December 2017

Prevalence and possible factors associated with anaemia, and vitamin B_{12} and folate deficiencies in women of reproductive age in Pakistan: Analysis of national-level secondary survey data

Sajid Bashir Soofi
Aga Khan University, sajid.soofi@aku.edu

Gul Nawaz Khan
Aga Khan University, gul.nawaz@aku.edu

Kamran Sadiq
Aga Khan University, kamran.sadiq@aku.edu

Shabina Ariff
Aga Khan University, shabina.ariff@aku.edu

Atif Habib
Aga Khan University, atif.habib@aku.edu

Authors
Sajid Bashir Soofi, Gul Nawaz Khan, Kamran Sadiq, Shabina Ariff, Atif Habib, Sumra Kureishy, Imtiaz Hussain, Muhammad Umer, Zamir Suhag, Arjumand Rizvi, and Zulfiqar Ahmed Bhutta

BMJ Open

Prevalence and possible factors associated with anaemia, and vitamin B_{12} and folate deficiencies in women of reproductive age in Pakistan: analysis of national-level secondary survey data

Sajid Soofi,[1] Gul Nawaz Khan,[1] Kamran Sadiq,[1] Shabina Ariff,[1] Atif Habib,[1] Sumra Kureishy,[1] Imtiaz Hussain,[1] Muhammad Umer,[1] Zamir Suhag,[1] Arjumand Rizvi,[1] Zulfiqar Bhutta[1,2]

To cite: Soofi S, Khan GN, Sadiq K, *et al*. Prevalence and possible factors associated with anaemia, and vitamin B_{12} and folate deficiencies in women of reproductive age in Pakistan: analysis of national-level secondary survey data. *BMJ Open* 2017;**7**:e018007. doi:10.1136/bmjopen-2017-018007

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2017-018007).

Received 7 June 2017
Revised 20 September 2017
Accepted 3 November 2017

[1]Department of Paediatrics and Child Health, Aga Khan University, Karachi, Pakistan
[2]Global Child Health at the Hospital for Sick Children, Toronto, Canada

Correspondence to
Dr Sajid Soofi;
sajid.soofi@aku.edu

ABSTRACT

Objective To determine the prevalence and possible factors associated with anaemia, and vitamin B_{12} and folate deficiencies in women of reproductive age (WRA) in Pakistan.

Methods A secondary analysis was conducted on data collected through the large-scale National Nutrition Survey in Pakistan in 2011. Anaemia was defined as haemoglobin levels <12 g/dL, vitamin B_{12} deficiency as serum vitamin B_{12} levels of <203 pg/mL (150 pmol/L) and folate deficiency as serum folate levels <4 ng/mL (10 nmol/L).

Results A total of 11 751 blood samples were collected and analysed. The prevalence of anaemia, vitamin B_{12} deficiency and folate deficiency was 50.4%, 52.4% and 50.8%, respectively. After adjustment, the following factors were positively associated with anaemia: living in Sindh province (RR 1.07; 95% CI 1.04 to 1.09) P<0.00, food insecure with moderate hunger (RR 1.03; 95% CI 1.00 to 1.06) P=0.02, four or more pregnancies (RR 1.03; 95% CI 1.01 to 1.05) P<0.00, being underweight (RR 1.03; 95% CI 1.00 to 1.05) P=0.02, being overweight or obese (RR 0.95; 95% CI 0.93 to 0.97) P<0.00 and weekly intake of leafy green vegetables (RR 0.98; 95% CI 0.95 to 1.00) P=0.04. For vitamin B_{12} deficiency, a positive association was observed with rural population (RR 0.81; 95% CI 0.66 to 1.00) P=0.04, living in Khyber Pakhtunkhwa province (RR 1.25; 95% CI 1.11 to 1.43) P<0.00 and living in Azad Jammu and Kashmir (RR 1.50; 95% CI 1.08 to 2.08) P=0.01. Folate deficiency was negatively associated with daily and weekly intake of eggs (RR 0.89; 95% CI 0.81 to 0.98) P=0.02 and (RR 0.88; 95% CI 0.78 to 0.99) P=0.03.

Conclusions In Pakistan, anaemia, and vitamin B_{12} and folate deficiencies are a severe public health concern among WRA. Our findings suggest that further research is needed on culturally appropriate short-term and long-term interventions within communities and health facilities to decrease anaemia, and vitamin B_{12} and folate deficiencies among Pakistani women.

Strengths and limitations of this study

- This study was national in scope and included all districts of Pakistan.
- This is the first study to examine the prevalence and possible factors associated with anaemia, and folate and vitamin B_{12} deficiencies in Pakistan.
- The study sheds light on possible factors associated with anaemia, and folate and vitamin B_{12} deficiencies from a large-scale data.
- Due to our study focusing on secondary data, causality cannot be determined.

INTRODUCTION

In low-income and middle-income countries, anaemia is a major public health problem.[1] The populations most affected by anaemia are women of reproductive age (WRA) (15–49 years of age), pregnant women and children.[2] It is estimated that anaemia affects 29% (496 million) of WRA, 38% of pregnant women and 43% of children worldwide.[3] Anaemia, assessed through haemoglobin concentrations, is an indicator of iron deficiency. However, iron deficiency is not the only underlying cause of anaemia, vitamin B_{12} and folate deficiencies also result in anaemia.[4]

Globally, iron deficiency is the most prevalent nutrient deficiency, affecting an estimated 2 billion people.[2] In Southeast Asia, it is estimated that iron deficiency anaemia affects about 50% of WRA.[3 5] According to WHO, the prevalence of folate and vitamin B_{12} deficiencies may be a public health concern affecting millions of people worldwide.[6] The prevalence of these deficiencies is derived from small local and national surveys. Therefore, due to limited population-based data, the global prevalence of folate and vitamin B_{12} deficiencies remain undetermined.

In Pakistan, previous national-level data on the prevalence of vitamin B_{12} and folate

deficiencies do not exist. However, a small-scale cross-sectional study in two urban settings found 6.8% of Pakistani male and female adults aged 46+10.5 years were vitamin B_{12} deficient, while 39.7% were folate deficient in 2009.[7] According to the National Nutrition Survey (NNS) 2001, the prevalence of anaemia was 28.4% in WRA.[8] The NNS had covered all provinces of Pakistan with representative population-based samples.

The presence of these micronutrient deficiencies in WRA can result in adverse birth outcomes,[9] increasing the risk of morbidity and mortality during pregnancy,[10–13] neural tube defects (NTDs),[10] low birth weight (LBW)[10–13] and premature birth.[14] Anaemia is also associated with an elevated risk of infections, impaired physical and cognitive development, and poor school performance among offspring.[14] Folic acid deficiency, specifically, is associated with megaloblastic anaemia, NTDs and a higher risk of LBW babies.[10] Vitamin B_{12} deficiency has also been identified as a risk factor for NTDs, early fetal loss, failure-to-thrive, stunting, poor neurocognitive function and developmental delays.[15 16]

Currently, none of the wheat flour millers in Pakistan are fortifying their products on a voluntary basis. Except for Punjab province, there is no current, mandatory legislation in Pakistan for wheat flour fortification (ie, with iron and folic acid). In 1965, fortification of edible oil was mandated by legislation at the federal level and has been retained in the provincial food laws in all four provinces, but it is inadequately enforced. The prevalence and local factors associated with anaemia, and vitamin B_{12} and folate deficiencies in women have not been previously identified in literature. To identify the prevalence and possible factors, we conducted a secondary analysis of data collected through a large-scale national survey in 2011.[17]

METHODS

Data source

The present study used the data subset of the 2011 NNS. The NNS is a cross-sectional survey that used a mixed-methods approach to collect representative data on the health and nutritional status of women and children in Pakistan.[17] The survey components were selected from previous NNS and similar international surveys. Trained female data collectors administered the surveys through face-to-face interviews. A structured questionnaire was used to obtain information on household characteristics, food security, maternal and child health, and nutrition status. For qualitative data collection, semistructured interviews and focus-group discussions were used to gain information on food consumption patterns, nutrition and food behaviour, and other factors affecting decision-making. A cluster sampling design was used to select a representative sample size of households in Pakistan. The survey data are publicly available.

Ethical considerations

Informed consent was obtained from all study participants prior to data and blood sample collection.

Sampling

Sampling frame of Federal Bureau of Statistics (FBS) was used for 2011 NNS. FBS has its own sampling frame for all urban and rural areas of Pakistan in the form of enumeration block. Each enumeration block consists of about 200–250 households with well-defined boundaries. There are 26 753 enumeration blocks in all urban areas of the country. One thousand five hundred enumeration blocks were provided by FBS for the survey from all provinces of Pakistan. Each enumeration block was demarcated, mapped and listed before the actual data collection, and from each enumeration block 20 households were selected randomly through a computer programme. A two-staged sampling technique was used to select the households for interview and blood sampling. In the first stage of sampling, enumeration blocks in urban and rural areas were taken as primary sampling units (PSUs). In the second stage, 20 households with target population from each urban and rural sample PSU were selected with equal probability using a systematic sampling technique with a random start.

Study population

In total, 27 963 households responded to the 2011 NNS. The survey participants were WRA aged 15–49 years, children under 5 years of age, children between 6 and 12 years of age and older adults (men and women aged 50 years or older). For the present study, the participants were limited to WRA, resulting in a final sample size of 22 278 women.

Sample size

A sample size of 30 000 households was calculated to provide representative results of NNS 2011. For biochemical analysis, 51% prevalence of anaemia in women from NNS 2001 was taken as an indicator for sample-size estimation with a precision of 2%, design effect of 1.6% and 90% power of the study. The final sample size for WRA was 9836 with 15% attrition rate. We selected households, which were having a pair of mother and under-five children, for blood sampling.

Assessment of anaemia, and vitamin B_{12} and folate deficiencies

Blood samples were collected from WRA for the assessment of anaemia (n=10 787), vitamin B_{12} deficiency (n=8400) and folate deficiency (n=8371). Sample centrifugation and serum separation were conducted at the field site within 30 min of sample collection. Serum vitamin B_{12} and folate were measured with an electrochemiluminescence immunoassay method using Elecsys 2010 (Roche diagnostics, Bernried, Germany). Vitamin B_{12} deficiency was defined as serum vitamin B_{12} levels of <203 pg/mL (150 pmol/L).[18] Folate deficiency was defined as serum folate levels <4 ng/mL (10 nmol/L).[19] Haemoglobin concentration was measured with a HemoCue microcuvette machine. In WRA, anaemia was defined as haemoglobin levels <12 g/dL.[20]

A countrywide network of laboratories and collection centres was used to maintain the blood cold chain and ensure the viability of samples. The samples were transported through the countrywide network of Aga Khan University (AKU) laboratories and collection centres to the Nutrition Research Laboratory (NRL) at AKU in Karachi for analysis. Quality control of collected samples was monitored through the National Institute of Standards and Technology Standard Reference Materials. For external quality control, the NRL verified the quality of the collected samples through the Center of Disease Control proficiency programme. Blood samples were collected between January and June 2011, and analysed between July and September 2011.

Assessment of possible factors

Possible factors for analysis were selected based on literature and availability in the dataset; age, residence area, household socioeconomic status (SES), household food insecurity status, literacy status, employment status, number of pregnancies, birth interval, worm infestation, body mass index (BMI) and dietary intake. As part of survey administration, anthropometric measurements were conducted by trained female data collectors. The measurements were (height, weight and mid-upper arm circumference) obtained using standard protocol. BMI was calculated as weight in kilograms divided by height in metre squared and categorised into underweight (<18.5 kg/m^2), normal weight (18.5–24.99 kg/m^2), overweight (25–34.99 kg/m^2) and obese (≥35 kg/m^2). Dietary intake was assessed using 24-hour dietary recalls. A Food Frequency Questionnaire was used to estimate the consumption of various food groups. The respondents were asked to report all of the food, beverages and/or supplements that they have consumed during the past 24 hours. Food frequency per day, per week and per month was estimated. Information on other factors—age, literacy status, SES, food insecurity, employment status, number of pregnancies and worm infestation—was obtained through the survey.

Description of variables

The analysis reported is based on a sample of 22 278 non-pregnant women. We modelled three outcomes: (1) anaemia, (2) folate and (3) vitamin B_{12} deficiency. All outcome variables are dichotomous in nature. The selection of explanatory variables for analysis was informed by the literature and their availability in the dataset. Common predictors in each model were area of residence, economic status of the household (wealth quintile), food insecurity status, age, education, work status, number of living children (parity), birth interval, worm infestation, dietary intake and BMI of women

Statistical analysis

Data analyses was conducted by using STATA V.15 (Stata) with 'Svy' commands to allow for adjustments for the multistage sampling design used in the survey. The frequencies along with weighted percentage were calculated for the selected variables.

To estimate dietary intake, the following food groups were included in the analysis: green leafy vegetables, red meat, regular consumption of tea, dairy products and eggs. The household food security was determined on the basis of four categories: food secure, food insecure without hunger, food insecure with moderate hunger and food insecure with severe hunger.

As the prevalence of all of the outcomes is high, prevalence risk ratio (RR) was calculated using generalised linear model approach with Poisson distribution and log link function. The independent variables were grouped in three broad categories: (1) area, (2) household characteristics and (3) women level indicators.

All variables with a P value ≤0.25 at univariate level were included in a multivariate model, which were constructed using stepwise backward elimination procedure. However, area and demographic variables, that is, household SES and food insecurity, were considered for adjustment in all models irrespective of their statistical significance. A sensitivity analysis is also performed after removing these factors if they are not significantly associated with the outcomes. The results show no difference with removing these variables. Prevalence RRs were estimated as the exponential of the regression coefficients, and 95% CI for the RRs was calculated. A P value ≤0.10 was considered significant for multivariate analysis. The analysis was adjusted for the survey design, that is, stratification, clustering and probability of selection. The sampling weights were applied at cluster level.

RESULTS

Of the 22 278 WRA, 13 188 (69%) were from rural areas and 9090 (31%) were from urban areas. Majority of these women were from the provinces of Punjab (51.5%) and Sindh (22.3%), with a mean age of 31.80±7.76 years and literacy rate of 59.4%. The SES quintiles (poorest, poorer, middle, richer and richest) were almost equally represented in the sample. Among WRA, 5649 women (41.6%) were food secure, 3626 women (28.3%) were food insecure without hunger, 2659 (20.2%) were food insecure with moderate hunger and 1284 (9.9%) were food insecure with severe hunger. Approximately 94% of the women were unemployed, and 52% were of normal BMI (18.5–24.99 kg/m^2) (table 1). The overall prevalence among WRA was 50.4% for anaemia, 50.8% for folate deficiency and 52.4% for vitamin B_{12} deficiency (table 2).

After multivariable adjustment between anaemia and participant characteristics, women living in Sindh province (RR 1.07; 95% CI 1.04 to 1.09; P<0.00), households identified as food insecure with moderate hunger (RR 1.03; 95% CI 1.00 to 1.06; P-0.02), having four or more pregnancies (RR 1.03; 95% CI 1.01 to 1.05; P-0.00) and being underweight (RR 1.03; 95% CI 1.00 to 1.05; P-0.02) were significantly more likely to be anaemic. Alternatively, women living in Khyber Pakhtunkhwa (RR 0.93; 95% CI

Table 1 Background characteristics of women of reproductive age (n=22 278)

Key variables	N	Per cent
Area of residence		
Urban	9090	31.0
Rural	13 188	69.0
Distribution by province		
Punjab	10 156	51.5
Sindh	4805	22.3
KPK	2893	14.8
Balochistan	1786	5.0
FATA	850	3.4
AJK	1173	2.5
Gilgit	615	0.6
Household wealth quintiles		
Poorest	4400	20.7
Poorer	4592	21.6
Middle	4514	20.4
Richer	4491	19.5
Richest	4281	17.7
Food security (n=13 218)		
Food secure	5649	41.6
Food insecure without hunger	3626	28.3
Food insecure with moderate hunger	2659	20.2
Food insecure with severe hunger	1284	9.9
Age		
15–19 years	331	1.6
20–29 years	8513	37.9
30–39 years	9702	43.3
40–49 years	3732	17.3
Education (n=22 063)		
Illiterate	9165	40.6
Literate	12 898	59.4
Occupation		
Employed	1357	6.1
Unemployed	20 921	93.9
No of pregnancies (n=21 720)		
≤4	13 663	63.5
>4	8057	36.5
Birth interval (n=8926)		
≥36 months	1095	11.9
<36 months	7831	88.1
BMI of women (n=21 677)		
Underweight (<18.5)	3024	14.3
Normal (18.5–24.99)	11 057	51.9
Overweight (25.0–34.9)	6936	30.9
Obesity (>=35)	660	2.9

AJK, Azad Jammu and Kashmir; BMI, body mass index; FATA, Federally Administered Tribal Areas; KPK, Khyber Pakhtunkhwa.

Table 2 Prevalence of micronutrient deficiencies in women of reproductive age

Micronutrient deficiencies	%	SE	95% CI Lower	95% CI Upper	n
Anaemia	50.4	0.5	49.4	51.5	10 787
Folate deficiency	50.8	0.6	49.7	51.9	8371
Vitamin B_{12} deficiency	52.4	0.6	51.3	53.5	8400

0.89 to 0.96; P<0.00), Azad Jammu and Kashmir (RR 0.93; 95% CI 0.88 to 0.98; P-0.00) and Gilgit-Baltistan (RR 0.81; 95% CI 0.76 to 0.85; P<0.00), and weekly intake of leafy green vegetables (RR 0.98; 95% CI 0.95 to 1.00; P-0.04), and overweight or obese women (RR 0.95; 95% CI 0.93 to 0.97; P<0.00) were significantly less likely to be anaemic. All other factors (residence area, wealth quintiles, age, education, occupation, birth interval, worm infestation, use of iron folate/folic acid/multiple micronutrients (MMN) during last pregnancy and dietary intake) were not significantly associated with anaemia (table 3).

Women living in provinces Khyber Pakhtunkhwa (RR 0.77; 95% CI 0.66 to 0.91; P<0.00) and Azad Jammu and Kashmir (RR 0.52; 95% CI 0.37 to 0.75; P<0.00) were less likely to be folate deficient as compared with other provinces in the country. Daily and weekly intake of eggs (RR 0.89; 95% CI 0.81 to 0.98; P-0.02, and RR 0.88; 95% CI 0.78 to 0.99; P-0.03, respectively) were less likely to be folate deficient as compared with egg intake on monthly basis (table 3).

Women living in rural areas (RR 0.81; 95% CI 0.66 to 1.00; P-0.04) were less likely to be vitamin B_{12} deficient compared with those living in urban areas. Furthermore, women living in provinces Khyber Pakhtunkhwa (RR 1.25; 95% CI 1.11 to 1.43; P<0.00) and Azad Jammu and Kashmir (RR 1.50; 95% CI 1.08 to 2.08; P-0.01) were more likely to be vitamin B_{12} deficient compared with other provinces in the country. Age, education, use of iron folate/folic acid/MMN during last pregnancy, birth interval, having worm infestation, intake of red meat, regular consumption of tea and intake of dairy products were not significantly associated with vitamin B_{12} and folate deficiencies (table 3).

DISCUSSION

We estimated the prevalence of anaemia, and folate and vitamin B_{12} deficiencies among WRA from a nationally representative sample in Pakistan. Substantially, more than half of Pakistani WRA were found to be anaemic, and vitamin B_{12} and folate deficient in 2011. Based on literature, a prevalence of ≥40.0% is indicative of a severe public health problem.[21] Thus, our findings suggest that these deficiencies are a severe public health problem in Pakistani WRA.

Similar to our findings, previous surveys also found a high prevalence of anaemia among WRA.[8 22] Generally,

Table 3 Multivariate relative risks and 95% CIs of anaemia, folate deficiency and vitamin B_{12} deficiency among women of reproductive age

	Anaemia				Folate deficiency				Vitamin B_{12} deficiency			
	Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
Predictors	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**
Area of residence												
Rural	1.01 (0.99 to 1.02)	0.222	0.99 (0.97 to 1.02)	0.614	0.94 (0.87 to 1.02)	0.192	0.95 (0.85 to 1.05)	0.304	0.99 (0.92 to 1.08)	0.973	0.81 (0.66 to 1.00)	0.048
Urban	Ref		Ref		Ref		Ref		Ref		Ref	
Province												
Punjab	Ref		Ref		Ref		Ref		Ref		Ref	
Sindh	1.09 (1.07 to 1.11)	<0.001	1.07 (1.04 to 1.09)	<0.001	1.01 (0.92 to 1.12)	0.768	0.94 (0.84 to 1.06)	0.328	0.89 (0.80 to 0.98)	0.022	1.25 (1.01 to 1.55)	0.043
Khyber Pakhtunkhwa	0.91 (0.88 to 0.94)	<0.001	0.93 (0.89 to 0.96)	<0.001	0.67 (0.58 to 0.79)	<0.001	0.77 (0.66 to 0.91)	0.002	1.26 (1.13 to 1.41)	<0.001	1.25 (1.11 to 1.43)	<0.001
Balochistan	1.00 (0.96 to 1.05)	0.912	0.97 (0.92 to 1.02)	0.262	1.06 (0.92 to 1.21)	0.413	1.05 (0.90 to 1.24)	0.536	0.80 (0.69 to 0.93)	0.004	1.33 (0.47 to 3.77)	0.587
Federally Administered Tribal Areas	1.00 (0.95 to 1.05)	0.924	0.99 (0.93 to 1.06)	0.825	0.75 (0.54 to 1.04)	0.083	0.85 (0.60 to 1.20)	0.349	1.06 (0.87 to 1.29)	0.556	1.04 (0.86 to 1.27)	0.554
Azad Jammu and Kashmir	0.95 (0.91 to 0.99)	0.008	0.93 (0.88 to 0.98)	0.005	0.52 (0.37 to 0.74)	<0.001	0.52 (0.37 to 0.75)	<0.001	1.17 (1.03 to 1.32)	0.018	1.50 (1.08 to 2.08)	0.015
Gilgit-Baltistan	0.83 (0.79 to 0.87)	<0.001	0.81 (0.76 to 0.85)	<0.001	0.97 (0.81 to 1.17)	0.761	0.96 (0.74 to 1.23)	0.733	1.05 (0.94 to 1.18)	0.374	1.28 (0.79 to 2.09)	0.317
Household SES quintiles												
Poorest	1.09 (1.06 to 1.12)	<0.001	1.03 (0.99 to 1.07)	0.172	1.07 (0.96 to 1.19)	0.176	1.08 (0.92 to 1.26)	0.328	0.88 (0.80 to 0.98)	0.021	1.21 (0.83 to 1.77)	0.329
Poorer	1.03 (1.01 to 1.06)	0.005	1.02 (0.98 to 1.05)	0.318	0.98 (0.88 to 1.09)	0.731	1.05 (0.91 to 1.21)	0.497	0.93 (0.85 to 1.03)	0.208	1.37 (0.96 to 1.94)	0.080
Middle	1.03 (1.00 to 1.05)	0.016	1.01 (0.98 to 1.04)	0.500	0.97 (0.88 to 1.06)	0.571	1.02 (0.91 to 1.14)	0.78	1.03 (0.95 to 1.12)	0.418	1.21 (0.88 to 1.68)	0.241
Richer	1.01 (0.99 to 1.04)	0.215	0.99 (0.96 to 1.02)	0.429	0.97 (0.89 to 1.06)	0.621	0.99 (0.89 to 1.09)	0.774	0.96 (0.89 to 1.05)	0.464	1.10 (0.81 to 1.50)	0.542
Richest	Ref		Ref		Ref		Ref		Ref		Ref	
Food security												
Food secure	Ref		Ref		Ref		Ref		Ref		Ref	
Food insecure without hunger	1.03 (1.01 to 1.05)	<0.001	1.01 (0.99 to 1.04)	0.290	1.02 (0.95 to 1.09)	0.536	0.97 (0.89 to 1.06)	0.562	0.93 (0.87 to 0.99)	0.026	1.05 (0.85 to 1.30)	0.622
Food insecure with hunger moderate	1.07 (1.05 to 1.09)	<0.001	1.03 (1.00 to 1.06)	0.027	1.10 (1.03 to 1.19)	0.005	1.02 (0.92 to 1.13)	0.71	0.90 (0.84 to 0.97)	0.007	1.09 (0.89 to 1.35)	0.397
Food insecure with hunger severe	1.07 (1.05 to 1.10)	<0.001	1.01 (0.98 to 1.04)	0.555	1.09 (1.00 to 1.19)	0.046	0.99 (0.87 to 1.13)	0.901	0.88 (0.81 to 0.96)	0.005	1.07 (0.80 to 1.43)	0.651
Age												
15–19 years	0.99 (0.93 to 1.06)	0.943	–		1.15 (0.97 to 1.37)	0.103	–		1.07 (0.89 to 1.28)	0.431	–	
20–29 years	Ref				Ref				Ref			
30–39 years	0.99 (0.98 to 1.01)	0.649	–		0.98 (0.93 to 1.03)	0.494	–		1.00 (0.95 to 1.05)	0.965	–	
40–49 years	1.03 (1.01 to 1.06)	0.004	–		1.00 (0.92 to 1.09)	0.916	–		0.98 (0.90 to 1.06)	0.655	–	
Education												
Illiterate	1.02 (1.01 to 1.04)	<0.001	–		1.00 (0.94 to 1.06)	0.976	–		0.97 (0.91 to 1.03)	0.390	–	
Literate	Ref				Ref				Ref			
Occupation												

Continued

Table 3 Continued

	Anaemia				Folate deficiency				Vitamin B_{12} deficiency			
	Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
Predictors	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**	**RR (95% CI)**	**P value**
Employed	0.97 (0.94 to 0.99)	0.049	–		0.85 (0.78 to 0.93)	<0.001	–		1.07 (0.96 to 1.19)	0.205	–	
Unemployed	Ref				Ref				Ref			
No of pregnancies												
≤4	Ref.		Ref.		Ref.				Ref.			
>4	1.03 (1.02 to 1.05)	<0.001	1.03 (1.01 to 1.05)	0.002	1.05 (1.00 to 1.10)	0.034	–		0.92 (0.87 to 0.97)	0.002	–	
Use of iron folate/folic acid/ MMN during last pregnancy												
Yes	0.96 (0.95 to 0.97)	<0.001	–		0.98 (0.92 to 1.03)	0.519	–		0.98 (0.93 to 1.03)	0.526	–	
No	Ref				Ref				Ref			
Birth interval												
≥36 month	Ref				Ref				Ref			
<36 month	1.00 (0.98 to 1.03)	0.516	–		1.02 (0.93 to 1.13)	0.562	–		0.96 (0.88 to 1.06)	0.523	–	
Worm Infestation												
Yes	1.02 (0.97 to 1.06)	0.332	–		1.07 (0.92 to 1.23)	0.335	–		0.89 (0.75 to 1.05)	0.194	1.44 (0.95 to 2.17)	0.087
No	Ref				Ref				Ref		Ref	
Intake of green leafy vegetables												
Daily	0.93 (0.90 to 0.96)	<0.001	0.98 (0.94 to 1.02)	0.363	0.78 (0.68 to 0.89)	<0.001	0.86 (0.74 to 1.01)	0.924	1.07 (0.96 to 1.20)	0.187	–	
Weekly	0.98 (0.96 to 1.00)	0.105	0.98 (0.95 to 1.00)	0.043	0.97 (0.89 to 1.05)	0.471	1.00 (0.92 to 1.10)	0.062	0.98 (0.91 to 1.05)	0.594	–	
Monthly	Ref		Ref		Ref		Ref		Ref			
Intake of red meat												
Daily	0.96 (0.88 to 1.03)	0.306	–		1.05 (0.83 to 1.32)	0.670	–		0.80 (0.60 to 1.07)	0.134	–	
Weekly	0.97 (0.95 to 0.99)	0.003	–		0.99 (0.93 to 1.05)	0.857	–		1.00 (0.94 to 1.07)	0.783	–	
Monthly	Ref				Ref				Ref			
Regular consumption of tea												
<3 times per day	Ref				Ref				Ref		Ref	
≥3 times per day	0.97 (0.92 to 1.01)	0.228	–		0.94 (0.79 to 1.12)	0.514	–		0.87 (0.73 to 1.04)	0.150	0.78 (0.59 to 1.04)	0.094
Intake of dairy products												
Daily	1.03 (0.99 to 1.07)	0.123	1.03 (0.98 to 1.08)	0.234	0.90 (0.78 to 1.03)	0.141	–		1.09 (0.92 to 1.28)	0.287	–	
Weekly	1.04 (1.00 to 1.09)	0.031	1.04 (0.99 to 1.09)	0.087	0.94 (0.81 to 1.07)	0.384	–		1.12 (0.95 to 1.31)	0.156	–	
Monthly	Ref		Ref		Ref				Ref			
Intake of egg												
Daily	0.97 (0.94 to 1.00)	0.155	–		0.80 (0.72 to 0.89)	<0.001	0.89 (0.81 to 0.98)	0.022	1.08 (0.9 to 1.21)	0.148	–	
Weekly	0.98 (0.95 to 1.00)	0.183	–		0.85 (0.78 to 0.93)	<0.001	0.88 (0.78 to 0.99)	0.032	1.01 (0.92 to 1.11)	0.785	–	
Monthly	Ref				Ref				Ref			

Continued

Table 3 Continued

Predictors	Anaemia				Folate deficiency				Vitamin B_{12} deficiency			
	Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
	RR (95% CI)	P value	RR (95% CI)	P value	RR (95% CI)	P value	RR (95% CI)	P value	RR (95% CI)	P value	RR (95% CI)	P value
BMI of women												
Underweight (<18.5)	1.03 (1.01 to 1.05)	<0.001	1.03 (1.00 to 1.05)	0.023	1.07 (1.00 to 1.14)	0.032	–		0.92 (0.85 to 0.99)	0.031	–	
Normal (18.5–24.99)	Ref		Ref		Ref				Ref			
Overweight/obesity (>25)	0.93 (0.91 to 0.94)	<0.001	0.95 (0.93 to 0.97)	<0.001	1.00 (0.94 to 1.07)	0.851	–		1.07 (1.01 to 1.13)	0.010	–	

BMI, body mass index; MMN, multiple micronutrients; SES, socioeconomic status.

the prevalence estimates ranged from 42% to 55% among South Asian countries.[23–26] The prevalence of anaemia among WRA in India was 52%. However, a lower prevalence of anaemia (33.2%) was found in Bangladesh. Although nationally representative information regarding vitamin B_{12} and folate deficiencies in low-income and middle-income countries is scarce, a number of subnational studies have shown high levels of folate and B_{12} deficiencies among women.[21] A community-based study in India, where vegetarianism is high, found prevalence of vitamin B_{12} deficiency to be 33% (24% vegetarian vs 9% non-vegetarian) among women.[27] Similarly, B_{12} deficiency level of 45.5% and folate deficiency level of 14.7% among Chinese women have been documented in another study.[28] A study of non-pregnant women of childbearing age in Guatemala found a prevalence of 19% B_{12} deficiency and 5.1% folate deficiency.[9] A large-scale cross-sectional household survey in Ethiopia documented 67% folate deficiency (≤6.6 ng/mL) among WRA.[29] According to Abdollahi and his colleagues, in Iran, 14.3% and 22.7% of women of childbearing age have low folic acid levels (<6.7 nmol/L) and vitamin B_{12} levels (<110 pmol/L), respectively.[30]

Furthermore, we examined the possible factors associated with anaemia, and vitamin B_{12} and folate deficiencies in Pakistani WRA. Among low-income and middle-income countries, women living in rural areas, having a lower SES and being malnourished and underweight were more likely to have increased risk of anaemia.[30 31] These findings were also evident in our study. As seen in previous studies, a higher than normal BMI[31] and increased intakes of leafy green vegetables[32] were found to have a protective effect against anaemia in WRA. We also found that those having a high BMI have a protective effect against anaemia compared with those with low BMI levels. The risk of anaemia posed by high parity was also noted in US women. Women with parity of 2 or more were found to be at a higher risk of anaemia than women with lower parity.[33] In literature, other factors found to increase the likelihood of anaemia were lower levels of education, inadequate diet and poverty.

Women from low SES were at a higher risk of folate deficiency in our study compared with relatively affluent women. Dietary habits such as low intake of fresh foods and raw vegetables may be one of the reasons leading to the higher odds of folate deficiency among less affluent women. These dietary habits could be the result of financial constraints or cultural dietary practices, such as overcooking food. Lack of knowledge about folate is also a possible contributor to the higher odds of anaemia among lower SES women.[34] Women living in rural areas, with employment and who have daily intake of leafy green vegetables have a lower likelihood of being folate deficient compared with urban unemployed women with less frequent intake. As one of the best sources of dietary folate, it is evident that a more frequent intake of leafy green vegetables would lower the likelihood of folate deficiency among women.

Additionally, the difference among employed women living in rural areas may be attributed to higher accessibility to foods rich in folate.[35] The possibility also exists of community-based supplementation programme contributing to the inverse association between folate deficiency and rural areas.

There is limited research available on vitamin B_{12} deficiency in WRA. However, previous research focusing on pregnant women has shown a high prevalence of B_{12} deficiency and a higher risk of deficiency among those with less frequent intake of dairy products.[36] This could point to a precarious vitamin B_{12} balance among Pakistani women, especially during high demand states such as pregnancy. Our study identified women from the poorest households as having a lower likelihood of deficiency compared with those living in the richest households. Nevertheless, the lack of research on vitamin B_{12} deficiency among WRA impairs our ability to further explore our findings.

The role of gender and intrahousehold food distribution may be another possible contributing factor to these deficiencies among women. The association of gender with intrahousehold food distribution among adults in Pakistan has not been studied in detail. However, gender bias in favour of male children related to food allocation and care-seeking behaviours is widely present in Pakistan.[37] It is possible that food allocation, in terms of quality and quantity, is biased against women putting them at higher risk for these micronutrient deficiencies. In this context, it is also pertinent to note that household food security does not necessarily mean food security for all household members.[38] Thus even in food-secure households, inequitable food allocation may put women at risk of these deficiencies. We also not assessed intrahousehold food distribution for men to assess inequity in proportion of food consumed.

We also found interprovincial differences in anaemia, vitamin B_{12} and folate deficiency. This can be due to difference in SES of households, exposure to available interventions in each province and food consumption practices at household levels.

The potential consequences of these deficiencies are considerable at the individual and population level. These deficiencies may result in risk of infertility, congenital malformations, neurological abnormalities, poor intrauterine growth, abortion, perinatal mortality, miscarriages and stillbirths.[39–41] Therefore, appropriate short-term and long-term interventions that decrease micronutrient deficiencies need to be implemented for Pakistani women. Interventions focused on enhancing parental health education, supporting household livelihoods, ensuring dietary diversity in poor households and increasing appropriate supplementation and food fortification must be implemented to decrease the burden of deficiencies.[42] Other contributory factors such as high fertility must also be tackled for sustainable improvement in anaemia and micronutrient deficiencies among Pakistani women.

CONCLUSION

Our study draws attention to several areas where further research is warranted. There is a need for an in-depth study on community level and intrahousehold risk factors of food distribution, in terms of quality and quantity, with respect to gender. Provincial strategies to overcome anaemia, and vitamin B_{12} and folate deficiencies can be feasible. Further research is also needed on culturally appropriate short-term and long-term interventions within communities and health facilities to decrease anaemia, and vitamin B_{12} and folate deficiencies among Pakistani women.

Acknowledgements We are grateful to Pakistan Medical Research Council and the Federal Ministry of Health for their collaboration during the design and implementation of the survey. We are thankful to all field staff who involved in data collection, supervision, monitoring and data management.

Contributors ZB conceptualised the idea. ZB and SS conceptualised the study design, oversaw data analyses and contributed to drafting the initial and final manuscript. GNK contributed in the revised version of the manuscript. AR carried out the data cleaning and analysis. SS, GNK, KS, SA, AH, SK, IH, MU and ZS critically reviewed the initial and final manuscript. All authors approved the final version of the manuscript for publication.

Funding The national nutrition survey was funded by Unicef, grant number SSA/PAKA/2011/00000660-4. The Global Alliance for Improved Nutrition (GAIN), grant number 391-G-11-00001-00, provided funding for additional micronutrient assessments, which included assessment of vitamin B_{12} and folate concentrations.

Competing interests None declared.

Ethics approval Ethical review committee of Aga Khan University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement We have shared data with funding agency and data can be accessed by requesting to Unicef, Pakistan.

REFERENCES

1. World Health Organization (WHO) and United Nations Children's Fund (UNICEF). Focusing on anaemia: towards an integrated approach for effective anaemia control. 2004 http://www.who.int/nutrition/publications/micronutrients/WHOandUNICEF_statement_anaemia_en.pdf (accessed 31 Mar 2017).
2. Guilbert JJ. The world health report 2002 - reducing risks, promoting healthy life. *Educ Health* 2003;16:230 http://www.who.int/whr/2002/en/whr02_en.pdf.
3. Stevens GA, Finucane MM, De-Regil LM, *et al*. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. *Lancet Glob Health* 2013;1:e16–e25.
4. Kraemer K, Zimmermann MB. Nutritional anemia. 2007 http://www.sightandlife.org/fileadmin/data/Books/Nutritional_anemia_book.pdf (accessed 31 Mar 2017).
5. Ramakrishnan U. Prevalence of micronutrient malnutrition worldwide. *Nutr Rev* 2002;60:i–S52.
6. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. *Food Nutr Bull* 2008;29:S238–S244.
7. Iqbal MP, Lindblad BS, Mehboobali N, *et al*. Folic acid and vitamin B6 deficiencies related hyperhomocysteinemia in apparently healthy Pakistani adults; is mass micronutrient supplementation indicated in this population? *J Coll Physicians Surg Pak* 2009;19:308–12.

8. Aga Khan University Hospital (AKUH)Pakistan Institute of Development Economics. Pakistan national nutrition survey 2001-2002. accessed 31 Mar 2017 http://ghdx.healthdata.org/record/pakistan-national-nutrition-survey-2001-2002.
9. Rosenthal J, Lopez-Pazos E, Dowling NF, *et al*. Folate and Vitamin B12 Deficiency Among Non-pregnant Women of Childbearing-Age in Guatemala 2009-2010: Prevalence and Identification of Vulnerable Populations. *Matern Child Health J* 2015;19:2272–85.
10. Black RE, Allen LH, Bhutta ZA, *et al*. Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;371:243–60.
11. Peña-Rosas JP, De-Regil LM, Gomez Malave H, *et al*. Intermittent oral iron supplementation during pregnancy. *Cochrane Database Syst Rev* 2015;19:CD009997.
12. World Health Organization. *WHA global nutrition targets 2025: anaemia policy brief*: World Health Organization, 2014. http://www.who.int/nutrition/publications/globaltargets2025_policybrief_anaemia/en/. (accessed 31 Mar 2017).
13. Black RE, Victora CG, Walker SP, *et al*. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet* 2013;382:427–51.
14. Peña-Rosas JP, De-Regil LM, Garcia-Casal MN, *et al*. Daily oral iron supplementation during pregnancy. *Cochrane Database of Syst Rev* 2015;22:CD004736.
15. Ray JG, Wyatt PR, Thompson MD, *et al*. Vitamin B12 and the risk of neural tube defects in a folic-acid-fortified population. *Epidemiology* 2007;18:362–6.
16. Fawzi WW, Msamanga GI, Urassa W, *et al*. Vitamins and perinatal outcomes among HIV-negative women in Tanzania. *N Engl J Med* 2007;356:1423–31.
17. Aga Khan University, Pakistan Medical Research Council. *National nutrition survey Pakistan 2011*. Pakistan: Aga Khan University, 2011. https://www.humanitarianresponse.info/system/files/documents/files/59_National%20Nutrition%20Survey-2011.pdf (accessed 31 Mar 2017).
18. World Health Organization (WHO), Food and Agricultural Organization of the United Nations (FAO). *Guidelines on food fortification with micronutrients*. Geneva, Switzerland and Rome, Italy: WHO and UNICEF, 2006. http://www.who.int/nutrition/publications/micronutrients/9241594012/en/ (accessed 31 Mar 2017).
19. World Health Organization. *Serum and red blood cell folate concentrations for assessing folate status in populations*. Switzerland: World Health Organization, 2012. http://apps.who.int/iris/bitstream/10665/75584/1/WHO_NMH_NHD_EPG_12.1_eng.pdf (accessed 12 Sep 2017).
20. World Health Organization. *Hemoglobin concentrations for the diagnosis of anemia and assessment of severity*. Switzerland: Vitamin and Mineral Nutrition Information System (VMNIS), WHO, 2011. http://www.who.int/vmnis/indicators/haemoglobin.pdf (accessed 22 Jun 2016).
21. Hamedani P, Hashmi KZ, Manji M. Iron depletion and anaemia: prevalence, consequences, diagnostic and therapeutic implications in a developing Pakistani population. *Curr Med Res Opin* 1987;10:480–5.
22. McLean E, de Benoist B, Allen LH. Review of the magnitude of folate and vitamin B12 deficiencies worldwide. *Food Nutr Bull* 2008;29:S38–51.
23. National Institute of Population Research and Training (NIP), Mitra and Associates, ICF International. *Bangladesh demographic and health survey 2011*. Dhaka, Bangladesh, and Calverton, Maryland, USA: NIP and ICF International, 2013. http://www.dhsprogram.com/publications/publication-FR265-DHS-Final-Reports.cfm (accessed 31 Mar 2017).
24. National Institute of Statistics (NIS), Directorate General for Health, ICF International. *Cambodia demographic and health survey 2014*. Phnom Penh, Cambodia and Rockville, Maryland, USA: NIS and ICF International, 2015. http://www.dhsprogram.com/publications/publication-FR312-DHS-Final-Reports.cfm (accessed 31 Mar 2017).
25. Ministry of Health and Population (MHP), New ERA, ICF International. *Nepal demographic and health survey 2011*. Kathmandu, Nepal and Calverton, Maryland, USA: MHP and ICF International, 2012. http://www.dhsprogram.com/publications/publication-FR257-DHS-Final-Reports.cfm (accessed 22 Jun 2016).
26. International Institute for Population Sciences (IIPS) and Macro International. *National Family Health Survey (NFHS-3) 2005-06*. Mumbai: IIPS, 2007. http://www.dhsprogram.com/publications/publication-FRIND3-DHS-Final-Reports.cfm (accessed 22 Jun 2016).
27. Gammon CS, von Hurst PR, Coad J, *et al*. Vegetarianism, vitamin B12 status, and insulin resistance in a group of predominantly overweight/obese South Asian women. *Nutrition* 2012;28:20–4.
28. Dang S, Yan H, Zeng L, *et al*. The status of vitamin B12 and folate among Chinese women: a population-based cross-sectional study in northwest China. *PLoS One* 2014;9:e112586.
29. Haidar J, Melaku U, Pobocik R. Folate deficiency in women of reproductive age in nine administrative regions of Ethiopia: an emerging public health problem. *South African Journal of Clinical Nutrition* 2010;23:132–7.
30. Abdollahi Z, Elmadfa I, Djazayeri A, *et al*. Folate, vitamin B12 and homocysteine status in women of childbearing age: baseline data of folic acid wheat flour fortification in Iran. *Ann Nutr Metab* 2008;53:143–50.
31. Bentley ME, Griffiths PL. The burden of anemia among women in India. *Eur J Clin Nutr* 2003;57:52–60.
32. Panigrahi A, Sahoo PB. Nutritional anemia and its epidemiological correlates among women of reproductive age in an urban slum of Bhubaneswar, Orissa. *Indian J Public Health* 2011;55:317–20.
33. Mei Z, Cogswell ME, Looker AC, *et al*. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999-2006. *Am J Clin Nutr* 2011;93:1312–20.
34. Deepti DK, *et al*. Determinants of knowledge regarding folic acid in periconceptional use among pregnant mothers in Southern India. *IOSR Journal of Dental and Medical Sciences* 2013;4:25–9.
35. Thoradeniya T, Wickremasinghe R, Ramanayake R, *et al*. Low folic acid status and its association with anaemia in urban adolescent girls and women of childbearing age in Sri Lanka. *Br J Nutr* 2006;95:511–6.
36. Park H, Kim YJ, Ha EH, *et al*. The risk of folate and vitamin B(12) deficiencies associated with hyperhomocysteinemia among pregnant women. *Am J Perinatol* 2004;21:469–75.
37. Nazli H, Hamid S. *Concerns of food security, role of gender and intrahousehold dynamics in Pakistan*. Pakistan: Pakistan Institute of Development Economics. http://www.pide.org.pk/Research/Report175.pdf. (accessed 20 Jun 2016).
38. Alderman H, Garcia M. *Poverty, household food security, and nutrition in rural Pakistan*. USA: The International Food Policy Research Institute, 1993. http://www.ifpri.org/publication/poverty-household-food-security-and-nutrition-rural-pakistan (accessed 31 Mar 2017).
39. Smith CA. Effects of maternal under nutrition upon the newborn infant in Holland (1944-1945). *J Pediatr* 1947;30:229–43.
40. Stein Z, Susser M, Saeger G, *et al*. *Famine and human development: dutch hunger winter of 1944-45*. New York: Oxford University Press, 1975.
41. Ebbs JH, Tisdall FF, Scott WA. The influence of prenatal diet on the mother and child. *J Nutr* 1941;22:515–21.
42. Ruel MT, Alderman H. Maternal and Child Nutrition Study Group. Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition? *Lancet* 2013;382:536–51.