factors on hemoglobin measurement: A comparison of

two HemoCue® point-of-care analyzers. *Clinical Biochemistry, 50*(9), 513-520. doi:https://doi.org/10.1016/j.clinbiochem.2017.04.006

WHO. (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization. Retrieved from (http://www.who.int/vmnis/indicators/haemoglobin.pdf

Yasuda, H., & Tsutsui, T. (2016). Infants and elderlies are susceptible to zinc deficiency. *Scientific reports, 6*, 21850-21850. doi:10.1038/srep21850