Prevalence and possible factors associated with anaemia, and vitamin B₁₂ and folate deficiencies in women of reproductive age in Pakistan: analysis of national-level secondary survey data

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ABSTRACT

Objective To determine the prevalence and possible factors associated with anaemia, and vitamin B₁₂ deficiency 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 B₁₂ deficiency as serum vitamin B₁₂ 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 11751 blood samples were collected and analysed. The prevalence of anaemia, vitamin B₁₂ 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 (OR 1.07; 95% CI 1.04 to 1.09) <P=0.00, food insecure with moderate hunger (OR 1.03; 95% CI 1.00 to 1.06) <P=0.02, four or more pregnancies (OR 1.03; 95% CI 1.01 to 1.05) <P=0.00, being overweight or obese (OR 0.95; 95% CI 0.93 to 0.97) <P<0.00, and weekly intake of leafy green vegetables (OR 0.98; 95% CI 0.95 to 1.00) <P=0.04. For vitamin B₁₂ deficiency, a positive association was observed with rural population (OR 0.81; 95% CI 0.66 to 1.00) <P=0.04, living in Khyber Pakhtunkhwa province (OR 1.25; 95% CI 1.11 to 1.43) <P<0.00, and living in Azad Jammu and Kashmir (OR 1.50; 95% CI 1.08 to 2.08) <P=0.01. Folate deficiency was negatively associated with daily and weekly intake of eggs (OR 0.89; 95% CI 0.81 to 0.98) <P=0.02 and (OR 0.88; 95% CI 0.78 to 0.99) <P=0.03.

Conclusions In Pakistan, anaemia, and vitamin B₁₂ 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 B₁₂ and folate deficiencies among Pakistani women.

INTRODUCTION

In low-income and middle-income countries, anaemia is a major public health problem.⁴

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 B₁₂ and folate deficiencies also result in anaemia.³

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 According to WHO, the prevalence of folate and vitamin B₁₂ 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 B₁₂ deficiencies remain undetermined.

In Pakistan, previous national-level data on the prevalence of vitamin B₁₂ 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 B₁₂ deficient, while 39.7% were folate deficient in 2009. According to the National Nutrition Survey (NNS) 2001, the prevalence of anaemia was 28.4% in WRA. 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, increasing the risk of morbidity and mortality during pregnancy, neural tube defects (NTDs), low birth weight (LBW), and premature birth. Anaemia is also associated with an elevated risk of infections, impaired physical and cognitive development, and poor school performance among offspring. Folic acid deficiency, specifically, is associated with megaloblastic anaemia, NTDs and a higher risk of LBW babies. Vitamin B₁₂ deficiency has also been identified as a risk factor for NTDs, early fetal loss, failure-to-thrive, stunting, poor neurocognitive function and developmental delays.

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 B₁₂ 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.

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. 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 26753 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, 27963 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 22278 women.

Sample size

A sample size of 30000 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 B₁₂ and folate deficiencies

Blood samples were collected from WRA for the assessment of anaemia (n=10787), vitamin B₁₂ 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 B₁₂ and folate were measured with an electrochemiluminescence immunoassay method using Elecsys 2010 (Roche diagnostics, Bernried, Germany). Vitamin B₁₂ deficiency was defined as serum vitamin B₁₂ levels of <203 pg/mL (150 pmol/L). Folate deficiency was defined as serum folate levels <4 ng/mL (10 nmol/L). Haemoglobin concentration was measured with a HemoCue microcuvette machine. In WRA, anaemia was defined as haemoglobin levels <12 g/dL.
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/m²), normal weight (18.5–24.99 kg/m²), overweight (25–34.99 kg/m²) and obese (≥35 kg/m²). 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 B₁₂ 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 (60%) 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/m²) (table 1). The overall prevalence among WRA was 50.4% for anaemia, 50.8% for folate deficiency and 52.4% for vitamin B₁₂ 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                                | 13188| 69.0     |
| Distribution by province              |     |          |
| Punjab                               | 10156| 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                             | 12898| 59.4     |
| Occupation                           |     |          |
| Employed                             | 1357 | 6.1      |
| Unemployed                           | 20921| 93.9     |
| No of pregnancies (n=21 720)         |     |          |
| ≤4                                   | 13 663| 63.5     |
| >4                                   | 8057 | 36.5     |
| Birth interval (n=8 926)             |     |          |
| ≥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)                 | 11057| 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 B₁₂ 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 anemic. 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 B₁₂ 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 B₁₂ 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 B₁₂ and folate deficiencies (table 3).

DISCUSSION

We estimated the prevalence of anaemia, and folate and vitamin B₁₂ deficiencies among WRA from a nationally representative sample in Pakistan. Substantially, more than half of Pakistani WRA were found to be anaemic, and vitamin B₁₂ 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 B₁₂ deficiency among women of reproductive age

| Predictors                     | Anaemia Unadjusted | Anaemia Adjusted | Folate deficiency Unadjusted | Folate deficiency Adjusted | Vitamin B₁₂ Unadjusted | Vitamin B₁₂ 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   |
| 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                |
| Urban                          | Ref                | Ref              | 0.95 (0.85 to 1.05)         | 0.304                      | 0.99 (0.92 to 1.08)    | 0.048                |
| Province                       |                    |                  |                             |                            |                        |                      |
| Punjab                         | 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)    | 0.328                |
| 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)    | 0.002                |
| 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)    | 0.536                |
| 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)    | 0.349                |
| 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               |
| 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)    | 0.733                |
| 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                |
| 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                |
| 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                |
| 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                |
| Richest                        | Ref                | Ref              | 0.99 (0.89 to 1.09)         | 0.774                      | 0.96 (0.89 to 1.05)    | 0.464                |
| Food security                  |                    |                  |                             |                            |                        |                      |
| Food secure                    | 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                |
| Food insecure with hunger      | 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                |
| Food insecure with hunger      | 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                |
| 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                    | 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                |
| Education                      |                    |                  |                             |                            |                        |                      |
| Illiterate                     | 1.02 (1.01 to 1.04) | <0.001           | 1.02 (0.94 to 1.06)         | 0.976                      | 0.97 (0.91 to 1.03)    | 0.390                |
| Literate                       | Ref                | Ref              |                             |                            | Ref                    | Ref                  |
| Occupation                     |                    |                  |                             |                            |                        |                      |
| Continued                       |                    |                  |                             |                            |                        |                      |
| Predictors | Anaemia Unadjusted | RR (95% CI) | P value | Anaemia Adjusted | RR (95% CI) | P value | Folate deficiency Unadjusted | RR (95% CI) | P value | Folate deficiency Adjusted | RR (95% CI) | P value | Vitamin B<sub>12</sub> deficiency Unadjusted | RR (95% CI) | P value | Vitamin B<sub>12</sub> deficiency Adjusted | 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.        |             |         | 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                          |             |         |                           |             |         |                           |             |         |                           |             |         |
| 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                          |             |         |                           |             |         |                           |             |         |                           |             |         |
| 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                          |             |         |                           |             |         |                           |             |         |                           |             |         |
| ≥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                          |             |         |                           |             |         |                           |             |         |                           |             |         |
| 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 (0.99 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 | –               |             |         |                           |             |         |                           |             |         |
### Table 3 Continued

| Predictors | Anaemia | | | Folate deficiency | | | Vitamin B₁₂ deficiency | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Unadjusted | Adjusted | P value | Unadjusted | Adjusted | P value | Unadjusted | Adjusted | 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 of anaemia among WRA in India was 52%. However, a lower prevalence of anaemia among WRA in India was found in Bangldesh (33.2%). The prevalence of anaemia among WRA in South Asian countries ranged from 42% to 55%. The prevalence of folate deficiency among WRA in India was 19%. B₁₂ deficiency among WRA in India was 14.3%.
Additionally, the difference among employed women living in rural areas may be attributed to higher accessibility to foods rich in folate. 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 B₁₂ deficiency in WRA. However, previous research focusing on pregnant women has shown a high prevalence of B₁₂ deficiency and a higher risk of deficiency among those with less frequent intake of dairy products. This could point to a precarious vitamin B₁₂ 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 B₁₂ 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. 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. 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 B₁₂, 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. 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 introducing appropriate supplementation and food fortification must be implemented to decrease the burden of deficiencies. 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 B₁₂ 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 B₁₂ and folate deficiencies among Pakistani women.

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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.

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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.

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