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Factors affecting anaemia among women of reproductive age in Nepal: a multilevel and spatial analysis

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ABSTRACT

Objective: The main objective of this study was to explore the factors affecting anemia among women of reproductive age (WRA) in Nepal using spatial and multilevel epidemiological analysis.

Design: This cross-sectional study analyzed the data from the 2016 Nepal demographic and health survey (NDHS 2016). The spatial analysis was performed using ArcGIS software V.10.8 to identify the hot and cold spots of anaemia among WRA (15 to 49 years). Data were analyzed using multilevel mixed-effect logistic regression analysis.

Setting: Nepal

Participants: A total of 6,414 WRA were included in the analysis.

Main outcome measure: Anaemia defined by World Health Organization (WHO) as hemoglobin level less than 12g/dL in non-pregnant women and less than 11g/dL in pregnant women.

Results: The spatial analysis showed that statistically significant hotspots of anaemia were in the southern Terai region (4 districts in Province-1, 8 districts in Province-2, 1 district in Bagmati province, and 3 districts in Province-5) of Nepal. At the individual level, women with no education (aOR: 2.07 95% CI: 1.21-3.54), and from middle socio-economic class families (aOR: 1.66, 95% CI: 1.02-2.68) were more likely to be anemic, whereas, older women (≥35 years) (aOR: 0.48, 95% CI: 0.26-0.91), and those women who were using hormonal contraceptives (aOR: 0.57, 95% CI: 0.39-0.84) were less likely to be anaemic. At the community level, women from Province-2 (aOR: 3.12, 95% CI: 1.58-6.14) had higher odds of being anaemic.

Conclusion: WRA had higher odds of developing anemia and it varied by the geographical regions. High prevalence of anaemia were observed in the southern Terai. The multi-pronged approaches including nutritional, and non-nutritional anaemia prevention and control strategies can benefit by tailoring the programs to the high risk provinces and districts with high burden of anemia.

Strengths and limitations of this study

- Used comprehensive, nationally representative data with haemoglobin level.
- The combined statistical methods including multilevel and spatial analysis were applied, which takes into account the role of geographical risk profile and determinants of anaemia among WRA in Nepal.
- Due to the cross-sectional design, it was difficult to determine the cause-and-effect relationships between the predictors and outcome variable (anaemia).
- Other potential confounding factors of anaemia such as nutrient intake, worm infestations, malaria and other non-modifiable risk factors were beyond the scope of this study.
- This study could not distinguish the types of anaemia such as nutritional, genetic and infectious.

INTRODUCTION

Anaemia remains a significant public health problem in developing countries despite advances in health science.[1] Approximately one-third of the population is affected by anaemia globally.[2,3] South Asian accounts for the largest burden of anaemia with estimated prevalence of 49% in 2016 among women of reproductive age (WRA).[4] The highest prevalence over the past 26 years was 55.2% in 1990.[4] Despite the implementation of a '1000 days nutrition program' among various other programs, targeted to a mother with newborn babies in South Asia, the reduction in anemia among pregnant women has not been significant.[5] To accelerate reduction of anaemia, the World Health Assembly has set a target of achieving a 50% reduction of anaemia among WRA by 2025 relative to 2010.[6] However, not a single South Asian country is on the way to achieve the 2025 targets.[7] Despite the historic efforts in preventing anaemia through the implementation of national nutrition programs and policies including iron-folic acid supplementation across the country, the prevalence of anaemia among WRA has been steadily increasing from 35% in 2011 to 41% in 2016.[8,9] These figures suggest that anaemia continues to be a severe public health problem in Nepal where the prevalence rate is ≥40%.[10]

Anaemia in WRA is associated with multiple conditions and consequences such as preterm delivery,[11] miscarriage,[12] low birth weight,[13] child growth faltering, impairment of cognitive function, increased susceptibility to infection, and poverty.[14,15] It is also associated with increased risk of prenatal and maternal mortality.[13,16] Approximately 20% of maternal deaths are caused by anaemia and it is also considered as an additional risk factor for 50% of all maternal deaths.[17,18]

Contributing factors and distribution of anaemia include a complex interplay of political, ecological, social, and biological factors.[19] In Nepal, anaemia among WRA is associated with various socio-ecological factors. In the southern Terai of Nepal, low community education status, gender based-inequality, poor health seeking behavior,[20–22] inadequate dietary intake during pregnancy,[23–25] lack of diversified diet,[9,24,26] high burden of hookworm infection and malaria,[27–29] and high amount of arsenic in potable water[30] were identified as factors contributing to anaemia. On the other hand, high prevalence of anaemia in the mountainous region were attributed to among others, food insecurity, and low dietary diversity,[9,24,31] poor health services,[22] illiteracy, and gender based-inequality.[20,21]

To the best of our knowledge, this is the first study to explore the geographical regions with hotspots (high prevalence rate) and factors affecting anaemia among WRA using cluster sampling of the nationally representative data of Nepal. Exploring spatial patterns and factors affecting anaemia by geographical region is crucial to inform the planning for targeted anaemia control and prevention programs.[32] The main objective of this study was to explore the spatial distribution and contributing factors of anaemia among WRA in Nepal.

METHODS

Patient and public involvement

This study utilized a publicly available data set (NDHS); therefore, there was no patient involved.

Data source

This study was based on the data from Nepal Demographic and Health Survey (NDHS) 2016, a nationally representative cross-sectional survey. This survey was carried out as part of the DHS program by New ERA under the guidance of the Ministry of Health, Government of Nepal and was supported by ICF international and United States Agency for International Development

(USAID). The study population for this study was WRA from the Nepal Demographic and Health Survey 2016.

Sampling strategy

The NDHS 2016 utilized a stratified, two-stage cluster sampling design to provide representative estimates for seven provinces, three ecological zones, and urban and rural areas. The survey used enumeration areas (EAs) which is a primary sampling unit (PSU) and was selected from 383 wards in both rural (n=199) and urban (n=184) areas with probability proportional to size method. In the second stage, 30 households on average within EAs were selected using a systematic sampling technique. A more detailed methodology of the NDHS has been published elsewhere.[9] All WRA (pregnant and non-pregnant) with available data on anaemia, complete socio-demographic and nutritional characteristics who were resident or who had slept in the selected households on the night before the survey were eligible for the survey. The details of the sample size and exclusion criteria for the selection of the samples are presented in Figure 1.

Study variables

Outcome variables

Haemoglobin level was measured using capillary blood by a battery-operated portable HemoCue rapid testing machine and was adjusted for altitude and smoking status.[9] According to the WHO, for non-pregnant and pregnant women aged 15-49 years, any form of anaemia is defined as haemoglobin concentration <12.0g/dL and 11g/dL respectively.[33] The categories of anaemia were further dichotomized into 'anaemic' and 'not anaemic'.

Predictor's variables

Predictors of anaemia were selected based on the literature review.[14,34–36] The wide range of socio-demographic, individual, household and community factors were hypothesized to increase the likelihood of anaemia. The predictors of anaemia including both individual-level and community-level factors were included in the analysis. The coding strategy of individual-level and community-level factors are presented in online supplementary Table 1.

Individual-level factors

A total of 11 individual-level factors were identified, and that included for example, respondent's age, education level, occupation, wealth index, nutritional status, pregnancy status, and minimum dietary diversity for WRA (MDD-W). The minimum Dietary Diversity for Women of Reproductive age (MDD-W) was calculated using the guideline of the modified FANTA III tool developed by the Food and Agriculture Organization (FAO) of the United Nations.[37]

Community-level factors

The six different factors were included in the community-level factors that included place of residence, province, community wealth, community female education, community safe water access, and community toilet facility. The selection of community-level factors were based on the group of women living in similar settings.[32] If more women have shared features such as place of residence, province, type of water source, and toilet facility; it was considered to have the same effect on anaemia among WRA.[32] For community level wealth and female education, we constructed the aggregate continuous community-level predictor-variables by aggregating individual-level characteristics at the community (cluster) level. We dichotomized the aggregate variables into "high" or "low" based on the distribution of the proportion values calculated for each community.[38] The mean value was used as a cut-off point of the proportion values for the categorization of community-level variables. The community wealth was categorized as 'high' if the proportion of women from richest (rich, richest) households in the community was above 21% and 'low' if the proportion was 0 to 21%. Community female education was defined as the

137 mean percentage of women in the community with at least primary education and above.[39]
138 Water supply and sanitation guidelines based on the WHO and UNICEF Joint Monitoring
139 Progamme (JMP) were used to define improved toilet facility and improved water source.[40]

140 Data analysis

141 Statistical analysis

142 Data were analyzed using Stata/MP version 14.1 (StataCorp LP, College Station, Texas). Data
143 were adjusted for enumeration areas (EAs) and disproportionate sampling and non-response.
144 Weighted frequencies, weighted percentage, mean and SD were used for the descriptive analysis.
145 The multilevel mixed-effect logistic regression analysis was performed to estimate the adjusted
146 odds ratio and to estimate the extent of random variations between communities.

147 Four models were created and were fitted. Model 1 (empty model) was fitted without predictor
148 variable to test random variability in the intercept and to estimate the intra-class correlation
149 (ICC).[41] Model 2 examined the effects of individual-level characteristics, model 3 examined
150 the effects of community-level variables, and model 4 examined the effects of both individual
151 and community-level characteristics simultaneously. In the multilevel mixed-effect logistic
152 regression models, the fixed effects estimated the association between the likelihood of anaemia
153 among WRA and the individual-level and community-level factors, and the findings are reported
154 in terms of adjusted odds ratio (aOR) with p-values <0.05 and 95% confidence intervals (CIs).
155 To prevent statistical bias in the multilevel logistic regression model, we examined and reported
156 multicollinearity among the predictor variables using variation inflation factors (VIF). In this
157 study, we used "10" as a cut-off value for the maximum level of VIF.[42] The random effects are
158 expressed as ICC,[41] and proportional change in variance (PCV).[43] The ICC was calculated
159 to evaluate the cluster variability; and PCV can measure the total variation due to factors at the

individual- and community- levels.[41] Models fit were assessed using Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Considering the nested structure of the survey data, a multilevel model is considered to be appropriate than ordinary single-level regression model because it provides correct parameter estimates by handling the cluster data.[44,45]

Spatial analysis

Spatial analysis were performed using ArcGIS software V.10.8, and base files of the administrative provinces and districts of Nepal were obtained from Government of Nepal, Ministry of Land Management, [46] and Natural Earth.[47] The global positioning system (GPS) data set for NDHS was obtained from the DHS website after receiving the approval letter. The prevalence of anaemia and standardized prevalence ratio were computed for both the districts and provinces in Stata/MP version 14.1 software and were later transferred to Excel spreadsheet. These data were imported into the ArcGIS software to link the reported anaemia prevalence for each cluster to the corresponding geographical location (survey cluster data). The spatial variations of the prevalence of anaemia among WRA by both districts and provinces were visualized. Similarly, the standardized prevalence ratio of anaemia among WRA (normalized to the national prevalence of 41%) for both districts and provinces were visualized.

The spatial analysis was performed to explore the clustering of anaemia among WRA using the Getis-Ord G-statistic tool in ArcGIS software. The Getis-Ord G-statistics identifies statistically significant spatial clusters of high values (hot spots) and low values (cold spots).[48] The statistical significance of autocorrelation was determined by z-scores and p-value ≤0.05 with a 95% Confidence Intervals (CIs).[32,48] An autocorrelation can be categorized into positive and negative using Getis-Ord G-statistics. For statistically significant positive autocorrelation, larger z-score, meant more intense clustering of high values (hot spots) and for statistically significant negative autocorrelation, smaller z-score meant more intense clustering of low values (cold spot) which allowed us to identify the spatial variability of anaemia among WRA.[9] An anaemia hot spot was defined as the occurrence of high prevalence rates of anaemia clustered together on the map. Anaemia cold spot was referred to the occurrence of low prevalence rates of anaemia clustered together on the map.[32,48] The spatial pattern and distribution of anaemia prevalence rates among WRA in Nepal are visualized on the map (Figure 2)

Ethical consideration

The DHS survey protocols were approved by the institutional review boards of ICF, the DHS program, and the Nepal Health Research Council (NHRC). The details on ethical procedures used in this survey have been published elsewhere.[9] We registered and requested for access to both main data and GPS data from the DHS website[49] and received an approval to access and download the DHS data file. DHS program collected data following a written informed consent from each individual. All individual identifiers were precluded from the final dataset in this study.

RESULTS

Socio-demographic characteristics of study participants

In this study, a total of 6,414 WRA were included in the analysis (Table 1). The mean (±SD) age of the study participants was 29 (±9.7) years. More than one third (38.1%) of the participants from age group 15-24 years, and women (35.5%) had attended secondary level of education. Nearly one quarter (22.4%) of study participants were from richer wealth quintiles and more than half (59.7%) of the women were not using any contraceptive methods, and a half (50.6%) of the participants consumed more than five food types. High proportion of women (68.8%) belonged to urban areas, and were from Bagmati province (21.9%). More than half (54.2%) of the women had the highest percentage of community female education, whilst, nearly two-thirds (64%) of the women belonged to a low percentage of community female wealth index. Majority of the participants had improved sources of drinking water (91%) and type of toilet facility (84.1%). (Table 1)

Prevalence of anaemia among women of reproductive age

In the current study, the overall prevalence of any anaemia across Nepal was 41% (95% CI: 38.5-42.9%). At the individual level, the prevalence of anaemia was higher among younger age groups (43.5%), and women who attended at least secondary level education (42.6%). The higher cases of anaemia were found in middle socio-economic class families (48.9%). The prevalence of anaemia was more in who used various contraceptive methods (43.1%), and those who consumed less than five food groups (51%). At the community-level, the prevalence of anaemia was higher in women who came from Province-2 (57.7%). The high proportion of anaemia was found in community with low female education (47.8%), female wealth index (44.3%), and who did not have improved toilet facility (51.4%) (Table 1).

Factors affecting anemia among women of reproductive age

The fixed effects (a measure of association) and the random-effects for the risk of developing anaemia among WRA are presented in Table 2. The results of the empty model (model 1) showed that there was statistically significant variability in the odds of anaemia between communities (τ=0.627, $p<0.001$). The ICC in the empty model implied that 16% of the total variance for the risk of developing anaemia was attributed to differences between the communities. In individual-level factors (model 2), women who did not have formal education (aOR=2.23, 95% CI: 1.35-3.67) compared to those with higher education, and who belonged to middle-class families (aOR=1.81, 95% CI: 1.17-2.75) compared to the family from poorest families were found to have higher odds of anemia. Older women had 54% lower (aOR=0.46, 95% CI: 0.24-0.88) odds of developing anaemia compared to younger women. Women who used hormonal contraceptive methods had 39% lower (aOR=0.61, 95% CI: 0.42-0.86) odds of anaemia compared to who did not use contraceptive methods. The ICC in model 2 indicated that 13.9% of the variation in WRA anaemia was attributable to differences across communities. The PCV indicated that 15.3% of the variance in WRA anaemia across communities was explained by the individual-level characteristics.

The community-level (model 3) showed that women from Province-2 had 2.5 times higher (aOR=2.51, 95% CI: 1.79-3.53) odds of anaemia compared to women from Bagmati province. Women who belonged to communities with a high percentage of the wealthy households had 1.48 times higher (aOR=1.48, 95% CI: 1.21-1.80) odds of anaemia compared to those coming from low percentage of the wealthy household; and women residing in communities with a high percentage of community female education had 1.39 times higher (aOR=1.39, 95% CI: 1.15-1.68) odds of anaemia compared to those coming from the communities with a low percentage of education. The ICC in model 3 showed that differences between communities accounted for about 11.1% of the variation in anaemia among WRA. In addition, the PCV indicated that 33.9% of the variation in WRA anaemia between communities was explained by community-level characteristics.

In model 4, women who had no formal education were found to have 2 times higher (aOR=2.07, 95% CI: 1.21-3.54) odds of anaemia compared to women who had higher education. Women from middle socio-economic family had higher (aOR=1.66, 95% CI: 1.02-2.68) odds of anaemia compared to poorest counterparts. Women who came from Province-2 (aOR=3.12, 95% CI:

1.1.58-6.14) had higher odds of anaemia compared to Bagmati province. The older women had 52% lower (aOR=0.48, 95% CI: 0.26-0.91) odds of anaemia compared to younger women, and women who used hormonal contraceptive methods had 43% lower (aOR=0.57, 95% CI: 0.39-0.84) odds of anaemia compared to who did not use contraceptive methods. After the inclusion of both the individual and community-level variables in model 4, the ICC indicated that 10% of the variability in anaemia among WRA was attributable to the difference between communities. Furthermore, the PCV indicated that 39.5% of the variation in anaemia among WRA between communities was explained by both individual and community-level characteristics (Table 2).

Spatial data analysis

Figure 3(a) shows the prevalence of anaemia among WRA across provinces in Nepal. A severe anaemia prevalence (≥40%) among WRA was seen in Province-2, followed by Province-1 and 5. The prevalence of moderate anaemia (20-39.9%) was observed in Bagmati, Gandaki, Karnali, and Sudurpaschim province. Mild anaemia (prevalence<19.9%) was not found in any provinces. Figure 3(b) shows the prevalence of anaemia among WRA across 75 districts in Nepal. The prevalence rate of severe anaemia was observed in 29 out of 75 districts, and mild anaemia was found in only eight districts (Figure 3).

The standardized prevalence ratio by provinces (standardized to the national mean prevalence of 41%) are shown in Figure 4 (a), and ranged from 0.68 to 1.4. The higher prevalence ratio of anaemia was found in Province-2, whereas a lower prevalence ratio was observed in Bagmati, Gandaki, and Karnali province. Figure 4 (b) shows the standardized prevalence ratio across the 75 districts, and ranged from 0.76 to 1.59. The higher standardized prevalence ratio of anaemia was observed in 17 out of 75 districts across the country (Figure 4).

The spatial pattern and distribution of anaemia among WRA at the cluster level are displayed in Figure 5. The spatial analysis at the cluster level showed that statistically significant high hotspots of anaemia were observed in the southern Terai of the country bordering India: Province-2 (8 districts), Province-1 (4 districts), southern plain of the Bagmati province (1 district), and southwestern region of Province-5 (3 districts). Whilst, cold spots of anaemia were observed in most of the hilly regions of the country (Figure 5).

DISCUSSION

Overall findings

In this study, more than 40% of WRA were anaemic which implies that anaemia is still a serious public health problem in Nepal.[33] Geographical pattern showed that anaemia is a serious public health problem in three of the seven provinces and 29 out of the 75 districts in Nepal. The higher prevalence of anaemia was observed in the southern Terai bordering India particularly in Province-2, and the upper Himalayan region of the country. The spatial analysis at the cluster level showed that high hot spots of anaemia were observed in Terai region especially in Province-1, 2 and 5. A possible reason could be that WRA from Terai region and high mountainous regions are more likely to be of a lower socioeconomic status and thus can afford less diversified diet compared to other provinces and districts.[9,26]

In the southern Terai of Nepal, despite ecological richness, people have long suffered from a deficiency of micronutrients such as Vitamin A, iron and zinc,[23] and a high burden of hookworm infestation and a high prevalence of malaria which are established to contribute to anemia.[27] Diet of women in the Terai lack diversity and, nutrient adequacy which pose an increased risk of anaemia.[24] Furthermore, the Terai region of Nepal is considered to be high malaria-endemic region,[28,29,50] and higher risk of malaria is associated with a higher risk of anaemia.[27] The majority (90%) of the population from Terai region rely on groundwater especially shallow tube well for domestic purposes including drinking.[30] Higher arsenic concentration can inhibit haem iron metabolism and increase erythrocyte hemolysis.[51] Consequently, drinking arsenic-containing water poses an increased risk of anaemia among women.[52] Arsenic exposure was more likely to cause anemia among women in Bangladesh.[53]

The spatial analysis at the cluster level found that high hotspots of anaemia were also observed in southwestern region (Province-5), particularly Banke, Bardiya and Kanchanpur district. A previous study highlighted that Glucose 6 phosphate dehydrogenase deficiency (G6PDd), sickle cell trait (SCT), and sickle cell anaemia (SCA) as the most common disorder in Tharu community living in southwestern Province-5.[54,55] G6PDd and haemoglobinopathies are established to cause anaemia among WRA.[36]

The geographical variance of high cases of anaemia across the high mountainous region could be attributed to food insecurity, low dietary diversity, [9,24,31] less calorie diet,[24] poor health service coverage,[22] high illiteracy, gender based-inequality, and poor health seeking behaviour.[20,21]

Socio-demographic characteristic among women and anaemia

Women who had no formal education, and those who came from middle socio-economic households, and from younger age group were at increased risk of anaemia. These findings corroborate with previous studies from low and middle-income countries (LMICs) including Ethiopia,[32] India,[56] Tanzania,[57] Rwanda,[58] Timor-Leste,[59] and Bangladesh.[60] Studies from Ethiopia and Tanzania suggest that higher-level education might enable women to gain knowledge and improve attitude which in turn can promote them to adopt healthier lifestyle including good nutrition habits, better health-seeking behavior, and good hygiene practices that can prevent anaemia.[32,57]

Anaemia is a multifaceted problem where nutrition and household economic status are considered to have a synergistic association.[58] Women belonging to poorer households are more likely to be anaemic compared to those living in middle or richer households in most of the countries.[14,32,58] Contrastingly, this study revealed that poorer Nepalese women were less likely to be anaemic. In this study, prevalence of anaemia was 32.3% in poorest, 41.5% in poorer, 48.9% in middle, 43.4% in richer, and 35.9% in richest households. These results are in agreement with a previous study that used the data from the 2016 Nepal National Micronutrient Status Survey (NNMSS).[36] The possible reason could be Nepal is an agrarian-based country and the staple diet may be similar for most of the households.[61] Most of the Nepalese people consume iron-rich staple foods including cereal, grains, lentils and animal source food regardless of wealth status.[61] Future research are critical to explore the association of household economic status and anaemia among WRA in Nepal.

In this study, the prevalence of anaemia was found to decrease with increasing age. These findings are consistent with studies from Ethiopia,[32] Demographic Republic of Congo,[62] and Benin.[52] The possible reason could be that the low fertility rates are high among older women.[32] Relatively higher prevalence of anaemia in a younger age could be because of lower dietary iron intake and the additional demand for iron to compensate iron loss during menstruation, pregnancy, and lactation.[1] High prevalence of anaemia among younger women could also be attributed to teenage pregnancy.[35]

Effect of contraceptive use on anaemia

In this study, the use of hormonal contraceptive methods was less likely to be associated with anaemia among WRA which is consistent with previous studies conducted in Nepal,[35] Rwanda,[58] Tanzania,[57] and Ethiopia.[63] This could be due to multiple reasons. For instance, the plausible primary mechanism could be the use of hormonal contraceptive lead to less blood loss during the menstruation.[64,65] Almost 100-150 mg of iron is lost during menstrual bleeding.[66] Subsequently, this may directly or indirectly furnish iron loss among women at high risk for iron-deficiency anaemia.[65,66] A previous study also suggested that Depo-Provera injections were more likely to increase haemoglobin concentration among WRA in Nepal.[67]

Community-level factors and anaemia

In this study, estimated intra-class correlation (ICC) shows that about 10.3% of the community level variability was attributable to the difference between communities among WRA. The PCV indicated that 39.5% of the variation in WRA anaemia between communities was explained by both individual and community-level characteristics. This finding is in line with the study conducted in Ethiopia, where both individual- and community-level factors accounted for about 43% of the variability of anaemia among WRA.[32] Women who came from Province-2 had more than three times higher odds of anaemia compared to Bagmati province. A possible reason could be due to the fact that WRA from Province-2 are more likely to be of a lower socioeconomic status and are less likely to afford a diverse diet.[9,26] In addition, compliance rate of recommended dose of iron tablets amongst the pregnant mothers in Province-2 was also low (28%) compared to other provinces.[9] This study showed that promoting community female education has a potential role in lowering the likelihood of anaemia which echoes with a study from Malawi.[38] This could be explained by the fact that higher community education

366 provides a context where women are enabled to gain nutritional knowledge and material
367 resources[25] that can increase consumption of iron absorption enhancers such as vitamin C,
368 phytates (whole grains, legumes), and calcium (dairy products). With increase knowledge,
369 women may also reduce the intake of tea, coffee, and some spices which are known to inhibit the
370 iron absorption.[68]

371 Strength and limitation

372 This is the first study to explore the spatial pattern and multilevel analysis of anaemia among
373 WRA in Nepal using stringent cluster sampling of a comprehensive nationally representative
374 data. The combined statistical methods including multilevel and spatial analysis used in this
375 study provides important insights on the role of contextual factors and geographical patterns in
376 the occurrences of anaemia among WRA in Nepal. This study has some limitations. The cross-
377 sectional design of the study does not allow us to establish the causality. This study relied on
378 haemoglobin as the measure of anaemia; further studies should consider other indices that
379 include total ferritin and total iron binding capacity to differentiate the types of anemia. Since
380 this study is based on the secondary data analysis, we are unable to incorporate potential
381 confounding factors of anaemia such as nutrient intake, worm infestations, malaria and other
382 non-modifiable risk factors.

383 CONCLUSION

384 This study highlighted a high prevalence of anaemia among WRA across Nepal. At an individual
385 level, women who had no formal education, those who came from middle socio-economic class
386 families were more likely to be anaemic, whereas, older women, and those who used hormonal
387 contraceptive were less likely to be anaemic. At the community level, low community female
388 education, and women living in Province-2 were associated with increased odds of anaemia. In

the spatial analysis, our study found statistically significant hot spots in the southern Terai region particularly in Province-1 (4 districts), Province-2 (8 districts), Bagmati province (1 district), and Province-5 (3 districts). Multi-pronged approaches including nutritional, and non-nutritional anaemia prevention and control strategies can benefit by tailoring the programs to the high risk provinces and districts with high burden of anemia.

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Contributors

DRSu designed, conceptualized the study, data extraction and analysis, interpreted the results, generate the map, writing an original draft, reviewing, editing, and overall supervision of the research. SS generate the map, and writing an original draft. DRSi, BA, and PMSP writing an original draft, reviewing and editing, overall supervision of the research. All authors read and approved the final manuscript.

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Competing interests

None declared

Patient consent for publication

Not required

Ethical approval

The NDHS 2016 was approved by the Nepal Health Research Council (NHRC) and ICF Macro.

Data sharing statement

Dataset used in this study is publicly available from the DHS website (URL: https://www.dhsprogram.com/data/available-datasets.cfm).

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Figure 1 Flow chart for sample size selection

Figure 2 Study area map and observed anaemia prevalence among women of reproductive age for the NDHS survey clusters

Table 1 Socio-demographic characteristics and prevalence of anaemia among women of reproductive age by determining factors (n=6,414)

Table 2 Multilevel mixed-effects logistic regression for individual and community-level factors affecting anaemia among women of reproductive age (n=6,414)

Figure 3 (a) Prevalence of anaemia among women of reproductive age across provinces. 3 (b) Prevalence of anaemia among women of reproductive age across districts

Figure 4 (a) Standardized prevalence ratio of anaemia among women of reproductive age across provinces. 4 (b) Standardized prevalence ratio of anaemia among women of reproductive age across districts (standardized to the national mean prevalence of 41%)

Figure 5 Spatial pattern and distribution of hot and cold spots of anaemia among women of reproductive age at cluster level in Nepal

Table 1 Socio-demographic characteristics and prevalence of anaemia among women of reproductive age by determining factors (n=6,414)

Variables	Total sample	Prevalence of any anemia		p-value[1, 2]
	Frequency (%)	Frequency (%)	95% CI	
Overall prevalence	6414	2614 (40.7)	[38.5-42.9]	
Severe anemia	17 (0.3)			
Moderate anemia	450 (7)			
Mild anemia	2147 (33.4)			
Hemoglobin level, mean (±SD)	12.3 ± 1.5	10.8 ± 1.1		<0.001*
Individual-level factors				
Age in years, mean (±SD)	29.1 ± 9.7	28.4 ± 9.5		<0.001*
Age				<0.001*
15-24	2443 (38.1)	1065 (43.5)	[40.6-46.5]	
25-34	1971(30.7)	814 (41.3)	[38.4-44.2]	
≥35	2000(31.1)	735 (36.7)	[34-39.6]	
Education				0.086
No education	2144 (33.4)	892 (41.5)	[38.2-44.9]	

Primary	1069 (16.6)	411 (38.4)	[34.2-42.8]	
Secondary	2277 (35.5)	972 (42.6)	[39.6-45.7]	
Higher	924 (14.4)	339 (36.7)	[32.3-41.3]	
Occupation				0.084
Not working	2096 (32.6)	899 (42.9)	[39.6-46.2]	
Working	4318 (67.3)	1715 (39.7)	[37.2-42.2]	
Wealth index				<0.001*
Poorest	1093 (17)	353 (32.3)	[28.7-36.1]	
Poorer	1225 (19.1)	508 (41.5)	[37.9-45.2]	
Middle	1317 (20.5)	645 (48.9)	[45.1-52.8]	
Richer	1441 (22.4)	626 (43.4)	[38.8-48.1]	
Richest	1338 (20.8)	482 (35.9)	[32.1-40.1]	
BMI, mean (±SD)	22 ± 3.8	21.4 ± 3.6		<0.001*
BMI (n=6411)				<0.001*
Normal	3925 (61.2)	1673 (42.6)	[39.7-45.5]	
Underweight	1077 (16.8)	518 (48.1)	[44.6-51.6]	
Overweight/obesity	1408 (21.9)	423 (30)	[26.7-33.4]	
Currently pregnant				
No	6124 (95.4)	2481 (40.5)	[38.3-42.7]	0.081
Yes	290(4.5)	133 (46)	[39.4-52.7]	
Currently breastfeeding				
No	4988 (77.7)	1961 (39.3)	[37.1-41.6]	<0.001*
Yes	1426 (22.2)	653 (45.8)	[42.2-49.3]	
Total children ever born				0.612
No child	1842 (28.7)	770 (41.8)	[38.2-45.4]	
1-3 child	3386 (52.8)	1358 (40.1)	[37.7-42.5]	
4+ child	1186 (18.4)	487 (41)	[37.2-44.8]	
Current contraceptive use				<0.001*
Not using	3832 (59.7)	1625 (42.4)	[39.8-45]	
Natural/barrier/permanent	1676 (26.1)	723 (43.1)	[40.1-46.1]	
Hormonal	829 (12.9)	241 (29.1)	[25.2-33.3]	
IUD	77 (1.2)	25 (33)	[20.8-47.9]	
Cigarette/smoking				<0.001*
Smoking	573 (8.9)	156 (28.9)	[24.7-33.4]	
Not smoking	5841 (91.1)	2449 (41.9)	[39.7-44.1]	
MDD-W (n=1131)				0.025*
Not diverse	558 (49.3)	284 (51)	[45.8-56.1]	
Diverse	573 (50.6)	248 (43.1)	[38.2-48.2]	
Community-level factors				

Place of residence				0.202
Urban	4029 (68.8)	1596 (39.6)	[36.8-42.4]	
Rural	2385 (37.1)	1018 (42.6)	[39-46.3]	
Province				<0.001*
Province-1	1073 (16.7)	464 (43.2)	[37.9-48.7]	
Province-2	1285 (20)	742 (57.7)	[52.7-62.6]	
Bagmati	1408 (21.9)	409 (29)	[24.8-33.5]	
Gandaki	627 (9.7)	176 (28)	[23-33.6]	
Province-5	1086 (16.9)	472 (43.4)	[39.4-47.5]	
Karnali	369 (5.7)	129 (34.9)	[29.9-40.2]	
Sudurpaschim	566 (8.8)	222 (39.3)	[33.6-45.2]	
Community female education[a]				<0.001*
Low	2936 (45.7)	1406 (47.8)	[44.8-50.9]	
High	3478 (54.2)	1208 (34.7)	[31.9-37.6]	
Community female wealth index[b]				<0.001*
Low	4107 (64)	1820 (44.3)	[41.2-47.3]	
High	2307 (35.9)	795 (34.45)	[31.1-37.8]	
Source of drinking water (n=6084)				0.004*
Improved	5537 (91)	2286 (41.2)	[38.9-43.6]	
Not improved	547 (8.9)	184 (33.7)	[29.2-38.5]	
Type of toilet facility (n=6084)				<0.001*
Improved	5117 (84.1)	1972 (38.5)	[36.3-40.7]	
Not improved	967 (16)	498 (51.4)	[46-56.8]	

Frequency and percentage (%) are weighted
[1, 2]denotes Pearson Chi-Square test for categorical variables and independent t-test for continuous variables
*denotes statistically significant at p<0.05
[a]mean percent of households wealth quintiles categorized richer and richest and above
[b]mean percent of women with primary education level and above
BMI: Body Mass Index
MDD-W: Minimum Dietary Diversity for Women

Table 2 Multilevel mixed-effects logistic regression analysis for individual and community-level factors affecting anemia among women of reproductive age (n=6,414)

Variables	Model 1 Empty model	Model 2 Individual-level factors	Model 3 community-level factors	Model 4 Individual and community-level factors
		aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Fixed-effects model				
Individual-level factors				
Age				
15-24		0.76 (0.56-1.04)		0.73 (0.54-1.01)
25-34		Ref		Ref
≥35		0.46 (0.24-0.88)*		0.48 (0.26-0.91)*
Education				
No education		2.23 (1.35-3.67)**		2.07 (1.21-3.54)*
Primary		1.56 (0.96-2.56)		1.41 (0.84-2.38)
Secondary		1.63 (1.06-2.49)*		1.52 (0.96-2.38)
Higher		Ref		Ref
Occupation				
Not working		Ref		Ref
Working		1.01 (0.75-1.36)		0.91 (0.66-1.25)
Wealth index				
Poorest		Ref		Ref
Poorer		1.50 (1.00-2.24)		1.49 (0.97-2.29)
Middle		1.81 (1.18-2.78)**		1.66 (1.02-2.68)*
Richer		1.46 (0.92-2.30)		1.39 (0.84-2.32)
Richest		1.61 (0.90-2.87)		1.32 (0.69-2.52)
BMI (n=6411)				
Normal		Ref		Ref
Underweight		1.16 (0.82-1.64)		1.08 (0.74-1.56)
Overweight/obesity		0.90 (0.59-1.37)		1.03 (0.66-1.61)
Currently pregnant				
No		0.82 (0.42-1.62)		1.08 (0.53-2.20)
Yes		Ref		Ref
Currently breastfeeding				
No		Ref		Ref
Yes		1.38 (0.65-2.91)		1.59 (0.71-3.60)
Total children ever born				
No child		Ref		Ref

1-3 child		-		-
4+ child		1.18 (0.74-1.86)		1.02 (0.63-1.63)
Current contraceptive use				
Not using		Ref		Ref
Natural/barrier/permanent		1.26 (0.87-1.82)		1.26 (0.85-1.85)
Hormonal		0.61 (0.42-0.86)*		0.57 (0.39-0.84) *
IUD		2.54 (0.69-9.32)		2.07 (0.68-10.61)
Cigarette/smoking				
Smoking		0.81 (0.44-1.50)		1.21 (0.63-2.33)
Not smoking		Ref		Ref
MDD-W (n=1131)				
Not diverse		1.24 (0.93-1.64)		1.03 (0.76-1.40)
Diverse		Ref		Ref
Community-level factors				
Place of residence				
Urban			Ref	Ref
Rural			1.01 (0.83-1.21)	0.89 (0.64-1.24)
Province				
Province-1			1.70 (1.23-2.34)**	3.04 (1.59-5.82)**
Province-2			2.51 (1.79-3.53)***	3.12 (1.58-6.14)**
Bagmati			Ref	Ref
Gandaki			0.90 (0.64-1.26)	0.77 (0.39-1.51)
Province-5			1.60 (1.16-2.21)**	2.43 (1.31-4.55)**
Karnali			1.19 (0.85-1.66)	1.69 (0.88-3.19)
Sudurpaschim			1.54 (1.11-2.15)*	1.64 (0.84-3.17)
Community female education[a]				
Low			1.39 (1.15-1.68)***	-
High			Ref	-
Community wealth index[b]				
Low			1.48 (1.21-1.80)***	-
High			Ref	-
Source of drinking water (n=6084)				
Improved			Ref	Ref
Not improved			1.05 (0.84-1.30)	1.13 (0.68-1.86)

Type of toilet facility (n=6084)				
Improved			Ref	Ref
Not improved			1.03 (0.84-1.24)	0.95 (0.63-1.45)
Random-effects model				
Community-level variance (τ) (SE)	0.627 (0.073)***	0.531 (0.207)***	0.414 (0.056)***	0.379 (0.201)**
ICC (%)	16	13.9	11.1	10.3
PCV (%)	Ref	15.3	33.9	39.5
Model fit statistics				
AIC	8322.6	1558.6	7838.4	1397.9
BIC	8336.1	1669.6	7925.8	1551.4
Log-likelihood	4159.3	757.3	3906.2	667.9

Model 1 (empty model): without adjusted predictors variables; model 2: adjusted for individual-level factors; model 3: adjusted for community-level factors; model 4: adjusted for both individual-level and community-level factors.

*p<0.05, **p<0.01, ***p<0.001,

aOR=adjusted odds ratio, CI: confidence interval, Ref: reference category, SE: standard error,

ICC: intra-class correlation, PCV: percentage change in variation, AIC: Akaike information criterion, BIC: Bayesian information criterion

[a] mean percent of households wealth quintiles categorized richer and richest and above

[b] mean percent of women with primary education level and above

community female education and community wealth index is omitted in Model 4 due to collinearity

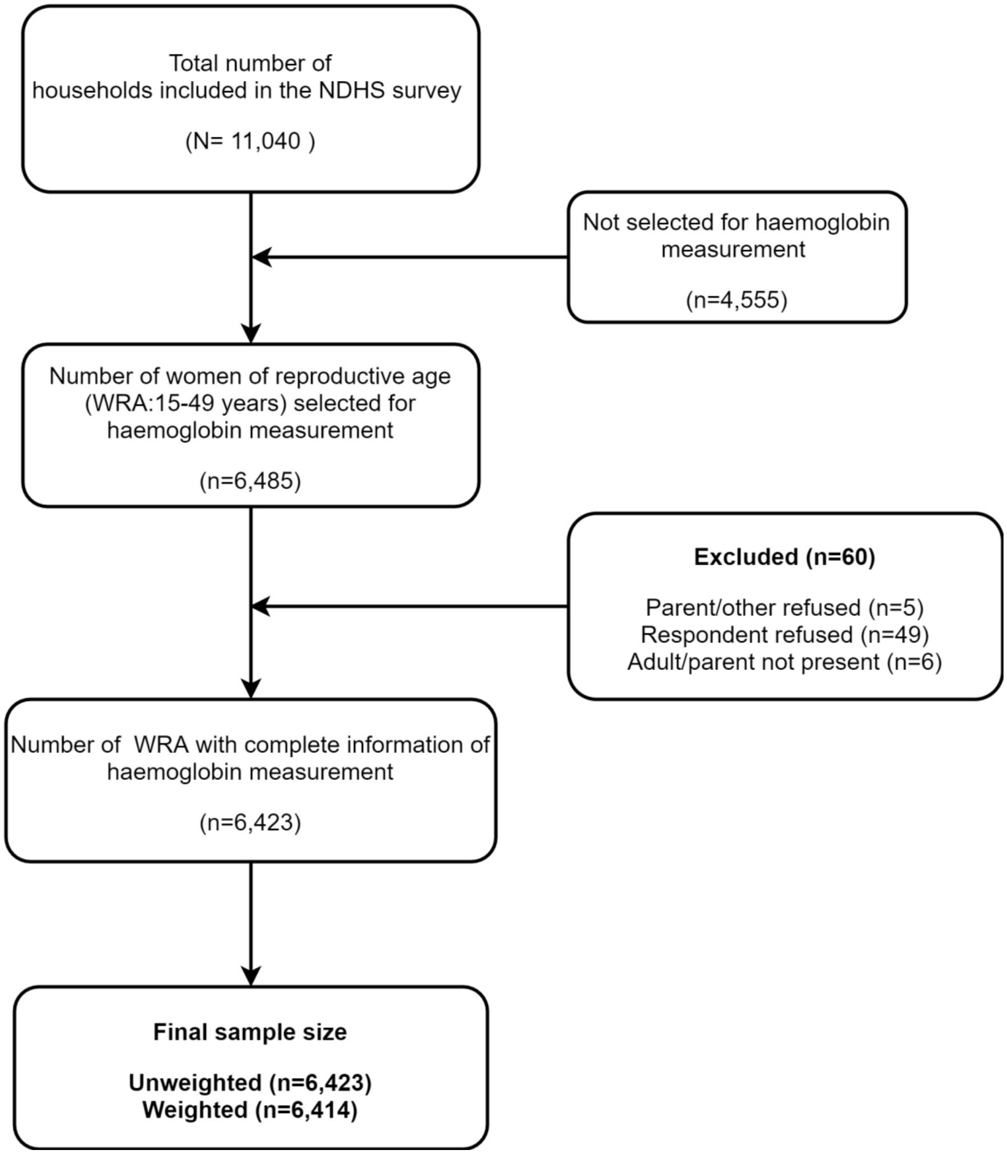

Figure 1 Flow chart for sample size selection

626x717mm (72 x 72 DPI)

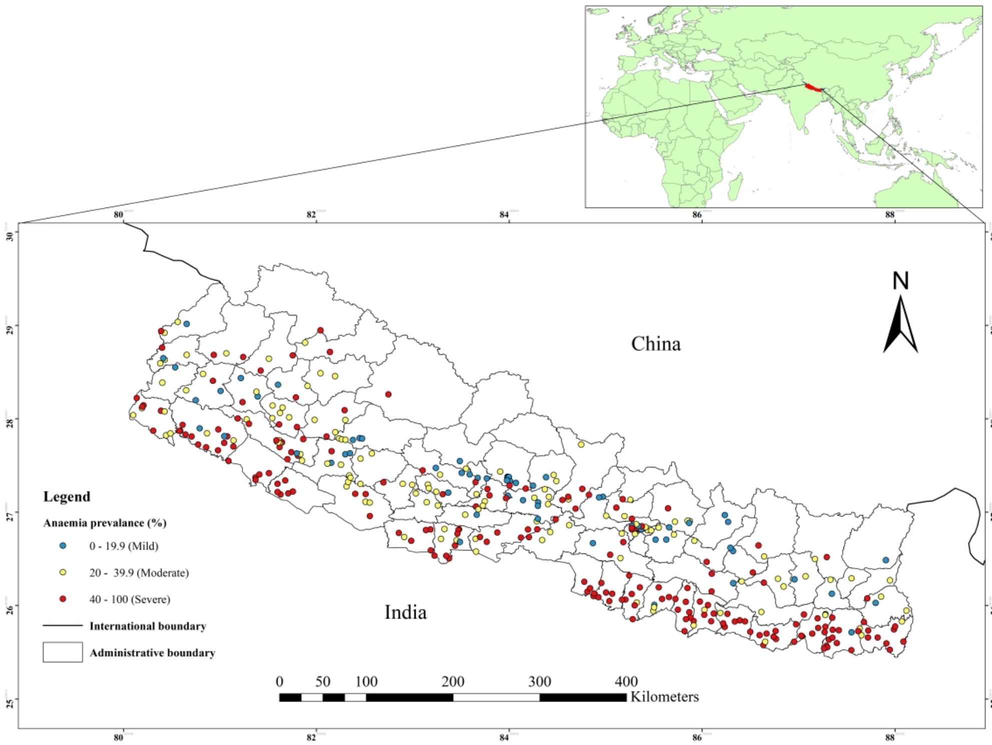

Figure 2 Study area map and observed anaemia prevalence among women of reproductive age for the NDHS survey clusters

609x457mm (300 x 300 DPI)

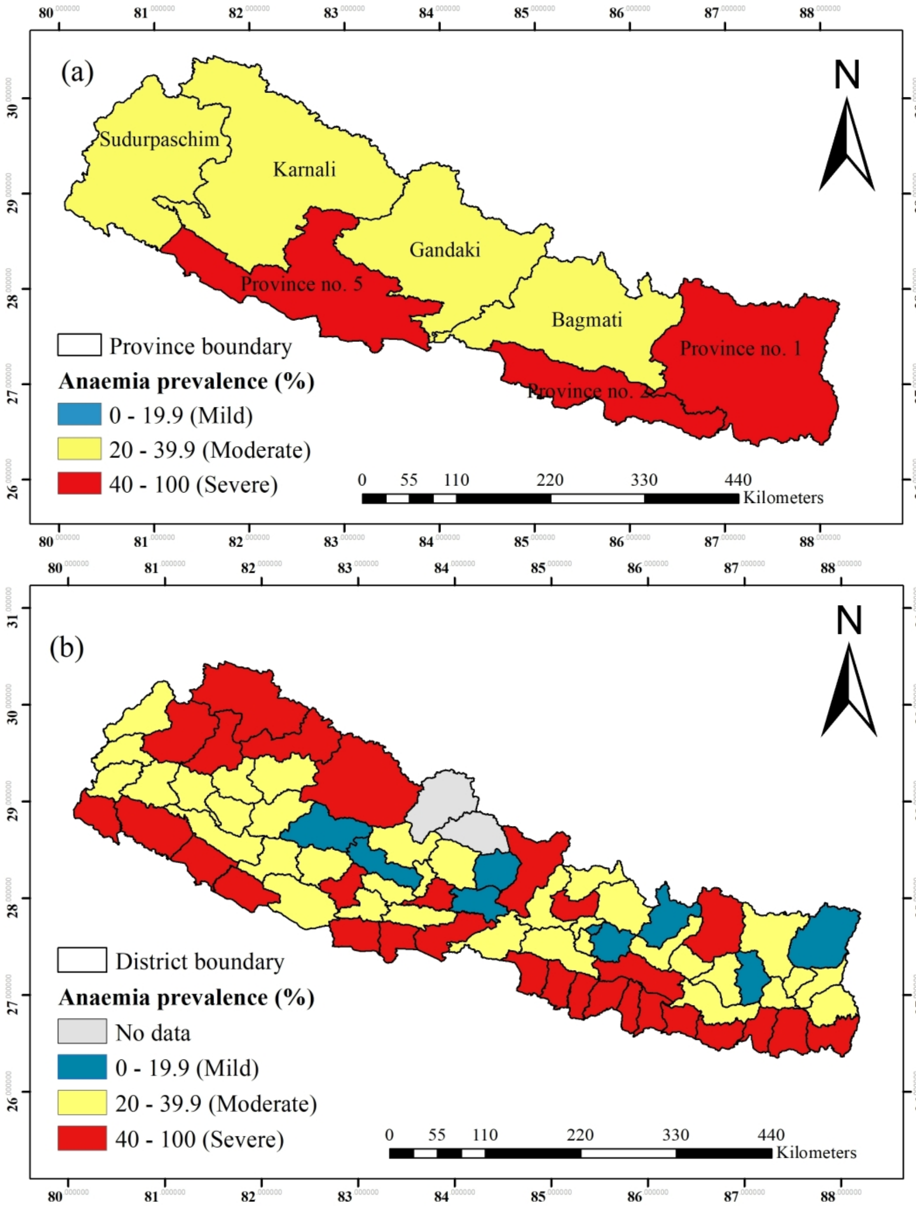

Figure 3 (a) Prevalence of anaemia among women of reproductive age across provinces. 3 (b) Prevalence of anaemia among women of reproductive age across districts

215x279mm (300 x 300 DPI)

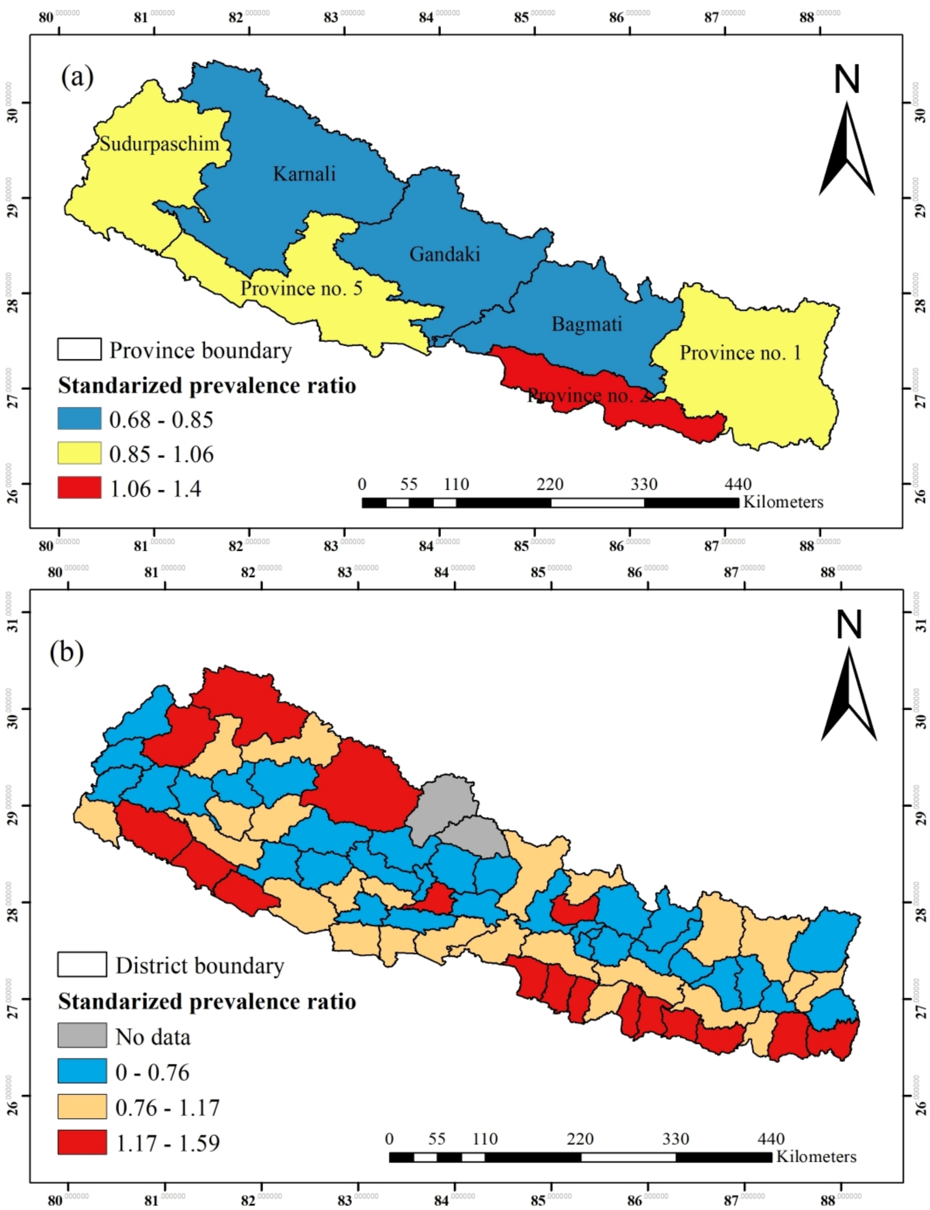

Figure 4 (a) Standardized prevalence ratio of anaemia among women of reproductive age across provinces. 4 (b) Standardized prevalence ratio of anaemia among women of reproductive age across districts (standardized to the national mean prevalence of 41%)

215x279mm (300 x 300 DPI)

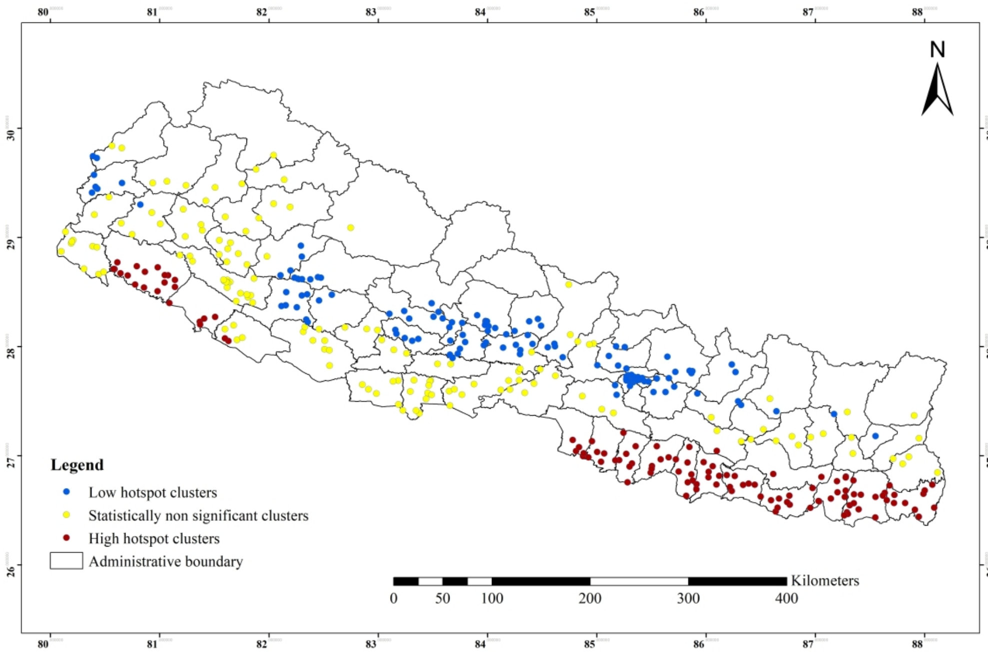

Figure 5 Spatial pattern and distribution of hot and cold spots of anaemia among women of reproductive age at cluster level in Nepal

457x304mm (300 x 300 DPI)

S1 Table Plan for data coding and description of the study variables

Study variables	Coding category for analysis
Age in years	1=15-24; 2=25-34; 3=35-49
Education	1=No education; 2=Primary; 3=Secondary; 4=Higher
Occupation	1= Not working; 2=Working (professional/technical/managerial, clerical, sales/services, agriculture-self-employed, skilled manual, unskilled manual, others)
Wealth index	1=Poorest; 2=Poorer; 3=Middle; 4=Richer; 5=Richest
Body mass index (BMI)	1=Normal (18.5-24.99); 2=Underweight(<18.5); 3=Overweight/Obesity(≥25)
Currently pregnant	1=No; 2=Yes
Currently breastfeeding	1=No; 2=Yes
Total children ever born	1= No child; 2=1-3 child; 3=4+ child
Current contraceptive use	1=Not using; 2=Natural/barrier/permanent (Male condom, female sterilization, male sterilization, periodic abstinence, withdrawal, other traditional, lactation amenorrhea); 3=Hormonal (Pill, Injections, Implants/Norplant's, Emergency contraception); 4=IUD
Cigarette/smoking	1= Smoking; 2=Not smoking
Minimum dietary diversity for women (MDD-W)	1=Not diverse (consume <5 food groups); 2=Diverse (Consume ≥5 food groups)
Residence	1=Urban; 2=Rural
Province	1=Provice-1; 2=Province-2, 3=Bagmati Province; 4=Gandaki Province; 5=Province-5; 6=Karnali Province; 7=Sudurpaschim Province
Community female education	1=Low (mean percent of women with lower primary education level was 0-25%); 2=High (mean percent of women with primary education level above 25%)
Community wealth index	1=Low (mean proportion of women from richest (rich, richest) households in the community was 0-21%); 2=High (mean

	proportion of women from richest (rich, richest) households in the community was above 21%)
Source of drinking water	1=Improved (piped water into dwelling, piped water to yard/plot, public tap or standpipe, tube well or borehole, protected dug well-protected spring and rainwater); 2=Not improved (Piped to neighbor, unprotected well, river, dam ponds, lake, and rainwater)
Type of toilet facility	1=Improved facility (flush toilet, piped sewer system, septic tank, flush/pour flush to pit latrine, ventilated improved pit latrine, pit latrine with slab, and composting toilet); 2=Not improved (flush to somewhere, flush don't know where, pit latrine without slab/open pit, and no facility/bush/field)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5-6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-10
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-10
		(*b*) Describe any methods used to examine subgroups and interactions	8-10
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-14
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-18
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Factors affecting anaemia among women of reproductive age in Nepal: a multilevel and spatial analysis

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ABSTRACT

Objective: The main objective of this study was to explore the factors affecting anaemia among women of reproductive age (WRA) in Nepal using spatial and multilevel epidemiological analysis.

Design: This cross-sectional study analyzed the data from the 2016 Nepal demographic and health survey (NDHS 2016). The spatial analysis was performed using ArcGIS software V.10.8 to identify the hot and cold spots of anaemia among WRA (15 to 49 years). Data were analyzed using multilevel mixed-effect logistic regression analysis.

Setting: Nepal

Participants: A total of 6,414 WRA were included in the analysis.

Main outcome measure: Anaemia defined by World Health Organization (WHO) as hemoglobin level less than 12g/dL in non-pregnant women and less than 11g/dL in pregnant women.

Results: The spatial analysis showed that statistically significant hotspots of anaemia were in the southern Terai region (4 districts in Province-1, 8 districts in Province-2, 1 district in Bagmati province, and 3 districts in Province-5) of Nepal. At the individual level, women who underwent female sterilization (aOR: 3.61, 95% CI: 1.10-11.84), with no education (aOR: 1.99, 95% CI: 1.17-3.39), and from middle socio-economic class families (aOR: 1.65, 95% CI: 1.02-2.68) were more likely to be anemic, whereas, older women (≥35 years) (aOR: 0.51, 95% CI: 0.26-0.97), and those women who were using hormonal contraceptives (aOR: 0.63, 95% CI: 0.43-0.90) were less likely to be anaemic. At the community level, women from Province-2 (aOR=2.97, 95% CI: 1.52-5.82) had higher odds of being anaemic.

Conclusion: WRA had higher odds of developing anaemia, and it varied by the geographical regions. Nutrition specific and nutrition sensitive interventions can tailor the interventions based on the factors identified in this study to curb the high burden of anaemia.

Strengths and limitations of this study

- This study utilizes comprehensive, and nationally representative data with haemoglobin level.
- The combined statistical methods including multilevel and spatial analysis were applied, which takes into account the role of geographical risk profile and determinants of anaemia among WRA in Nepal.
- Due to the cross-sectional design, it was difficult to determine the cause-and-effect relationships between the predictors and outcome variable (anaemia).
- Other potential confounding factors of anaemia such as nutrient intake, worm infestations, other non-modifiable risk factors, and other qualitative factors were beyond the scope of this study.
- This study could not distinguish the types of anaemia such as nutritional, genetic and infectious.

INTRODUCTION

Anaemia remains a significant public health problem in developing countries despite advances in health science.[1] Approximately one-third of the population is affected by anaemia globally.[2,3] South and Southeast Asian countries account for the largest burden of anaemia with estimated prevalence of 52.5% among women of reproductive age (WRA).[4] The highest prevalence over the past 26 years was 55.2% in 1990.[5] Despite the implementation of a '1000 days nutrition program' among various other programs, targeted to a mother with newborn babies in South Asia, the reduction in anaemia among pregnant women has not been significant.[6] To accelerate reduction of anaemia, the World Health Assembly has set a target of achieving a 50% reduction of anaemia among WRA by 2025 relative to 2010.[7] However, not a single South Asian country is on the way to achieve the 2025 targets.[8]

The Government of Nepal has set targets in line with various global and national indicators such as Multi-Sectoral Nutrition Plan II (2018–2022), and Sustainable Development Goals (SDGs) 2030 for the reduction of anaemia. [9,10] Despite the historic efforts in preventing anaemia through the implementation of national nutrition programs and policies including iron-folic acid supplementation across the country, the prevalence of anaemia among WRA has been increasing steadily from 35% in 2011 to 41% in 2016.[11,12] These figures suggest that anaemia continues to be a serious public health problem in Nepal.[13]

Anaemia in WRA is associated with multiple conditions and consequences such as preterm delivery,[14] miscarriage,[15] low birth weight,[16] child growth faltering, impairment of cognitive function, increased susceptibility to infection, and poverty.[17,18] It is also associated with increased risk of prenatal and maternal mortality.[16,19] Approximately 20% of maternal deaths are caused by anaemia and it is also considered as an additional risk factor for 50% of all maternal deaths.[20,21]

Contributing factors and distribution of anaemia include a complex interplay of political, ecological, social, and biological factors.[22] In Nepal, anaemia among WRA is associated with various socio-ecological factors. In the southern Terai of Nepal, low community education status, gender based-inequality, poor health seeking behavior,[23–25] inadequate dietary intake during pregnancy,[26–28] lack of diversified diet,[12,27,29] high burden of hookworm infection and malaria,[30–32] and high amount of arsenic in potable water[33] were identified as factors contributing to anaemia. On the other hand, high prevalence of anaemia in the mountainous region were attributed to among others, food insecurity, and low dietary diversity,[12,27,34] poor health services,[25] illiteracy, and gender based-inequality.[23,24]

To decrease the burden of anaemia, it is necessary to generate adequate evidence in terms of the role and contribution of individual, household and the community level factors along with the geographical risk profile of anaemia. Only a few studies in the past have explored factors affecting anaemia among WRA using nationally representative Nepal Demographic and Health Survey (NDHS) [35–37]. Till date, no studies have used spatial data to explore the geographical hotspots (high prevalence) of anaemia among WRA using cluster sampling of the NDHS data. In addition, population in Nepal has diverse characteristics in terms of their culture, ethnicity and geographical locations. Within the latitude of 193km (North to South), Nepal bears tropical/sub-tropical landscape on the south and temperate to alpine in the North, with an elevation ranging from 70m to the summit of Mount Everest (8848m).[38] The distinct characteristics such as dietary habit, lifestyle and socio-economic status linked to the geographical regions of Nepal are unique and pose risk of developing anaemia. Exploring spatial patterns and factors affecting anaemia by geographical region is therefore critical to inform the plans and policies for targeted anaemia control and prevention programs.[39] The main objective of this study was to explore the spatial distribution and contributing factors of anaemia among WRA in Nepal.

METHODS

Patient and public involvement

This study utilized a publicly available data set (NDHS); therefore, there were no patients involved.

Data source

This study was based on the data from Nepal Demographic and Health Survey (NDHS) 2016, a nationally representative cross-sectional survey. This survey was carried out as part of the DHS program by New ERA (a private non-profit research organization based in Kathmandu) under the guidance of the Ministry of Health, Government of Nepal and was supported by ICF international and United States Agency for International Development (USAID). The study population for this study was WRA from the Nepal Demographic and Health Survey 2016.

Study settings and Sampling strategy

The sample for the 2016 NDHS was designed to provide estimates of population health, and nutrition indicators including fertility and mortality rates for the overall country, provinces, development regions, urban and rural municipalities, and for the ecological zones: Terai, Hills, and Mountains. The NDHS 2016 utilized a stratified, two-stage cluster sampling design. The survey used enumeration areas (EAs) which is a primary sampling unit (PSU) and was selected from 383 wards in both rural (n=199) and urban (n=184) areas with probability proportional to size method. In the second stage, 30 households on average within EAs were selected using a systematic sampling technique. A more detailed methodology of the NDHS has been published elsewhere.[12] All WRA (pregnant and non-pregnant) with complete socio-demographic and nutritional characteristics who were residents or who had slept in the selected households on the night before the survey were eligible for the survey. The details of the sample size selection in the NDHS 2016 are presented in Figure 1.

Study variables

Outcome variables

Haemoglobin level was measured using capillary blood by a battery-operated portable HemoCue rapid testing machine and was adjusted for altitude and smoking status.[12] According to the WHO, for non-pregnant and pregnant women aged 15-49 years, any form of anaemia is defined as haemoglobin concentration <12 g/dL and <11g/dL respectively.[40] The categories of anaemia were further dichotomized into 'anaemic' and 'not anaemic'.

Predictor's variables

Predictors of anaemia were selected based on the literature review. [4,35–37,39] The wide range of socio-demographic, individual, household and community factors were hypothesized to increase the likelihood of anaemia. The predictors of anaemia including both individual-level and community-level factors were included in the analysis. The coding strategy of individual-level and community-level factors are presented in online supplementary Table 1.

Individual-level factors

A total of 11 individual-level factors were identified, and that included for example, respondent's age, education level, occupation, wealth index, nutritional status, pregnancy status, and minimum dietary diversity for WRA (MDD-W). The minimum dietary diversity for women of reproductive age (MDD-W) was calculated using the guideline of the modified FANTA III tool developed by the Food and Agriculture Organization (FAO) of the United Nations.[41]

Community-level factors

The six different factors were included in the community-level factors that included place of residence, province, community wealth, community female education, community safe water access, and community toilet facility. The selection of community-level factors were based on the group of women living in similar settings.[39] If more women have shared features such as place of residence, province, type of water source, and toilet facility; it was considered to have the same effect on anaemia among WRA.[39] For community level wealth and female education, we constructed the aggregate continuous community-level predictor-variables by aggregating individual-level characteristics at the community (cluster) level. We dichotomized the aggregate variables into "high" or "low" based on the distribution of the proportion values calculated for each community[42], but it was not applicable for the provinces and place of residence. Based on distribution of the aggregate variable (normal or non-normal), mean and median was used as a cut off point for the categorization of community-level variables respectively. The community wealth was categorized as 'high' if the proportion of women from richest (rich, richest) households in the community was above 21% and 'low' if the proportion was 0 to 21%. Community female education was defined as the mean percentage of women in the community with at least primary education and above.[43] Water supply and sanitation guidelines based on the WHO and UNICEF Joint Monitoring Progamme (JMP) were used to define improved toilet facility and improved water source.[44]

Data analysis

Statistical analysis

Data were analyzed using Stata/MP version 14.1 (StataCorp LP, College Station, Texas). The 'svy' command was used to account for sampling weights, clustering, and stratification in complex survey data. Weighted frequencies, weighted percentage, mean and SD were used for the descriptive analysis. Pearson Chi-Square test for categorical variables and independent t-test for continuous variables were used. The multilevel mixed-effect logistic regression analysis was performed to estimate the adjusted odds ratio and to estimate the extent of random variations between communities.

Four models were created and were fitted. Model 1 (empty model) was fitted without predictor variable to test random variability in the intercept and to estimate the intra-class correlation (ICC).[45] Model 2 examined the effects of individual-level characteristics, model 3 examined the effects of community-level variables, and model 4 examined the effects of both individual and community-level characteristics simultaneously. In the multilevel mixed-effect logistic regression models, the fixed effects estimated the association between the likelihood of anaemia among WRA and the individual-level and community-level factors, and the findings are reported in terms of adjusted odds ratio (aOR), and 95% confidence intervals (CIs). To prevent statistical bias in the multilevel logistic regression model, we examined and reported multicollinearity among the predictor variables using variation inflation factors (VIF). In this study, we used "10" as a cut-off value for the maximum level of VIF.[46] The random effects are expressed as ICC,[45] and proportional change in variance (PCV).[47] The ICC was calculated to evaluate the cluster variability; and PCV can measure the total variation due to factors at the individual- and community- levels.[45] Models fit were assessed using Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Considering the nested structure of the survey data, a multilevel model is considered to be appropriate than ordinary single-level regression model because it provides correct parameter estimates by handling the cluster data.[48,49]

Spatial analysis

Spatial analysis were performed using ArcGIS software V.10.8, and base files of the administrative provinces and districts of Nepal were obtained from Government of Nepal, Ministry of Land Management, [50] and Natural Earth.[51] The global positioning system (GPS) data set for NDHS was obtained from the DHS website after receiving the approval letter. The prevalence of anaemia and standardized prevalence ratio were computed for both the districts and provinces in Stata/MP version 14.1 software and were later transferred to Excel spreadsheet. These data were imported into the ArcGIS software to link the reported anaemia prevalence for each cluster to the corresponding geographical location (survey cluster data). The spatial variations of the prevalence of anaemia among WRA by both districts and provinces were visualized. To estimate the standardized prevalence ratio (SPR; ratio of observed prevalence to expected prevalence)[52] of anaemia among WRA, we first determined the prevalence of anaemia for both districts and provinces. District and province wise prevalence rate of anaemia among WRA was multiplied by the national prevalence rate of 41% (normalized to the national prevalence of 41%).

The Local Moran's I, Gettis-Ord G-statistics tool in ArcGIS software was used to compute to measure how spatial autocorrelation of anaemia among WRA varies across different locations in Nepal. The Getis-Ord G-statistics identifies statistically significant spatial clusters of hotspot clusters (High-High), and cold spot clusters (Low-Low).[53,54] Hotspot analysis computes Z-score and p-value to determine the statistical significance of the clustering of anaemia over the study area at different significance levels simultaneously. [54,55] The statistical significance of autocorrelation was determined by z-scores and p-value ≤0.05 with a 95% Confidence Intervals (CIs).[39,55] An anaemia hot spot was defined as the occurrence of high prevalence rates of anaemia clustered together on the map. Anaemia cold spot was referred to the occurrence of low prevalence rates of anaemia clustered together on the map.[39,55] The spatial pattern and distribution of anaemia prevalence rates among WRA in Nepal are visualized on the map (Figure 2)

Ethical consideration

The DHS survey protocols were approved by the institutional review boards of ICF, the DHS program, and the Nepal Health Research Council (NHRC). The details on ethical procedures used in this survey have been published elsewhere.[12] We registered and requested for access to both main data and GPS data from the DHS website [56] and received an approval to access and download the DHS data file. DHS program collected data following a written informed consent from each individual. All individual identifiers were precluded from the final dataset in this study.

RESULTS

Socio-demographic characteristics of study participants

In this study, a total of 6,414 WRA were included in the analysis (Table 1). The mean (±SD) age of the study participants was 29 (±9.7) years. More than one third (38.1%) of the participants were from age group 15-24 years, and women (35.5%) had attended secondary level of education. Nearly one quarter (22.4%) of study participants were from richer wealth quintiles and more than half (59.7%) of the women were not using any contraceptive methods, and a half (50.6%) of the participants consumed more than five food types. More than three quarters (77.1%) of participants had mosquitoes bed nets for sleeping. High proportion of women (68.8%) belonged to urban areas, and were from Bagmati province (21.9%). More than half (54.2%) of the women had the highest percentage of community female education, whilst, nearly two-thirds (64%) of the women belonged to a low percentage of community female wealth index. Majority of the participants had improved sources of drinking water (91%) and type of toilet facility (84.1%). (Table 1)

Prevalence of anaemia among women of reproductive age

In the current study, the overall prevalence of any anaemia across Nepal was 41% (95% CI: 38.5-42.9%). At the individual level, the prevalence of anaemia was higher among younger age groups (43.6 %), and women who attended at least secondary level education (42.7 %). The higher prevalence of anaemia were found in middle socio-economic class families (48.9%). The prevalence of anaemia was more among women who had undergone female sterilization (53.7%), and those who consumed less than five food groups (51%). At the community-level, the prevalence of anaemia was higher in women who came from Province-2 (57.7%). The high prevalence of anaemia was found in community with low female education (47.9 %), female wealth index (44.3%), and who did not have improved toilet facility (51.5 %) (Table 1).

Factors affecting anaemia among women of reproductive age

The fixed effects (a measure of association) and the random-effects for the risk of developing anaemia among WRA are presented in Table 2. The results of the empty model (model 1) showed that there was statistically significant variability in the odds of anaemia between communities ($\tau=0.627$, $p<0.001$). The ICC in the empty model implied that 16% of the total variance for the risk of developing anaemia was attributed to differences between the communities. In individual-level factors (model 2), women who did not have formal education (aOR=2.22, 95% CI: 1.35-3.82) compared to those with higher education, and who belonged to middle-class families (aOR=1.38, 95% CI: 0.86-2.20) compared to the family from poorest families were found to have higher odds of anaemia. Older women had 52% lower (aOR=0.48, 95% CI: 0.24-0.83) odds of developing anaemia compared to younger women. Women who used hormonal contraceptive methods had 35% lower (aOR=0.65, 95% CI: 0.46-0.92) odds of anaemia compared to who did not use contraceptive methods. The ICC in model 2 indicated that 11.8% of the variation in WRA anaemia was attributable to differences across communities. The PCV indicated that 29.3% of the variance in WRA anaemia across communities was explained by the individual-level characteristics.

The community-level (model 3) showed that women from Province-2 had 2.5 times higher (aOR=2.51, 95% CI: 1.79-3.53) odds of anaemia compared to women from Bagmati province. Women who belonged to communities with a low percentage of the wealthy households had 1.48 times higher (aOR=1.48, 95% CI: 1.21-1.80) odds of anaemia compared to those coming from high percentage of the wealthy household; and women residing in communities with a low percentage of community female education had 1.39 times higher (aOR=1.39, 95% CI: 1.15-1.68) odds of anaemia compared to those coming from the communities with a high percentage of education. The ICC in model 3 showed that differences between communities accounted for about 11.1% of the variation in anaemia among WRA. In addition, the PCV indicated that 33.9% of the variation in WRA anaemia between communities was explained by community-level characteristics.

In model 4, women who had undergone female sterilization were at higher (aOR: 3.61, 95% CI: 1.10-11.84) odds of anaemia compared to those who did not use contraceptive methods. Women with no formal education were found to have 2 times higher (aOR=1.99, 95% CI: 1.17-3.39) odds of anaemia compared to women who had higher education. Women from middle socio-economic family had higher (aOR=1.65, 95% CI: 1.02-2.68) odds of anaemia compared to poorest counterparts. Women who came from Province-2 (aOR=2.97, 95% CI: 1.52-5.82) had higher odds of anaemia compared to Bagmati province. The older women had 49% lower (aOR=0.51, 95% CI: 0.26-0.97) odds of anaemia compared to younger women, and women who used hormonal contraceptive methods had 37% lower (aOR=0.63, 95% CI: 0.43-0.90) odds of anaemia compared to who did not use contraceptive methods. After the inclusion of both the individual and community-level variables in model 4, the ICC indicated that 9.5% of the variability in anaemia among WRA was attributable to the difference between communities. Furthermore, the PCV indicated that 44.6% of the variation in anaemia among WRA between communities was explained by both individual and community-level characteristics (Table 2).

Spatial data analysis

Figure 3(a) shows the prevalence of anaemia among WRA across provinces in Nepal. A severe anaemia prevalence (≥40%) among WRA was seen in Province-2, followed by Province-1 and 5. The prevalence of moderate anaemia (20-39.9%) was observed in Bagmati, Gandaki, Karnali, and Sudurpaschim province. Mild anaemia (prevalence<19.9%) was not found in any provinces. Figure 3(b) shows the prevalence of anaemia among WRA across 75 districts in Nepal. The prevalence rate of severe anaemia was observed in 29 out of 75 districts, and mild anaemia was found in only eight districts (Figure 3).

The standardized prevalence ratio by provinces (standardized to the national mean prevalence of 41%) are shown in Figure 4 (a), and ranged from 0.68 to 1.4. The higher prevalence ratio of anaemia was found in Province-2, whereas a lower prevalence ratio was observed in Bagmati, Gandaki, and Karnali province. Figure 4 (b) shows the standardized prevalence ratio across the 75 districts, and ranged from 0.76 to 1.59. The higher standardized prevalence ratio of anaemia was observed in 17 out of 75 districts across the country (Figure 4).

The spatial pattern and distribution of anaemia among WRA at the cluster level are displayed in Figure 5. The spatial analysis at the cluster level showed that statistically significant high hotspots of anaemia were observed in the southern Terai of the country bordering India: Province-2 (8 districts), Province-1 (4 districts), southern plain of the Bagmati province (1 district), and southwestern region of Province-5 (3 districts). Whilst, cold spots of anaemia were observed in most of the Hilly regions of the country (Figure 5).

DISCUSSION

In this study, more than 40% of WRA were anaemic which implies that anaemia is still an important public health problem in Nepal.[40] Geographical pattern showed that anaemia is an important public health problem in three of the seven provinces and 29 out of the 75 districts in Nepal. The higher prevalence of anaemia was observed in the southern Terai bordering India particularly in Province-2, and the upper Himalayan region of the country. The spatial analysis at the cluster level showed that high hot spots of anaemia were observed in Terai region especially in Province 1, 2 and 5. These findings are consistent with a previous study [57] which was based on the analysis of Nepal National Micronutrient Status Survey 2016 and found WRA living in the Terai ecological zone had higher odds of anaemia relative to women living in the Mountain and Hill regions. A possible reason could be that in the Terai region, there is a shortage of safe and adequate drinking water supply and the risks of malaria and hookworm infestation are high. [30]

In the southern Terai of Nepal, despite ecological richness, people have long suffered from a deficiency of micronutrients such as Vitamin A, iron and zinc,[26] and a high burden of hookworm infestation and malaria which can contribute to development of anaemia.[30] Diet of women in the Terai lack diversity and, nutrient adequacy which pose an increased risk of anaemia.[27] Terai region of Nepal is endemic to malaria in contrast to Hill and Mountain region of Nepal, [31,32,58] and thus poses higher risk of anaemia.[30] The majority (90%) of the population from Terai region rely on groundwater especially shallow tube well for domestic purposes including drinking whereas most of the people from Mountain and Hill region rely on municipal taps, spring water source, and stone spouts as drinking water sources. Terai region is the most densely populated region compared to Hill and Mountain regions of Nepal. The population of Terai increased dramatically after the 1970s because people from the Mountains and Hills migrated to Terai for permanent settlement. [33] Most of the water wells were installed to meet the growing population demand. However, well water in the Terai consists of high arsenic concentrations (>10 μg/ L) beyond the WHO recommendation. [33] A previous study also explained the high burden of anaemia might be due to chronic exposure to arsenic through contaminated groundwater in Terai region. [57] Higher arsenic concentration can inhibit haem iron metabolism and increase erythrocyte hemolysis.[59] Consequently, drinking arsenic-containing water poses an increased risk of anaemia among women.[60] Arsenic exposure was more likely to cause anaemia among women in Bangladesh.[61] The spatial analysis at the cluster level found that high hotspots of anaemia were also observed in southwestern region (Province-5, and southern plain of Sudurpaschim province), particularly Banke, Bardiya and Kanchanpur district. A previous study highlighted that Glucose 6 phosphate dehydrogenase deficiency (G6PDd), sickle cell trait (SCT), and sickle cell anaemia (SCA) as the most common disorder in Tharu communities living in southwestern province-5, and sudurpaschim province [62,63]. Study based on the Nepal National Micronutrient Status Survey 2016 also found the G6PD and haemoglobinopathies had strong association with anaemia among WRA.[57]

The geographical variance of high cases of anaemia across the high Mountainous region could be attributed to food insecurity, low dietary diversity, [12,27,34] less calorie diet,[27] poor health service coverage,[25] high illiteracy, gender based-inequality, and poor health seeking behaviour.[23,24]

Socio-demographic characteristic among women and anaemia

Women who had no formal education, and those who came from middle socio-economic households, and from younger age group were at increased risk of anaemia. These findings are in line with previous studies from low and middle-income countries (LMICs) including Ethiopia,[39] India,[64] Tanzania,[65] Rwanda,[66] Timor-Leste,[67] and Bangladesh.[68] Studies from Ethiopia and Tanzania suggest that higher-level education might enable women to gain knowledge and improve attitude which in turn can promote them to adopt healthier lifestyle including good nutrition habits, better health-seeking behavior, and good hygiene practices that can prevent anaemia.[39,65]

Anaemia is a multifaceted problem where nutrition and household economic status are considered to have a synergistic association.[66] Women belonging to poorer households are more likely to be anaemic compared to those living in middle or richer households in most of the countries.[17,39,66]. Contrastingly, this study revealed that poorer Nepalese women were less likely to be anaemic. Similar findings were reported by a previous study which was based on the analysis of the 2016 NDHS dataset.[36] The possible reason could be first, Nepal is an agrarian-based country and the staple diet may be similar for most of the households.[69] Second, poorer Nepalese women being at lower risk of anaemia might be due to the nationwide open defecation free (ODF) campaign initiated after 2011, which was targeted at poor households which contributed to the reduction of anaemia through the reduction in prevalence of helminthiasis.

[36] Third, there was various ongoing health and nutrition intervention program targeted at poorer households. For instance, consumption of iron-rich dark green leafy vegetables available from their kitchen garden,[70] consumption of animal source foods, and dietary iron consumption. Interventions such as Suaahara-I (from 2011 to 2016), and Sunaula Hazar Din (from 2014 to 2017), which provided financial and technical support to poor households for poultry farming, and contributed to increased consumption of meat and eggs among WRA.[71,72] Future studies are critical to explore the association of household economic status and anaemia among WRA in Nepal.

In this study, the prevalence of anaemia was found to be decreasing with increasing age. These findings are consistent with studies from Nepal, [36] Ethiopia,[39] Demographic Republic of Congo,[73] and Benin.[60] The possible reason could be that the low fertility rates are high among older women.[39] Also, it might be due to young girls being under-represented in the public health programs that aim to prevent anaemia in Nepalese context. [36] However, Government of Nepal has started weekly iron folic acid supplementation to adolescent girls aged 10–19 years only after 2016.[74] In contrast, few studies from Nepal did not show any association of age with anaemia.[35,37] The discrepancies might be due to the nature of statistical model used in the analysis.

Effect of contraceptive use on anaemia

In this study, those using hormonal contraceptive were less likely to be anaemic among WRA which is consistent with previous studies conducted in Nepal,[35,36] Rwanda,[66] Tanzania,[65] and Ethiopia.[75] This could be due to multiple reasons. For instance, use of hormonal contraceptive can reduce the blood loss during the menstruation.[76,77] Almost 100-150 mg of iron is lost during menstrual bleeding.[78] Subsequently, this may directly or indirectly furnish iron loss among women at high risk for iron-deficiency anaemia.[77,78] A previous study also suggested that Depo-Provera injections were more likely to increase haemoglobin concentration among WRA in Nepal.[79] Interestingly, in this study women who had undergone female sterilization were at increased risk of anaemia. These findings are in line with the similar study conducted in Nepal.[36,80]

Community-level factors and anaemia

In this study, estimated intra-class correlation (ICC) shows that about 9.5% of the community level variability was attributable to the difference between communities among WRA. The PCV indicated that 44.6% of the variation in WRA anaemia between communities was explained by both individual and community-level characteristics. This finding is in line with the study conducted in Ethiopia, where both individual- and community-level factors accounted for about 43% of the variability of anaemia among WRA.[39] Women who came from Province-2 had more than two times higher odds of anaemia compared to Bagmati province. Previous studies reported that women from province number 2 were more likely to be coming from lower socioeconomic status and less diverse diet (an estimated 29% of minimum dietary diversity (MDD)). [12,29] Other evidence from Nepal also suggested that women in Province 2 have poor nutritional status.[81] In addition, compliance rate of recommended dose of iron tablets amongst the pregnant mothers in Province-2 was also low (28%) compared to other provinces.[12] This study showed that promoting community female education has a potential role in lowering the likelihood of anaemia which echoes with a study from Malawi.[42] This could be explained by the fact that higher community education provides a context where women are enabled to gain nutritional knowledge and material resources [28] that can increase consumption of iron-absorption-enhancers such as vitamin C, phytates (whole grains, legumes), and calcium (dairy products). Increasing community level education can play an important role in promoting knowledge and attitude which in turn can incite them to adopt healthier lifestyle including good nutrition habits that can ultimately prevent anaemia.[39,65,70]

Strength and limitation

This study is conducted based on the spatial pattern and multilevel epidemiological analysis of anaemia among WRA in Nepal using cluster sampling of nationally representative data. The combined statistical methods including multilevel and spatial analysis used in this study provides important insights on the role of contextual factors and geographical patterns in the occurrences of anaemia among WRA in Nepal. This study has some limitations. The cross-sectional design of the study does not allow us to establish the causality. This study relied on haemoglobin as the measure of anaemia; further studies should consider other indices that include total ferritin and total iron binding capacity to differentiate the types of anaemia. Since this study is based on the secondary data analysis, we are unable to incorporate potential confounding factors of anaemia such as nutrient intake, worm infestations, and other non-modifiable risk factors.

CONCLUSION

This study highlighted a high prevalence of anaemia among WRA across Nepal. At an individual level, women who had no formal education, those who came from middle socio-economic class families were more likely to be anaemic, whereas, older women, and those who used hormonal contraceptive were less likely to be anaemic. At the community level, low community female education, and women living in Province-2 were associated with increased odds of anaemia. In the spatial analysis, our study found statistically significant hot spots in the southern Terai region particularly in Province-1 (4 districts), Province-2 (8 districts), Bagmati province (1 district), and Province-5 (3 districts). Both nutrition specific and nutrition sensitive interventions such as social and behavioral programs can tailor their strategies based on the factors identified in this study to reduce the high burden of anaemia.

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Contributors

Dev Ram Sunuwar (DRSu), Devendra Raj Singh (DRSi), Bipin Adhikari (BA), Santosh Shrestha (SS), and Pranil Man Singh Pradhan (PMSP). DRSu designed, conceptualized the study, data extraction and analysis, interpreted the results, generate the map, writing an original draft, reviewing, editing, and overall supervision of the research. SS generate the map, and writing an original draft. DRSi, BA, and PMSP writing an original draft, reviewing and editing, overall supervision of the research. All authors read and approved the final manuscript.

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Competing interests

None declared

Patient consent for publication

Not required

Ethical approval

The NDHS 2016 was approved by the Nepal Health Research Council (NHRC) and ICF Macro.

Data sharing statement

Dataset used in this study is publicly available from the DHS website (URL: https://www.dhsprogram.com/data/available-datasets.cfm).

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Figure 1 Flow chart for sample size selection

Figure 2 Study area map and observed anaemia prevalence among women of reproductive age for the NDHS survey clusters

Table 1 Socio-demographic characteristics and prevalence of anaemia among women of reproductive age by determining factors (n=6,414)

Table 2 Multilevel mixed-effects logistic regression for individual and community-level factors affecting anaemia among women of reproductive age (n=6,414)

Figure 3 (a) Prevalence of anaemia among women of reproductive age across provinces.

3 (b) Prevalence of anaemia among women of reproductive age across districts

Figure 4 (a) Standardized prevalence ratio of anaemia among women of reproductive age across provinces. 4 (b) Standardized prevalence ratio of anaemia among women of reproductive age across districts (standardized to the national mean prevalence of 41%)

Figure 5 Spatial pattern and distribution of hot and cold spots of anaemia among women of reproductive age at cluster level in Nepal

Table 1 Socio-demographic characteristics and prevalence of anaemia among women of reproductive age by determining factors (n=6,414)

Variables	**Total sample**	**Anaemia status % (95% CI)**		**p-value[1, 2]**
	Frequency (%)	Not anaemic	Anaemic	
Overall prevalence	6414	59.3 (57.0-61.4)	40.7 (38.5-42.9)	
Severe anaemia	17 (0.3)			
Moderate anaemia	450 (7)			
Mild anaemia	2147 (33.4)			
Hemoglobin level, mean (±SD)	12.3 ± 1.5	13.2 ± 0.9	10.8 ± 1.1	<0.001*
Individual-level factors				
Age in years, mean (±SD)	29.1 ± 9.7	29.5 ± 9.7	28.4 ± 9.5	<0.001*
Age				<0.001*
15-24	2443 (38.1)	56.4 (53.4-59.3)	43.6 (40.6-46.5)	
25-34	1971 (30.7)	58.7 (55.7-61.5)	41.3 (38.4-44.2)	
≥35	2000 (31.1)	63.2 (60.3-66)	36.8 (34-39.6)	
Education				0.086
No education	2144 (33.4)	58.4 (55-61.7)	41.6 (38.2-44.9)	
Primary	1069 (16.6)	61.5 (57.1-65.7)	38.5 (34.2-42.8)	
Secondary	2277 (35.5)	57.3 (54.2-60.3)	42.7 (39.6-45.7)	

Higher	924 (14.4)	63.3 (58.7-67.6)	36.7 (32.3-41.3)	
Occupation				0.084
Not working	2096 (32.6)	57.1 (53.7-60.3)	42.9 (39.6-46.2)	
Working	4318 (67.3)	60.3 (57.7-62.7)	39.7 (37.2-42.2)	
Wealth index				<0.001*
Poorest	1093 (17)	67.7 (63.8-71.2)	32.3 (28.7-36.1)	
Poorer	1225 (19.1)	58.5 (54.8-62.1)	41.5 (37.9-45.2)	
Middle	1317 (20.5)	51.1 (47.2-54.8)	48.9 (45.1-52.8)	
Richer	1441 (22.4)	56.6 (51.8-61.1)	43.4 (38.8-48.1)	
Richest	1338 (20.8)	64.1 (59.9-67.9)	35.9 (32.1-40.1)	
BMI, mean (±SD)	22 ± 3.8	22.4 ± 3.9	21.4 ± 3.6	<0.001*
BMI (n=6411)				<0.001*
Normal	3925 (61.2)	57.4 (54.4-60.2)	42.6 (39.7-45.5)	
Underweight	1077 (16.8)	51.9 (48.3-55.4)	48.1 (44.6-51.6)	
Overweight/obesity	1408 (21.9)	69.9 (66.5-73.2)	30.1 (26.7-33.4)	
Currently pregnant				0.081
No	6124 (95.4)	59.5 (57.3-61.6)	40.5 (38.3-42.7)	
Yes	290 (4.5)	53.9 (47.2-60.5)	46.1 (39.4-52.7)	
Currently breastfeeding				<0.001*
No	4988 (77.7)	60.7 (58.4-62.9)	39.3 (37.1-41.6)	
Yes	1426 (22.2)	54.2 (50.6-57.7)	45.8 (42.2-49.3)	
Total children ever born				0.612
No child	1842 (28.7)	58.2 (54.5-61.7)	41.8 (38.2-45.4)	
1-3 child	3386 (52.8)	59.9	40.1	

		(57.4-62.2)	(37.7-42.5)	
4+ child	1186 (18.4)	58.9 (55.1-62.7)	41.1 (37.2-44.8)	
Current contraceptive use				<0.001*
Not using	3832 (59.7)	57.6 (54.9-60.1)	42.4 (39.8-45.1)	
Hormonal	905 (14.1)	70.5 (66.5-74.2)	29.5 (25.7-33.4)	
Female sterilization	730 (11.4)	46.3 (41.8-50.8)	53.7 (49.1-58.2)	
Male contraception	268 (4.2)	71.7 (61.9-79.8)	28.3 (20.1-38.1)	
Traditional	679 (10.6)	62.4 (58.1-61.4)	37.6 (33.3-41.9)	
Cigarette/smoking				<0.001*
Smoking	573 (8.9)	71.1 (66.5-75.2)	28.9 (24.7-33.4)	
Not smoking	5841 (91.1)	58.1 (55.8-60.3)	41.9 (39.7-44.1)	
MDD-W (n=1131)				0.025*
Not diverse	558 (49.3)	49 (43.8-54.1)	51 (45.8-56.1)	
Diverse	573 (50.6)	56.8 (51.7-61.7)	43.2 (38.2-48.2)	
Mosquitoes bed net for sleeping				0.024*
No	1412 (22.1)	68.9 (65.6-72.1)	31.1 (27.9-34.3)	
Yes	5002 (77.1)	56.5 (53.9-59)	43.5 (41-46.1)	
Place of residence				0.202
Urban	4029 (68.8)	60.4 (57.5-63.1)	39.6 (36.8-42.4)	
Rural	2385 (37.1)	57.3 (53.6-60.9)	42.7 (39.1-46.3)	
Province				<0.001*
Province-1	1073 (16.7)	56.7 (51.2-62.1)	43.3 (37.9-48.7)	
Province-2	1285 (20)	42.2	57.7	

		(37.3-47.2)	(52.7-62.6)	
Bagmati	1408 (21.9)	70.9 (66.4-75.1)	29.1 (24.8-33.5)	
Gandaki	627 (9.7)	71.9 (66.3-76.9	28.1 (23.1-33.6)	
Province-5	1086 (16.9)	56.5 (52.5-60.5)	43.5 (39.4-47.5)	
Karnali	369 (5.7)	65.1 (59.7-70.1)	34.9 (29.9-40.2)	
Sudurpaschim	566 (8.8)	60.7 (54.7-66.3)	39.3 (33.6-45.2)	
Community female education[a]				<0.001*
Low	2936 (45.7)	52.1 (49.1-55.2)	47.9 (44.8-50.9)	
High	3478 (54.2)	65.3 (62.3-68.1)	34.7 (31.9-37.6)	
Community female wealth index[b]				<0.001*
Low	4107 (64)	55.7 (52.6-58.7)	44.3 (41.2-47.3)	
High	2307 (35.9)	65.5 (62.1-68.8)	34.5 (31.1-37.8)	
Source of drinking water (n=6084)				0.004*
Improved	5537 (91)	58.7 (56.3-61.1)	41.3 (38.9-43.6)	
Not improved	547 (8.9)	66.3 (61.4-70.8)	33.7 (29.2-38.5)	
Type of toilet facility (n=6084)				<0.001*
Improved	5117 (84.1)	61.5 (59.3-63.6)	38.5 (36.3-40.7)	
Not improved	967 (16)	48.5 (43.1-53.9)	51.5 (46.1-56.8)	

Frequency and percentage (%) are weighted

[1, 2]denotes Pearson Chi-Square test for categorical variables and independent t-test for continuous variables

*denotes statistically significant at p<0.05

[a]mean percent of households wealth quintiles categorized richer and richest and above

[b]mean percent of women with primary education level and above BMI: Body Mass Index MDD-W: Minimum Dietary Diversity for Women

Table 2 Multilevel mixed-effects logistic regression analysis for individual and community-level factors affecting anaemia among women of reproductive age (n=6,414)

Variables	**Model 1 Empty model**	**Model 2 Individual-level factors**	**Model 3 community-level factors**	**Model 4 Individual and community-level factors**
		aOR (95% CI)	**aOR (95% CI)**	**aOR (95% CI)**
Fixed-effects model				
Individual-level factors				
Age				
15-24		0.80 (0.57-1.08)		0.75 (0.54-1.04)
25-34		Ref		Ref
≥35		0.48 (0.24-0.83)*		0.51 (0.26-0.97)*
Education				
No education		2.22 (1.35-3.82)**		1.99 (1.17-3.39)*
Primary		1.52 (0.99-2.63)		1.33 (0.79-2.23)
Secondary		1.61 (1.08-2.54)*		1.46 (0.93-2.29)
Higher		Ref		Ref
Occupation				
Not working		Ref		
Working		0.98 (0.73-1.32)		
Wealth index				
Poorest		Ref		Ref
Poorer		1.20 (0.79-1.86)		1.46 (0.95-2.25)
Middle		1.38 (0.86-2.20)**		1.65 (1.02-2.68)*
Richer		1.13 (0.68-1.82)		1.39 (0.84-2.32)
Richest		1.28 (0.69-2.30)		1.33 (0.70-2.52)
BMI (n=6411)				
Normal		Ref		Ref
Underweight		1.10 (0.80-1.60)		1.05 (0.72-1.51)
Overweight/obesity		0.89 (0.59-1.35)		1.03 (0.65-1.58)
Currently pregnant				
No		0.81 (0.42-1.58)		1.08 (0.53-2.19)
Yes		Ref		Ref
Currently breastfeeding				

No		Ref		Ref
Yes		1.34 (0.63-2.82)		1.60 (0.71-3.59)
Total children ever born				
No child		Ref		Ref
1-3 child		-		-
4+ child		1.15 (0.75-1.87)		1.02 (0.63-1.63)
Current contraceptive use				
Not using		Ref		Ref
Hormonal		0.65 (0.46-0.92)*		0.63 (0.43-0.90)*
Female sterilization		3.55 (1.21-10.44)*		3.61 (1.10-11.84)*
Male contraception		0.92 (0.17-4.79)		0.74 (0.14-3.77)
Traditional		1.10 (0.74-1.63)		1.12 (0.74-1.69)
Cigarette/smoking				
Smoking		0.89 (0.48-1.66)		1.21 (0.63-2.33)
Not smoking		Ref		Ref
MDD-W (n=1131)				
Not diverse		1.20 (0.91-1.59)		1.03 (0.76-1.40)
Diverse		Ref		Ref
Mosquitoes bed net for sleeping				
No		0.57 (0.38-0.86)		
Yes		Ref		
Community-level factors				
Place of residence				
Urban			Ref	Ref
Rural			1.01 (0.83-1.21)	0.89 (0.64-1.24)
Province				
Province-1			1.70 (1.23-2.34)**	2.07 (1.57-5.64)**
Province-2			2.51 (1.79-3.53)***	2.97 (1.52-5.82)**
Bagmati			Ref	Ref
Gandaki			0.90 (0.64-1.26)	0.76 (0.39-1.49)
Province-5			1.60 (1.16-2.21)**	2.42 (1.31-4.50)**
Karnali			1.19 (0.85-1.66)	1.66 (0.88-3.14)
Sudurpaschim			1.54 (1.11-2.15)*	1.62 (0.84-3.10)
Community female education[a]				
Low			1.39 (1.15-1.68)***	-
High			Ref	-

Community wealth index[b]				
Low			1.48 (1.21-1.80)***	-
High			Ref	-
Source of drinking water (n=6084)				
Improved			Ref	Ref
Not improved			1.05 (0.84-1.30)	1.13 (0.68-1.86)
Type of toilet facility (n=6084)				
Improved			Ref	Ref
Not improved			1.03 (0.84-1.24)	0.96 (0.63-1.45)
Random-effects model				
Community-level variance (τ) (SE)	0.627 (0.073)***	0.443 (0.192)***	0.414 (0.056)***	0.347 (0.194)**
ICC (%)	16	11.8	11.1	9.5
PCV (%)	Ref	29.3	33.9	44.6
Model fit statistics				
AIC	8322.6	1554.7	7838.4	1400.6
BIC	8336.1	1675.8	7925.8	1559.1

Model 1 (empty model): without adjusted predictors variables; model 2: adjusted for individual-level factors; model 3: adjusted for community-level factors; model 4: adjusted for both individual-level and community-level factors.

*p<0.05, **p<0.01, ***p<0.001,

aOR=adjusted odds ratio, CI: confidence interval, Ref: reference category, SE: standard error,

ICC: intra-class correlation, PCV: percentage change in variation, AIC: Akaike information criterion, BIC: Bayesian information criterion

[a] mean percent of households wealth quintiles categorized richer and richest and above

[b] mean percent of women with primary education level and above

community female education and community wealth index is omitted in Model 4 due to collinearity

719

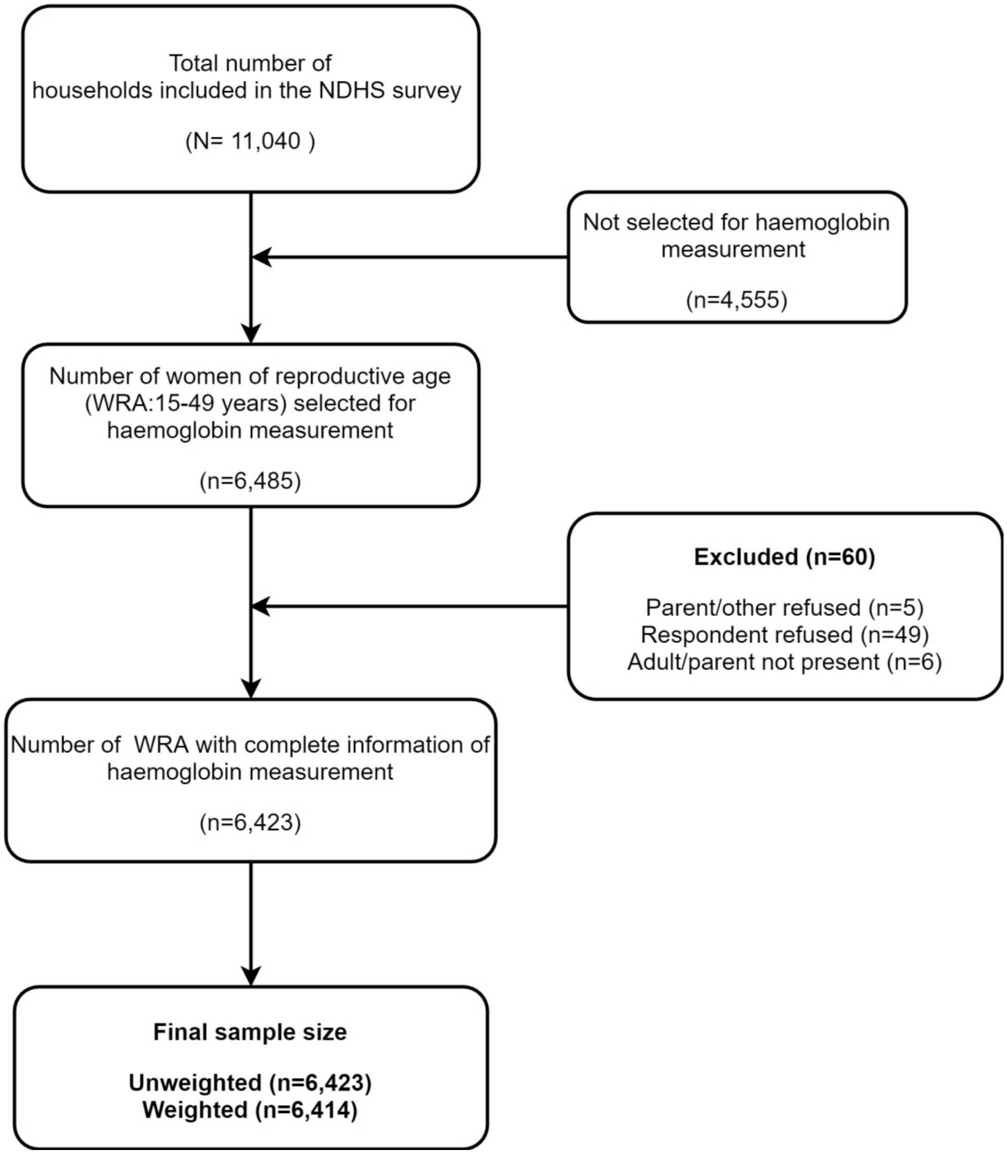

Figure 1 Flow chart for sample size selection

626x717mm (72 x 72 DPI)

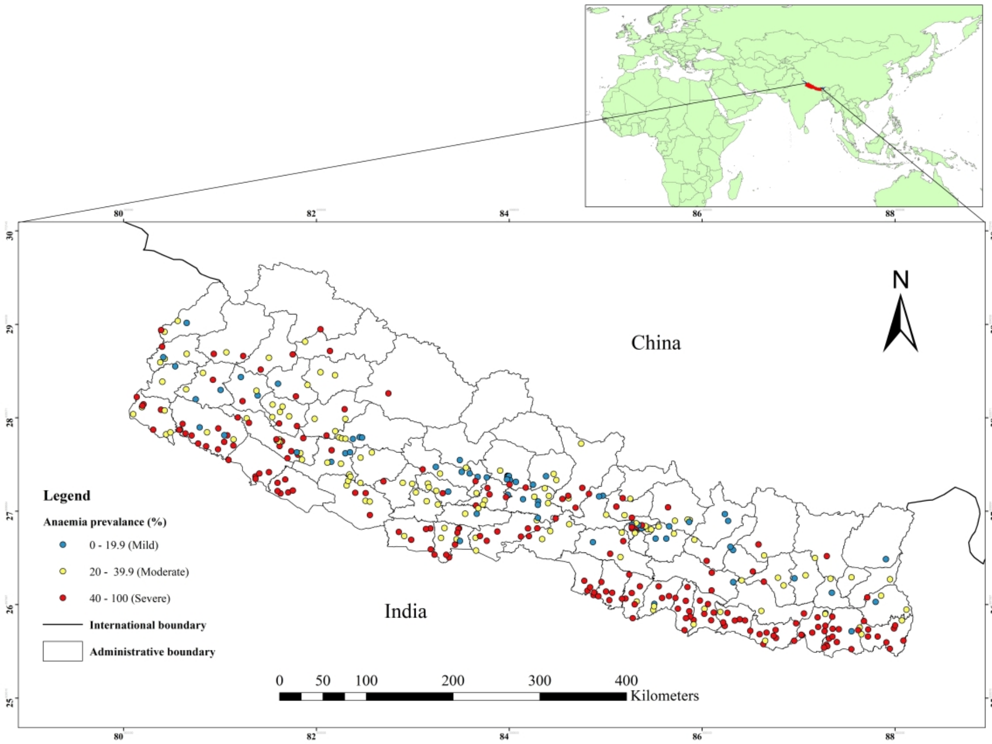

Figure 2 Study area map and observed anaemia prevalence among women of reproductive age for the NDHS survey clusters

609x457mm (300 x 300 DPI)

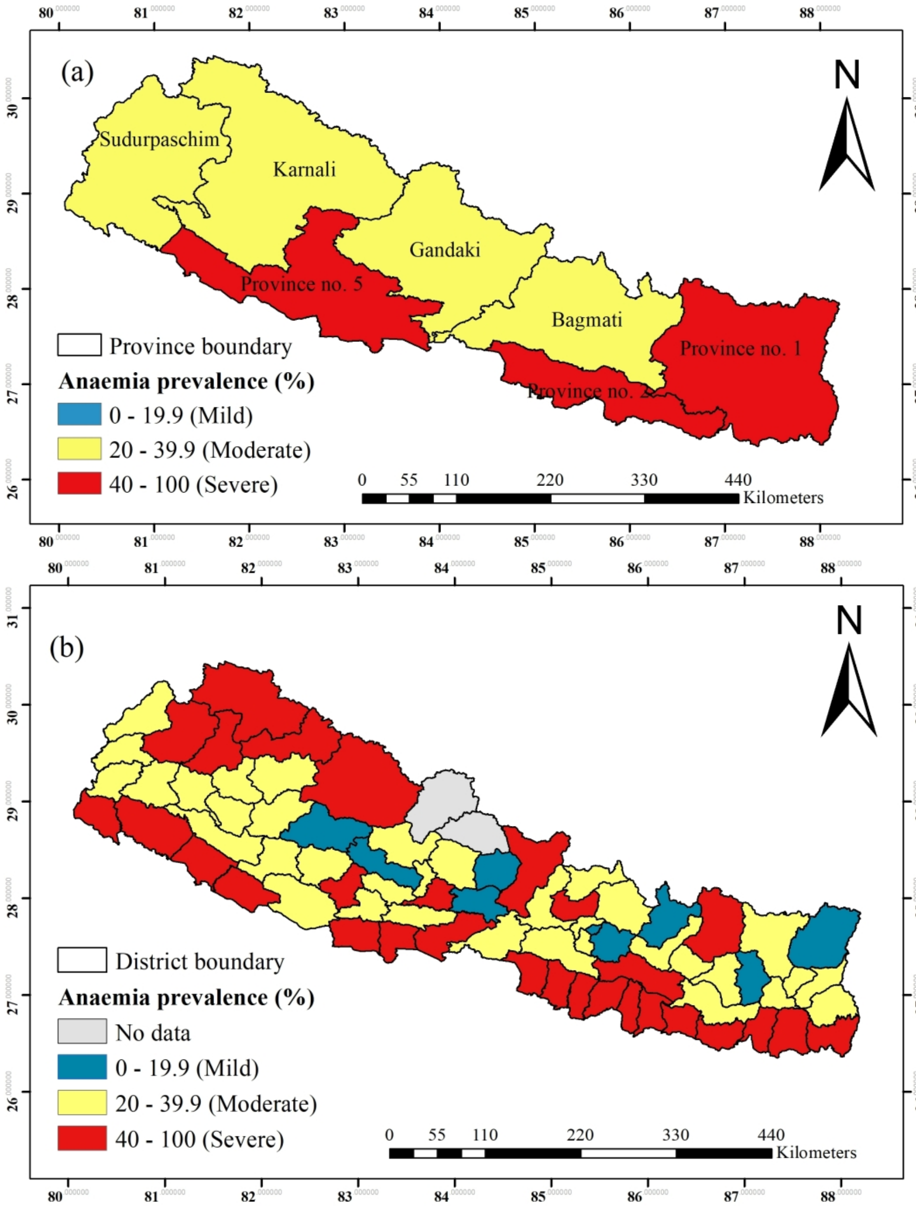

Figure 3 (a) Prevalence of anaemia among women of reproductive age across provinces. 3 (b) Prevalence of anaemia among women of reproductive age across districts

215x279mm (300 x 300 DPI)

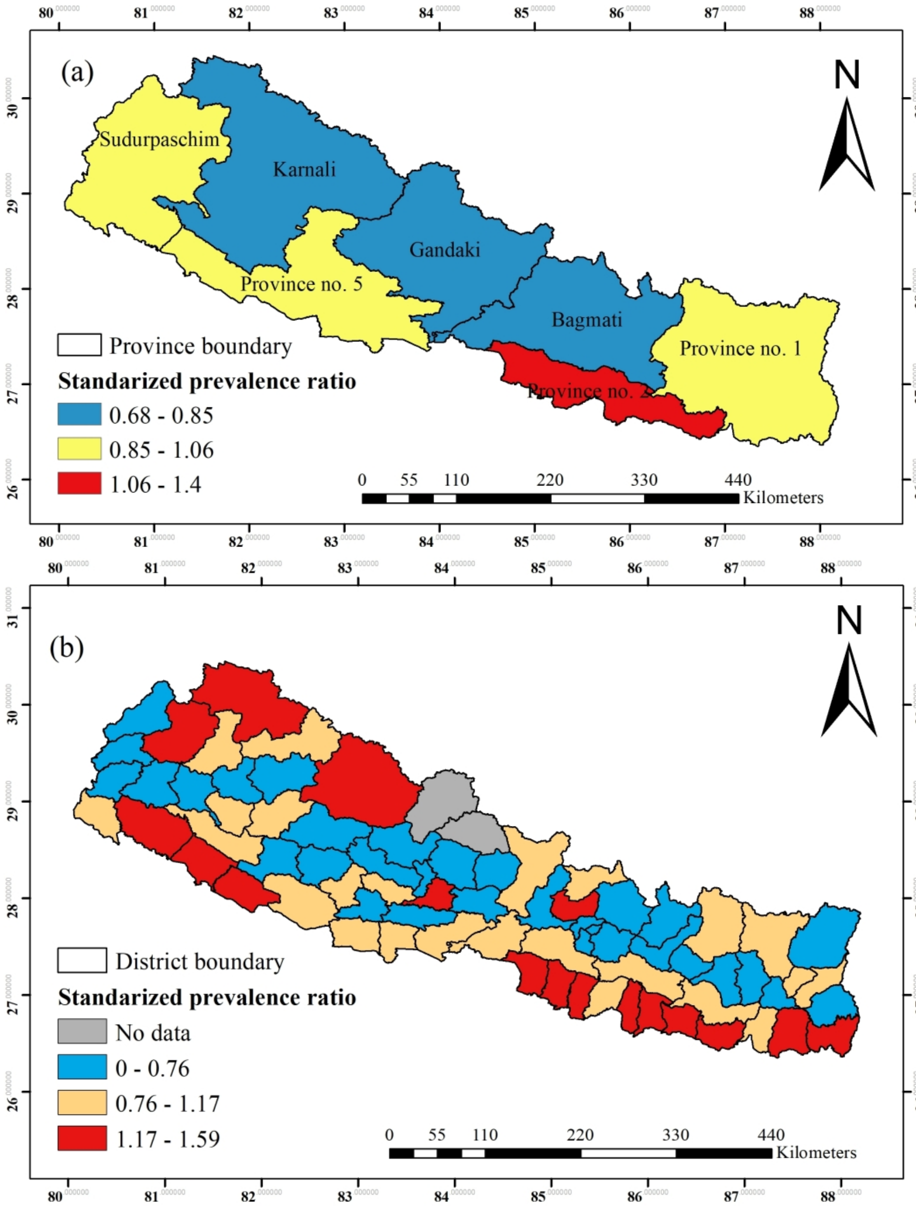

Figure 4 (a) Standardized prevalence ratio of anaemia among women of reproductive age across provinces. 4 (b) Standardized prevalence ratio of anaemia among women of reproductive age across districts (standardized to the national mean prevalence of 41%)

215x279mm (300 x 300 DPI)

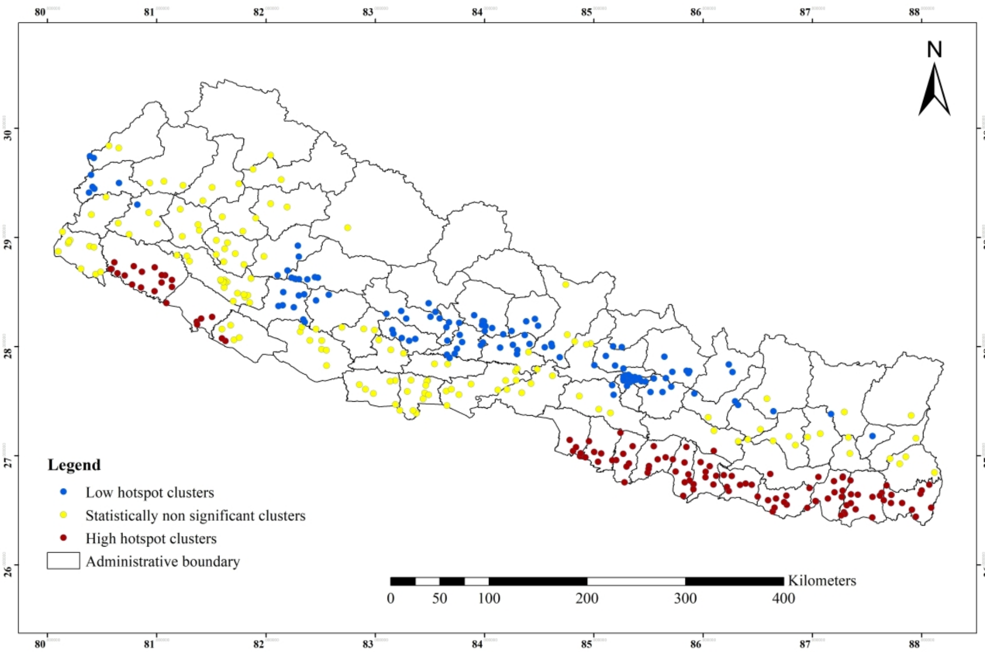

Figure 5 Spatial pattern and distribution of hot and cold spots of anaemia among women of reproductive age at cluster level in Nepal

457x304mm (300 x 300 DPI)

S1 Table Plan for data coding and description of the study variables

Study variables	Coding category for analysis
Age in years	1=15-24; 2=25-34; 3=35-49
Education	1=No education; 2=Primary; 3=Secondary; 4=Higher
Occupation	1= Not working; 2=Working (professional/technical/managerial, clerical, sales/services, agriculture-self-employed, skilled manual, unskilled manual, others)
Wealth index	1=Poorest; 2=Poorer; 3=Middle; 4=Richer; 5=Richest
Body mass index (BMI)	1=Normal (18.5-24.99); 2=Underweight(<18.5); 3=Overweight/Obesity(≥25)
Currently pregnant	1=No; 2=Yes
Currently breastfeeding	1=No; 2=Yes
Total children ever born	1= No child; 2=1-3 child; 3=4+ child
Current contraceptive use	1=Not using; 2=Hormonal (Pill, Injections, Implants/Norplant's, Emergency contraception); 3=Female sterilization; 4=Male contraception; 5= Traditional (Male condom, periodic abstinence, withdrawal, other traditional, lactational amenorrhea)
Cigarette/smoking	1= Smoking; 2=Not smoking
Minimum dietary diversity for women (MDD-W)	1=Not met (consume <5 food groups); 2= Met (Consume ≥5 food groups)
Mosquitoes bed net for sleeping	1= No; 2= Yes
Residence	1=Urban; 2=Rural
Province	1=Province-1; 2=Province-2, 3=Bagmati Province; 4=Gandaki Province; 5=Province-5; 6=Karnali Province; 7=Sudurpaschim Province
Community female education	1=Low (mean percent of women with lower primary education level was 0-25%); 2=High (mean percent of women with primary education level above 25%)
Community wealth index	1=Low (mean proportion of women from richest (rich, richest) households in the community was 0-21%); 2=High (mean

	proportion of women from richest (rich, richest) households in the community was above 21%)
Source of drinking water	1=Improved (piped water into dwelling, piped water to yard/plot, public tap or standpipe, tube well or borehole, protected dug well-protected spring and rainwater); 2=Not improved (Piped to neighbor, unprotected well, river, dam ponds, lake, and rainwater)
Type of toilet facility	1=Improved facility (flush toilet, piped sewer system, septic tank, flush/pour flush to pit latrine, ventilated improved pit latrine, pit latrine with slab, and composting toilet); 2=Not improved (flush to somewhere, flush don't know where, pit latrine without slab/open pit, and no facility/bush/field)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5-6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-10
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-10
		(*b*) Describe any methods used to examine subgroups and interactions	8-10
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-11
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-14
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-18
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.