Prevalence and Risk Factors of Vitamin B\textsubscript{12} Deficiency among Pregnant Women in Rural Bangladesh

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Abstract: Vitamin B\textsubscript{12} deficiency is associated with an increased risk of pregnancy complications and adverse birth outcomes. However, data on vitamin B\textsubscript{12} deficiency in pregnant Bangladeshi women are limited. This study examines vitamin B\textsubscript{12} deficiency and marginal deficiency in rural Bangladeshi women during early and late pregnancies. Some 522 women whose gestational age was <20 weeks were recruited. Serum vitamin B\textsubscript{12} concentrations were measured at baseline and after 14 weeks of iron-folate supplementation. Logistic regression analysis examined the association of various socio-demographic, dietary, and pregnancy-related factors with vitamin B\textsubscript{12} deficiency and marginal deficiency. Overall, 19\% of the women during early pregnancy had vitamin B\textsubscript{12} deficiency (serum vitamin B\textsubscript{12} concentration < 203 pg/mL) and nearly 40\% had marginal deficiency (serum vitamin B\textsubscript{12} concentration 203 to <300 pg/mL). Vitamin B\textsubscript{12} deficiency doubled to 38\% during late pregnancy, while marginal deficiency slightly increased to 41.7\%. The pregnant women with a gestational age of \geq 27 weeks had a higher risk of developing vitamin B\textsubscript{12} deficiency (OR = 2.61; 95\% CI = 1.096–6.214) than those of a gestational age of <27 weeks. Vitamin B\textsubscript{12} deficiency was significantly higher in pregnant women in rented accommodation (OR = 13.32; 95\% CI = 1.55–114.25) than in those living in their own house. Vitamin B\textsubscript{12} deficiency was significantly higher among women who consumed red or organ meat <3 times a week than in those who consumed it more often (OR = 2.327, 95\% CI = 1.194–4.536). None of these factors were significantly associated with marginal vitamin B\textsubscript{12} deficiency. In conclusion, vitamin B\textsubscript{12} deficiency and marginal deficiency among pregnant rural Bangladeshi women increased as their pregnancies progressed. Increasing gestational age, living in a rented house, and the consumption of red or organ meat <3 times a week were identified as the independent risk factors of vitamin B\textsubscript{12} deficiency in this population. Further research with more in-depth assessments of dietary vitamin B\textsubscript{12} intakes is needed to develop an intervention program preventing vitamin B\textsubscript{12} deficiency in this population.

Keywords: vitamin B\textsubscript{12} deficiency; marginal vitamin B\textsubscript{12} deficiency; pregnant women; Bangladesh

1. Introduction

A deficiency in vitamin B\textsubscript{12} (also known as cobalamin) has been identified as a significant public health problem globally [1,2]. While an impaired vitamin B\textsubscript{12} status can occur in individuals of all ages, pregnant women, particularly those in less industrialized countries, are at higher risk of developing vitamin B\textsubscript{12} deficiency [3–5].

Vitamin B\textsubscript{12}, an essential micronutrient in the human body, is required for the synthesis of DNA, RNA, phospholipids, and neurotransmitters [3,6]. It also helps to catabolize branched-chain and odd-chain fatty acids [3,6,7]. Thus, vitamin B\textsubscript{12} is crucial for cellular
growth, differentiation, and development [8]. During pregnancy, women are more vulnerable to a deficiency of vitamin B$_{12}$ due to increased metabolic demands imposed by physiological activities, such as the growth of the placenta, fetus, and maternal tissue [9]. Several studies have reported that the prevalence of vitamin B$_{12}$ deficiency increases as pregnancy progresses, with the highest prevalence during the third trimester [4,10–13].

Vitamin B$_{12}$ deficiency during pregnancy is associated with an increased risk of pregnancy complications, including spontaneous abortion [14], recurrent pregnancy loss [15,16], intrauterine growth restriction [17], low birth weight [17], and neural tube defects [5,18–20]. In a review of maternal vitamin B$_{12}$ status and perinatal health, Finkelstein et al. [3] reported a significant association between maternal and neonatal vitamin B$_{12}$ status at delivery. In a recent systematic review, Behere et al. [5] demonstrated an association between impaired maternal vitamin B$_{12}$ status and adverse longer-term health outcomes, such as cognitive functions, adiposity, and insulin resistance in children.

Although vitamin B$_{12}$ deficiency can be caused by malabsorption of food, the inadequate intake of animal-source foods is the main cause [21]. People who live in countries with restrictions on animal food consumption are susceptible to vitamin B$_{12}$ deficiency. These restrictions could result from cultural and religious practices, or because these countries have a low socioeconomic status, and the consumption of animal-source foods is limited because of cost, lack of availability, or poor access to fortified foods or supplements [1,2,22,23]. Individuals adhering to a vegetarian diet are at higher risk of developing vitamin B$_{12}$ deficiency [24], as their diet is predominantly plant-based, and the only food source rich in vitamin B$_{12}$ comes from animal products [25]. Vitamin B$_{12}$ deficiency is also caused by the malabsorption of food due to gastrointestinal infections, and nonspecific gastritis, including atrophy of the gastric mucosa, and gradual loss of gastric acid, particularly in the elderly [2,24].

In Bangladesh, some studies have revealed a high prevalence of vitamin B$_{12}$ deficiency among pregnant women [26–28]. However, these studies have used either a very small sample size (n = 68) [28], or have only focused on early pregnancy. In addition, to date, no studies have examined the changes in vitamin B$_{12}$ status with the progress of pregnancy. This is important as the requirement for vitamin B$_{12}$ significantly increases in late pregnancy when fetal growth is intense. Of note, one in five children in Bangladesh are born with a low birth weight [29]. Furthermore, none of the previous studies in Bangladesh has reported the risk factors of vitamin B$_{12}$ deficiency during pregnancy. Given the above limitations, and considering the importance of vitamin B$_{12}$ during pregnancy, this research aims to determine the prevalence of vitamin B$_{12}$ deficiency, and the associated risk factors of this deficiency, among rural Bangladeshi women during early and late pregnancy.

2. Materials and Methods

2.1. Study Population

The study group comprised 522 pregnant rural women with a gestational age of $\leq 20$ weeks (defined as early pregnancy) in Bangladesh. This longitudinal study was carried out between April and October 2015 while collecting data for an intervention study examining the effect of routine iron-folic acid supplementation during pregnancy.

2.2. Selection of Participants

The participants were selected purposively from four upazilas, or administrative subdistricts, Sharishabari, Pirgachha, Lalmohon, and Badarganj, covering three geographical regions (Northern, South-Central, and North-East) in Bangladesh. Twenty-four unions (administrative units consisting of a cluster of villages), six from each upazila, were randomly selected. Subsequently, an approximately equal number of participants was selected from each union using a convenience sampling technique. The selection of the participants is described in detail elsewhere [30].

Female field workers initially surveyed households in the study area to identify eligible study participants based on the date of their last menstrual period (LMP) and history of
antenatal clinic (ANC) visits during their current pregnancy. Women were eligible to participate if they had not visited an ANC for a check-up during their current pregnancy. All potential participants were provided with information about the purpose and the nature of the study before being invited to come to a designated ANC on a pre-set date for data collection. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh (on 16 April 2015; Ref No. Biol. Sci. 2014–2015).

2.3. Data Collection

On the day of data collection, after receiving informed consent from the women, all were tested for confirmation of pregnancy using a commercial pregnancy detection kit. The interviewer reconfirmed the time of their LMP. In total, 530 pregnant women were recruited for the study. The response rate was over 90%.

Structured interviews were conducted by trained interviewers to obtain socio-demographic and pregnancy-related information from the participants. A 7-day food frequency questionnaire (FFQ) was used to gather information on the usual dietary consumption pattern of selected food items rich in micronutrients (red meat (beef, goat, and liver); fish (small and big); dairy (milk and milk products) and eggs; leafy green vegetables; non-leafy vegetables; and seasonal fruits). The FFQ was adopted from the National Micronutrient Survey 2011–2012, modified, and pretested in the study population. Data on the consumption of any vitamin and mineral supplements were collected using a 30-day recall questionnaire. Following the interview, a disposable syringe was used to collect a 5 mL sample of venous blood from each woman. The serum was separated by centrifugation and serum specimens were taken in plastic microcentrifuge tubes and frozen in dry ice, before being transported to a laboratory in Dhaka, and stored at −20°C until analyzed.

A second blood sample was obtained from the pregnant women after approximately 14 weeks of routine iron (60 mg) and folic acid (400 ug) supplementation per day. Information was also obtained on dietary patterns and consumption of any vitamin or mineral supplements, other than those that were administered during the intervention.

2.4. Analytical Procedure

Serum vitamin B$_{12}$ concentrations were measured by electrochemiluminescence immunoassay (ECLIA) on a Roche automated immunoassay analyzer Cobas e601 using a commercial kit, Elecsys Vitamin B12 II (Roche Diagnostics, GmbH, 68305 Mannheim, Germany), according to the manufacturer’s instructions. Preci Control Varia 1 and 2 were used to check both accuracy and precision as an internal quality control material.

2.5. Statistical Analysis

Data were analyzed using the statistical software packages IBM SPSS Statistics version 28 (SPSS Inc., Chicago, IL, USA). Due to incomplete data or an insufficient blood sample for vitamin B$_{12}$ assay, eight participants were excluded. Thus, 522 pregnant women were included in the analysis. The distribution of serum vitamin B$_{12}$ concentration was checked by the Kolmogorov–Smirnov goodness of fit test and was normally distributed. The univariate analysis comprised a simple frequency distribution of selected variables. Vitamin B$_{12}$ deficiency was defined following the cut-off value suggested by the manufacturer of the kit used in the assay, as serum vitamin B$_{12}$ concentration < 203 pg/mL [31]. Marginal vitamin B$_{12}$ deficiency was defined using the Centers for Disease Control and Prevention (CDC) definition as serum vitamin B$_{12}$ concentration 203 to <300 pg/mL [32].

A paired t-test was applied to compare the difference of mean serum vitamin B$_{12}$ concentrations between women during early (at baseline) and late pregnancies (when they had the second blood sample; after 14 weeks of IFA supplementation). The differences in the prevalence of vitamin B$_{12}$ deficiency and marginal deficiency between early and late pregnancies were examined using a chi-squared test. The differences in marginal vitamin B$_{12}$
status and deficiency in late pregnancy were also compared between groups of various socio-demographic, pregnancy, and diet-related characteristics using a chi-squared test.

Finally, a logistic regression analysis was used to determine the independent association of selected socio-demographic, pregnancy, and diet-related variables with marginal vitamin B\textsubscript{12} status and vitamin B\textsubscript{12} deficiency separately among women during late pregnancy. The independent variables included in the analysis were age, parity, gestational age, participants, and their husband's education level and occupation, household size, home ownership, cultivable land ownership, taking vitamin and mineral supplements, and consumption of red or organ meat, fish, eggs, and dairy products. The odds ratio (OR) and 95% confidence interval (CI) were calculated. The $p$-value < 0.05 was considered statistically significant.

3. Results

A total of 522 participants enrolled during early pregnancy, and 405 completed the study protocol of 14 weeks follow up. Thus, the drop-out rate was 22.4%. There were no significant differences between the various socio-economic and pregnancy-related characteristics of the pregnant women who completed the study and those who did not (data not shown).

At the time of recruitment, the age of the participants ranged from 13–38 years (mean ± SD age 23.6 ± 4.8 years) and gestational age (GA) ranged from 7 to 20 weeks (mean ± SD GA 15.2 ± 2.7 weeks). A large majority of the participants (44%) and their husbands (57.5%) were functionally illiterate (had never been to school or had completed up to grade 5 only). Ninety-six percent of the participants were homemakers and about two-thirds of their husbands were either day laborers or farmers. Nine out of 10 participants owned their own homes, while over half (56.5%) of the participants had no cultivable land (Table 1).

Table 1. Socio-demographic and pregnancy-related characteristics of the women by early and late stages of pregnancy.

| Variable                  | Early Pregnancy ($n = 522$) | Late Pregnancy ($n = 404$) |
|---------------------------|-----------------------------|---------------------------|
|                           | (7–20 Weeks)                | (21–34 Weeks)             |
|                           | $n$  | %    | $n$  | %    |
| Age (Year)                |      |      |      |      |
| Adolescent (13–19)        | 126  | 24.1 | 97   | 24.0 |
| Young adult (20–24)       | 166  | 31.8 | 126  | 31.2 |
| Young adult2 (>25)        | 230  | 44.1 | 181  | 44.8 |
| Gestational Age (Week) *  |      |      |      |      |
| <13                       | 71   | 13.6 | 49   | 12.1 |
| 13 or more                | 451  | 86.4 | 355  | 87.9 |
| Parity                    |      |      |      |      |
| No living child           | 197  | 37.7 | 151  | 37.4 |
| Only one                  | 205  | 39.3 | 158  | 39.1 |
| Two or more               | 120  | 23.0 | 95   | 23.5 |
| Participant’s Education   |      |      |      |      |
| Functionally illiterate **| 230  | 44.1 | 174  | 43.1 |
| Grade 6 to 9              | 203  | 38.9 | 166  | 41.1 |
| SSC or above              | 89   | 17.0 | 64   | 15.8 |
| Husband’s Education       |      |      |      |      |
| Functionally illiterate   |      |      |      |      |
| Grade 6 to 9              | 300  | 57.5 | 234  | 57.9 |
| SSC or above              | 111  | 21.3 | 91   | 22.5 |
|                           | 111  | 21.3 | 79   | 19.6 |
Table 1. Cont.

| Variable                        | Early Pregnancy (n = 522) (7–20 Weeks) | Late Pregnancy (n = 404) (21–34 Weeks) |
|---------------------------------|----------------------------------------|-----------------------------------------|
|                                 | n           | %          | n          | %          |
| Husband’s Occupation            |             |            |             |            |
| Day laborer                     | 205         | 39.3       | 151        | 37.4       |
| Farmer                          | 136         | 26.1       | 115        | 28.5       |
| Business/service                | 181         | 34.6       | 138        | 34.1       |
| Participant’s Occupation        |             |            |             |            |
| Homemaker                       | 502         | 96.2       | 387        | 95.8       |
| Working                         | 20          | 3.8        | 17         | 4.2        |
| Family Size                     |             |            |             |            |
| Small family (up to 4)          | 315         | 60.3       | 239        | 59.2       |
| Large family (5 or more)        | 207         | 39.7       | 165        | 40.8       |
| Home Owner                      |             |            |             |            |
| No                              | 38          | 7.3        | 25         | 6.2        |
| Yes                             | 484         | 92.7       | 379        | 93.8       |
| Cultivable Land Ownership       |             |            |             |            |
| No land                         | 295         | 56.5       | 228        | 56.4       |
| Small landholding               | 227         | 43.5       | 176        | 43.6       |

* Gestational age at recruitment. ** No formal education or studies less than grade 5. Abbreviations: SSC—Secondary School Certificate.

The distributions of the participants by age, parity, and gestational age group between early and late pregnancies were not significantly different (Table 1). Similarly, the distributions of the participants by various socio-economic groups did not differ significantly between early and late pregnancies (Table 1). The mean serum vitamin B<sub>12</sub> concentration during late pregnancy was significantly lower (p-value = 0.0001) than during early pregnancy (Table 2). The mean (SD) difference of serum vitamin B<sub>12</sub> concentration between women in their early and late pregnancies was −56.2 (65.7) pg/mL.

Table 2. Mean difference in serum vitamin B<sub>12</sub> concentrations between women in their early and late pregnancies.

| Variables                   | n    | Mean (pg/mL) | SD  | p-Value * |
|-----------------------------|------|--------------|-----|-----------|
| Early pregnancy (7–20 weeks) | 522  | 299.9        | 121.1| 0.0001    |
| Late pregnancy (21–34 weeks) | 404  | 243.8        | 92.8 |           |
| Mean changes (late–early)    | 404  | −56.2        | 65.7 |           |

* Paired t-test.

Figure 1 shows the prevalence of vitamin B<sub>12</sub> deficiency and marginal deficiency with the progress of pregnancy. During the early pregnancy stage, nearly 40% of the women had a marginal vitamin B<sub>12</sub> status (serum vitamin B<sub>12</sub> concentration 203 to <300 pg/mL) and 19% had vitamin B<sub>12</sub> deficiency (serum vitamin B<sub>12</sub> concentration < 203 pg/mL). After 14 weeks of follow up, during the late pregnancy stage (mean ± SD GA 29 ± 2.6 weeks), the prevalence of marginal vitamin B<sub>12</sub> deficiency remained unchanged (41.7%), whereas vitamin B<sub>12</sub> deficiency rose significantly to 38%.

For the selected food items rich in vitamin B<sub>12</sub>, the distribution according to the frequency of consumption by women during their late pregnancy is shown in Table 3. Nearly 42% percent of the women did not consume red meat or organ meat at all in the 7 days preceding the interview, and another 32% of the women had it only once or twice a week. A large majority of the pregnant women (46%) had milk seven times or more per week; however, one in four women (23.5%) did not consume milk at all. About 30% of the women had eggs seven times or more per week, whereas 19% of the women did not consume eggs at all. It was also noted that one in four women (24.9%) did not consume fish at all.
Various foods rich in vitamin B12 were assessed, with about 30% of the study population consuming red or organ meat fewer than three times a week (adjusted OR = 1.689, 95% CI = 0.908–3.140; p = 0.03) compared to the nullipara pregnant women. The prevalence of vitamin B12 deficiency was significantly higher among pregnant women who did not have their own house (adjusted OR = 13.32; 95% CI = 1.55–114.25; p = 0.018). The pregnant women who usually consumed red or organ meat less than three times a week were more likely to have marginal deficiency than the nullipara pregnant women (adjusted OR = 2.33 times; 95% CI = 1.194–4.536; p = 0.013). However, the risk of marginal vitamin B12 deficiency was only 1.69 times higher among pregnant women who ate red or organ meat less than three times a week.

Table 4 illustrates the differences in the prevalence of vitamin B12 deficiency and marginal deficiency during late pregnancy by various socio-demographic, dietary, and pregnancy-related factors. Using bivariate analysis, the prevalence of both vitamin B12 deficiency and marginal vitamin B12 deficiency was higher among pregnant women with a gestational age of ≥27 weeks than in the pregnant women with a gestational age of <27 weeks. However, the difference was not statistically significant (p = 0.081). The prevalence of vitamin B12 deficiency was significantly higher (p = 0.005) among pregnant women who lived in a rented house compared to those who owned their own house. The prevalence of vitamin B12 deficiency and/or marginal deficiency during late pregnancy was not influenced by any other socio-demographic or pregnancy-related factors. The prevalence of vitamin B12 deficiency was significantly higher (p = 0.019) among pregnant women who consumed red or organ meat fewer than three times a week than in the pregnant women who consumed such meat three times or more per week.

The factors associated with vitamin B12 deficiency and marginal deficiency were separately examined using logistic regression analysis (Table 5). The pregnant women with a gestational age of ≥27 weeks had a higher risk of developing marginal vitamin B12 deficiency (adjusted OR = 1.98; 95% CI = 0.904–4.342; p = 0.088) and vitamin B12 deficiency (adjusted OR = 2.61; 95% CI = 1.096–6.214; p = 0.03) than the pregnant women with a gestational age of <27 weeks. The pregnant women with a parity of two or more were 2.74 times more likely to suffer from vitamin B12 deficiency than the nullipara pregnant women (adjusted OR = 2.744; 95% CI = 0.918–8.204; p = 0.07). The risk of vitamin B12 deficiency was significantly higher in pregnant women who did not have their own house (adjusted OR = 13.32; 95% CI = 1.55–114.25; p = 0.018). The pregnant women who usually consumed red or organ meat < 3 times a week were 2.33 times more likely to suffer from vitamin B12 deficiency compared with the pregnant women who consumed red or organ meat ≥ 3 times a week (adjusted OR = 2.327, 95% CI = 1.194–4.536; p = 0.013). However, the risk of marginal vitamin B12 deficiency was only 1.69 times higher among pregnant women who ate red or organ meat < 3 times a week (adjusted OR = 1.689, 95% CI = 0.908–3.140; p = 0.098).
Table 4. Prevalence of vitamin B\textsubscript{12} deficiency and marginal deficiency during late pregnancy by socio-demographic, dietary, and pregnancy-related factors.

| Factor                                      | Normal   | Marginally Deficient | Deficiency | p-Value * |
|---------------------------------------------|----------|----------------------|------------|-----------|
| | Total (n) | n %       | n %                  | n %        |           |
| Age (Year)                                 |          |                      |            |           |
| Adolescent (13–19)                         | 97       | 18 18.6              | 46 47.4    | 33 34.0   | 0.672     |
| Young adult (20–24)                        | 126      | 28 22.2              | 47 37.3    | 51 40.5   |           |
| Young adult2 (>25)                         | 181      | 37 20.4              | 74 40.9    | 70 38.7   |           |
| Gestational Age (Week)                     |          |                      |            |           |
| <27                                         | 49       | 16 32.7              | 18 36.7    | 15 30.6   | 0.081     |
| 27 or more                                  | 355      | 67 18.9              | 149 42.0   | 139 39.2  |           |
| Parity                                      |          |                      |            |           |
| No living child                             | 151      | 29 19.2              | 72 47.7    | 50 33.1   | 0.120     |
| Only one                                    | 158      | 38 24.1              | 61 38.6    | 59 37.3   |           |
| Two or more                                 | 95       | 16 16.8              | 34 35.8    | 45 47.4   |           |
| Participant’s Education                     |          |                      |            |           |
| Functionally illiterate                     | 174      | 36 20.7              | 70 40.2    | 68 39.1   | 0.805     |
| Grade 6 to 9                                | 166      | 32 19.3              | 74 44.6    | 60 36.1   |           |
| SSC or above                                | 64       | 15 23.4              | 23 35.9    | 26 40.6   |           |
| Husband’s Education                         |          |                      |            |           |
| Functionally illiterate                     | 234      | 43 18.4              | 102 43.6   | 89 38.0   | 0.655     |
| Grade 6 to 9                                | 91       | 21 23.1              | 33 36.3    | 37 40.7   |           |
| SSC or above                                | 79       | 19 24.1              | 32 40.5    | 28 35.4   |           |
| Husband’s Occupation                        |          |                      |            |           |
| Day laborer                                 | 151      | 31 20.5              | 56 37.1    | 64 42.4   | 0.685     |
| Farmer                                      | 115      | 23 20.0              | 50 43.5    | 42 36.5   |           |
| Business/service                           | 138      | 29 21.0              | 61 44.2    | 48 34.8   |           |
| Participant’s Occupation                    |          |                      |            |           |
| Homemaker                                   | 387      | 81 20.9              | 158 40.8   | 148 38.3  | 0.591 **  |
| Working                                     | 17       | 2 11.8               | 9 52.9     | 6 35.3    |           |
| Family Size                                 |          |                      |            |           |
| Small family (up to 4)                     | 239      | 48 20.1              | 99 41.4    | 92 38.5   | 0.956     |
| Large family (5 or more)                   | 165      | 35 21.2              | 68 41.2    | 62 37.6   |           |
| Home Owner                                  |          |                      |            |           |
| No                                          | 25       | 1 4.0                | 7 28.0     | 17 68.0   | 0.005 **  |
| Yes                                         | 379      | 82 21.6              | 160 42.2   | 137 36.1  |           |
| Cultivable Land Ownership                   |          |                      |            |           |
| No land                                     | 228      | 45 19.7              | 99 43.4    | 84 36.8   | 0.631     |
| Small landholding                           | 176      | 38 21.6              | 68 38.6    | 68 39.8   |           |
| Meat Intake (Frequency/Week) ***            |          |                      |            |           |
| <3                                          | 299      | 53 17.7              | 122 40.8   | 124 41.5  | 0.019     |
| 3 times or more                             | 105      | 30 28.6              | 45 42.9    | 30 28.6   |           |
| Fish Intake (Frequency/Week)                 |          |                      |            |           |
| <3                                          | 230      | 51 22.2              | 93 40.4    | 86 37.4   | 0.653     |
| 3 times or more                             | 174      | 32 18.4              | 74 42.5    | 68 39.1   |           |
| Milk Intake (Frequency/Week)                 |          |                      |            |           |
| <3                                          | 155      | 30 19.4              | 64 41.3    | 61 39.4   | 0.871     |
| 3 times or more                             | 249      | 53 21.3              | 103 41.4   | 93 37.3   |           |
| Eggs (Frequency/Week)                       |          |                      |            |           |
| <3                                          | 190      | 36 18.9              | 78 41.1    | 76 40.0   | 0.688     |
| 3 times or more                             | 214      | 47 22.0              | 89 42.6    | 78 36.4   |           |
| Vitamin/Mineral Supplement                  |          |                      |            |           |
| No                                          | 376      | 76 20.2              | 155 41.2   | 145 38.6  | 0.742     |
| Yes                                         | 28       | 7 25.0               | 12 42.9    | 9 32.1    |           |

* Chi-squared test  ** Exact test. *** Red or organ meat. Abbreviations: SSC—Secondary School Certificate.
Table 5. Logistic regression analysis for odds of vitamin B\textsubscript{12} deficiency and marginal deficiency by various factors among rural Bangladeshi women during late pregnancy.

| Variable                        | Marginal Deficient | Vitamin B\textsubscript{12} Deficiency | p-Value |
|---------------------------------|--------------------|----------------------------------------|---------|
|                                 | Exp (B)            | 95% CI for EXP(B)                      | p-Value |
|                                 | Lower | Upper | Lower | Upper | Lower | Upper | p-Value |
| Age (Year)                      |        |       |       |       |       |       |         |
| Adolescent (13–19) (Ref Cat **) | 1.0    | 1.0   |       |       |       |       |         |
| Young adult (20-24)             | 0.929  | 0.399 | 2.164 | 0.864 | 0.716 | 0.276 | 1.859 | 0.492 |
| Young adult2 (>25)              | 1.028  | 0.371 | 2.852 | 0.957 | 0.490 | 0.162 | 1.483 | 0.207 |
| Gestational Age (Week)          |        |       |       |       |       |       |         |
| <27 (Ref Cat)                   | 1.0    | 1.0   |       |       |       |       |         |
| 27 or more                      | 1.981  | 0.904 | 4.342 | 0.088 | 1.0   | 2.610 | 1.096 | 6.214 | 0.030 |
| Parity                          |        |       |       |       |       |       |         |
| No living child (Ref Cat)       | 1.0    | 1.0   |       |       |       |       |         |
| Only one                        | 0.598  | 0.264 | 1.356 | 0.218 | 1.397 | 0.519 | 1.799 | 0.651 | 4.967 | 0.257 |
| Two or more                     | 0.734  | 0.255 | 2.112 | 0.566 | 2.744 | 0.918 | 8.204 | 0.071 |
| Participant’s Education         |        |       |       |       |       |       |         |
| Functionally illiterate         | 0.911  | 0.301 | 2.759 | 0.870 | 0.519 | 0.171 | 1.572 | 0.246 |
| Grade 6 to 9                    | 1.408  | 0.549 | 3.614 | 0.477 | 0.667 | 0.254 | 1.756 | 0.413 |
| SSC or above (Ref Cat)          | 1.0    | 1.0   |       |       |       |       |         |
| Husband’s Education             |        |       |       |       |       |       |         |
| Functionally illiterate         | 1.723  | 0.648 | 4.579 | 0.275 | 1.799 | 0.651 | 4.967 | 0.257 |
| Grade 6 to 9                    | 0.976  | 0.394 | 2.417 | 0.958 | 1.559 | 0.577 | 4.214 | 0.381 |
| SSC or above (Ref Cat)          | 1.0    | 1.0   |       |       |       |       |         |
| Husband’s Occupation            |        |       |       |       |       |       |         |
| Day laborer                     | 0.682  | 0.332 | 1.402 | 0.298 | 1.003 | 0.468 | 2.150 | 0.993 |
| Farmer                          | 0.934  | 0.443 | 1.969 | 0.857 | 0.864 | 0.388 | 1.924 | 0.721 |
| Business/service (Ref Cat)      | 1.0    | 1.0   |       |       |       |       |         |
| Family Size                     |        |       |       |       |       |       |         |
| Small family (up to 4) (Ref Cat)| 1.0    | 1.0   |       |       |       |       |         |
| Large family (5 or more)        | 0.875  | 0.477 | 1.604 | 0.665 | 1.009 | 0.531 | 1.919 | 0.978 |
| Home Owner                      |        |       |       |       |       |       |         |
| No                              | 3.158  | 0.340 | 29.340| 0.312 | 13.32 | 1.55  | 114.25 | 0.018 |
| Yes (Ref Cat)                   | 1.0    | 1.0   |       |       |       |       |         |
| Cultivable Land Ownership       |        |       |       |       |       |       |         |
| No land                         | 1.367  | 0.702 | 2.659 | 0.358 | 1.223 | 0.623 | 2.400 | 0.558 |
| Sizeable land holding (Ref Cat) | 1.0    | 1.0   |       |       |       |       |         |
| Meat * Intake                   |        |       |       |       |       |       |         |
| <3 times                        | 1.689  | 0.908 | 3.140 | 0.098 | 2.372 | 1.194 | 4.536 | 0.013 |
| 3 times or more (Ref Cat)       | 1.0    | 1.0   |       |       |       |       |         |
| Fish Intake                     |        |       |       |       |       |       |         |
| <3                             | 0.640  | 0.357 | 1.147 | 0.134 | 0.628 | 0.339 | 1.162 | 0.138 |
| 3 times or more (Ref Cat)       | 1.0    | 1.0   |       |       |       |       |         |
| Milk Intake                     |        |       |       |       |       |       |         |
| <3 times                        | 0.948  | 0.509 | 1.764 | 0.866 | 0.919 | 0.482 | 1.754 | 0.798 |
| 3 times or more (Ref Cat)       | 1.0    | 1.0   |       |       |       |       |         |
| Egg Intake                      |        |       |       |       |       |       |         |
| <3 times                        | 1.138  | 0.635 | 2.041 | 0.664 | 1.235 | 0.664 | 2.297 | 0.506 |
| 3 times or more (Ref Cat)       | 1.0    | 1.0   |       |       |       |       |         |

* Red or organ meat. ** Reference Category. Abbreviations: SSC—Secondary School Certificate.

4. Discussion

This is first study that reports the changes in the prevalence of vitamin B\textsubscript{12} deficiency with the progress of pregnancy, and the factors associated with vitamin B\textsubscript{12} deficiency and marginal deficiency among pregnant women in rural Bangladesh. The majority of the participants in this study were functionally illiterate; they were homemakers who came from a low socio-economic background.

The study revealed that the prevalence of vitamin B\textsubscript{12} deficiency among these women increased significantly with the progress of their pregnancies. During early pregnancy,
19% of the women had vitamin B₁₂ deficiency, which doubled to 38% during late pregnancy. However, the prevalence of marginal vitamin B₁₂ deficiency remained unchanged. This could indicate that, as more women who were marginally deficient became deficient with the progression of pregnancy, there were additional women with B₁₂ sufficiency who gradually became marginally deficient, thus keeping the prevalence rate similar.

While there are limited data on vitamin B₁₂ deficiency and marginal deficiency among pregnant Bangladeshi women, one study conducted in rural north-western Bangladesh reported a 20% prevalence of vitamin B₁₂ deficiency during early (median GA of 10 weeks) pregnancy [26]. A randomized controlled trial with a small sample size conducted in Dhaka City reported a 26% prevalence of vitamin B₁₂ deficiency and another 40% with marginal vitamin B₁₂ deficiency during the early stage (GA of 11–14 weeks) of pregnancy [28]. Thus, the prevalence of vitamin B₁₂ deficiency in our study population was comparable to that observed in these two studies. However, an earlier study by Lindström et al. conducted in a sub-district of rural Bangladesh reported a 46% prevalence of vitamin B₁₂ deficiency during early pregnancy [27], which was more than double that in the present study. There can be several reasons for the differences in the prevalence of vitamin B₁₂ deficiency between the present study and the research conducted by Lindström et al. For instance, Lindström et al.’s study was conducted in only one sub-district of rural Bangladesh, while our study included pregnant women from four sub-districts from different geographical regions in rural Bangladesh. Furthermore, the study by Lindström et al. was conducted almost 18 years ago and did not reflect the present scenario. Similar to the situation in many resource poor countries, a low intake of animal source food is a major cause of poor vitamin B₁₂ status in Bangladesh. The available data indicate that there has been an overall increase in the consumption of animal foods in the country (26.2 g/capita per d in 2010 v. 20.8 g/capita per d in 2005) [33], which might have contributed to a variation in the prevalence of vitamin B₁₂ deficiency. In addition, Lindström et al. used radioimmunoassay (RIA) for the assessment of vitamin B₁₂ status, while we used electrochemiluminescence immunoassay, which is a more sensitive, reliable, and advanced method than RIA [34]. Thus, the methodological differences in the assessment of vitamin B₁₂ status between the two studies may also explain the differences in the prevalence of vitamin B₁₂ deficiency.

The present study reveals a significant increase in the prevalence of vitamin B₁₂ deficiency with the progress of pregnancy. A study carried out among pregnant women in Venezuela reported a 50% prevalence of vitamin B₁₂ deficiency in the first trimester, 59% in the second trimester, and 72.5% in the third trimester; the authors concluded that the prevalence of vitamin B₁₂ deficiency rises as pregnancy advances [35]. Another study conducted in Canada showed a 35% prevalence of vitamin B₁₂ deficiency during early pregnancy; as the pregnancy advanced, there was a significant rise in the prevalence of vitamin B₁₂ deficiency to 42.9% [36]. A systematic review, based on worldwide pooled trimester-wise estimates, reported a steady increase in the prevalence of vitamin B₁₂ deficiency with the progress of pregnancy [4]. Thus, the findings in the present study are consistent with the findings of previous studies.

Studies from the Netherlands [37], Spain [10], Canada [36], India [5], and in 12 out of 13 longitudinal studies included in a systematic review [4] reported a significant decline in the serum concentration of vitamin B₁₂ as a pregnancy progressed. The present study also found a significant decline in the mean serum vitamin B₁₂ concentration (p = 0.0001) from the early to late stages of pregnancy, where the mean concentration during early pregnancy was 300 pg/mL; with the advancement of pregnancy, the serum concentration fell by 56 pg/mL (19% decrease) during the late stage of pregnancy. There could be several possible reasons for the gradual decrease in serum vitamin B₁₂ concentration during pregnancy. For example, alterations in the concentration of vitamin B₁₂ binding proteins [38]. Grebe et al. [38], in their study, showed that the decline in serum vitamin B₁₂ concentration was closely related to the decline in the fraction of B₁₂ bound to haptocorrin (holo-haptocorrin) during pregnancy, while the fraction of B₁₂ bound to transcobalamin (holo-transcobalamin) remained unchanged. Furthermore, there were no changes in the
concentration of vitamin B\textsubscript{12} analogs bound to haptocorrin during pregnancy. The other reasons could be due to increased maternal nutritional and physiological demands as the pregnancy progressed, hemodilution due to plasma volume expansion, hormonal changes, and/or increased placental transfer of vitamin B\textsubscript{12} to the fetus [39–41]. In addition, low dietary intake of vitamin B\textsubscript{12} or lack of access to B\textsubscript{12} fortified foods or B\textsubscript{12} supplements further precipitates the decline in B\textsubscript{12} and could be an important but modifiable cause of poor vitamin B\textsubscript{12} status [21,23,41].

While we do not have quantitative estimates of dietary intake of vitamin B\textsubscript{12}, the present study collected data on the frequency of intake of selected animal source food rich in vitamin B\textsubscript{12}. Two in five women (41.7%) reported not consuming red or organ meat at all over a period of one week preceding the interview. Nearly a quarter of the women never had fish, eggs, or milk. Furthermore, nearly a third of the pregnant women had red meat, fish, and eggs only 1 to 2 times per week. Of note, vitamin B\textsubscript{12} fortified foods are not generally available in Bangladesh. In addition, none of the participants reported taking vitamin B\textsubscript{12} supplements. Therefore, it is highly likely that low dietary intake of vitamin B\textsubscript{12} might have contributed to the poor vitamin B\textsubscript{12} status in this population. Siddiqua et al. [28] in their study among pregnant women in Bangladesh also reported limited intake of animal-source food. A study by Herr\án et al. [23] also reported a low intake of animal-source foods in a Colombian population with a high prevalence of vitamin B\textsubscript{12} deficiency.

The present study explored the association of vitamin B\textsubscript{12} deficiency and marginal vitamin B\textsubscript{12} deficiency with various socio-economic, pregnancy, and diet-related factors. The results of bivariate analysis reveal a significantly higher prevalence of vitamin B\textsubscript{12} deficiency among pregnant women who lived in a rented house (\(p = 0.005\)) and those who consumed red or organ meat fewer than three times per week (\(p = 0.019\)).

We conducted separate logistic regression analysis to identify the factors that were independently associated with vitamin B\textsubscript{12} deficiency and marginal vitamin B\textsubscript{12} deficiency during late pregnancy by taking into account of potential confounders. We found that the women with a gestational age of \(\geq 27\) weeks had a 2.6 times higher risk of becoming vitamin B\textsubscript{12} deficient than the women with a gestational age of <27 weeks. While the risk of marginal vitamin B\textsubscript{12} deficiency in women with a gestational age of \(\geq 27\) weeks was nearly double that of women with a gestational age of <27 weeks, the difference was not statistically significant. Sukumar et al. [4], in their systematic review and meta-analysis of the prevalence of vitamin B\textsubscript{12} insufficiency in pregnancy examining worldwide pooled trimester wise estimates, also reported an increased prevalence of vitamin B\textsubscript{12} insufficiency with the increase in gestational age, thus supporting our findings. On the contrary, Barney et al. [42] conducted a study among pregnant rural South Indian women and reported nearly four-times higher odds of being B\textsubscript{12} deficient for women in the first trimester compared with those in the second trimester. The authors mentioned that the increased odds of vitamin B\textsubscript{12} deficiency in the first trimester could be due to decreased intake because of morning sickness.

Although not statistically significant (\(p = 0.07\)), women with parity of two or more had a 2.74 times higher odds of being vitamin B\textsubscript{12} deficient compared to nullipara pregnant women. On the contrary, a study conducted in South India reported that primipara women had a 1.4 times higher risk of developing impaired vitamin B\textsubscript{12} status defined by low serum B\textsubscript{12} concentration and elevated methyl malonic acid [43]. Another study conducted among pregnant women in Amsterdam showed that nulliparous women had a significantly lower concentration of vitamin B\textsubscript{12} compared with multiparous women and concluded that nulliparous women were more at risk of developing vitamin B\textsubscript{12} deficiency [44]. The discrepancy between the findings of the previous studies and present study could be due to the differences in pre-pregnancy vitamin B\textsubscript{12} status and/or dietary intake of vitamin B\textsubscript{12} during pregnancy.

The present study failed to show any association between various socio-economic factors and the risk of vitamin B\textsubscript{12} deficiency, except for home ownership. The logistic
regression revealed that the odds of developing vitamin B\textsubscript{12} deficiency were 13 times higher among pregnant women who lived in rented accommodation compared with those who lived in their own house. A study conducted among infants in Nepal also showed that families that lived in their own house had a higher concentration of serum vitamin B\textsubscript{12} than those living in a rented house [45]. A study conducted among Colombian women also found no association between socio-economic status and serum vitamin B\textsubscript{12} deficiency [46]. Another study of pregnant Colombian women reported a positive association between the education level of the household head and serum vitamin B\textsubscript{12} concentrations, but could not find any association with wealth index and/or food security [23].

The present study also found that the risk of vitamin B\textsubscript{12} deficiency was 2.33 times higher among women who consumed red or organ meat <3 times a week. While not statistically significant ($p = 0.098$), the risk of marginal vitamin B\textsubscript{12} deficiency in women who consumed red or organ meat <3 times a week was 1.68 times higher. A study conducted among pregnant women in South India examining the relationship between consumption of food rich in vitamin B\textsubscript{12} reported that the participants who consumed fish and yogurt more frequently had a higher concentration of serum vitamin B\textsubscript{12} and were less likely to develop a vitamin B\textsubscript{12} deficiency [43]. Another study among Dutch women during late pregnancy showed that vitamin B\textsubscript{12} intake from dairy, meat and fish, but not eggs was independently associated with plasma concentrations of total vitamin B\textsubscript{12} in a dose response manner. Furthermore, the intake of these foods was also independently associated with reduced odds of vitamin B\textsubscript{12} deficiency [47]. One of the reasons for the discrepancy between previous studies and our study could be due to the variations in the amount of vitamin B\textsubscript{12} intake from various animal source foods. Of note, in the present study, we collected data on the frequency of consumption, but without the portion size; thus, we were unable to determine the actual amount of vitamin B\textsubscript{12} intake from each of these animal source foods.

The strength of this study is that it represents a relatively large sample from different geographical areas in rural Bangladesh, and, for the first time, employed a longitudinal study design to assess the changes in the prevalence of vitamin B\textsubscript{12} deficiency with the progress of a pregnancy. However, this study also has some limitations. First, we used a convenience sampling method for selecting the study participants, and therefore the findings of this study may not be representative of the wider population from which the participants were drawn. Second, the dietary data focused on the frequency of consumption of various animal source foods, but not the specific amount of consumption. A more in-depth dietary assessment, including a quantitative estimation of vitamin B\textsubscript{12} intake, should be considered in future studies. Third, although serum vitamin B\textsubscript{12} concentrations are a commonly used biomarker for assessing vitamin B\textsubscript{12} status in population-based studies [22], it is not a reliable indicator of vitamin B\textsubscript{12} status during pregnancy. Thus, the findings of the study should be interpreted with caution. Other biomarkers, such as circulating holo-transcobalamin (refer to as active vitamin B\textsubscript{12}) and methylmalonic acid (MMA, functional biomarker), are more sensitive indicators of vitamin B\textsubscript{12} status during pregnancy than serum vitamin B\textsubscript{12} concentration [3,13]. Further, circulating holo-transcobalamin concentrations remain relatively unchanged during pregnancy [3,38] and it is a more sensitive indicator of vitamin B\textsubscript{12} status than the serum MMA concentration [48]. Future studies should include other markers, such as circulating holo-transcobalamin, which would enhance the accuracy in the assessment of vitamin B\textsubscript{12} status during pregnancy, and thus the interpretation of the findings.

5. Conclusions

In conclusion, we found a high prevalence of vitamin B\textsubscript{12} deficiency and marginal deficiency among pregnant rural women in Bangladesh, with a significant increase in the prevalence of vitamin B\textsubscript{12} deficiency with the progress of pregnancy. The finding raises concerns as it could impact on pregnancy outcomes. Furthermore, increasing gestational age, higher parity, living in a rented house, and consumption of red or organ meat fewer
than three times a week were identified as potential risk factors of vitamin B$_{12}$ deficiency in this population. Further research should focus on a more in-depth assessment of dietary vitamin B$_{12}$ intakes, along with identifying other non-dietary risk factors of vitamin B$_{12}$ deficiency, to develop an appropriate intervention program to prevent vitamin B$_{12}$ deficiency in this population.

**Author Contributions:** O.I.S. performed the preliminary data analysis and wrote the first draft of the paper. M.R.K. contributed to the study design and supervised fieldwork, data and blood collection. A.K.R. and R.R. were responsible for laboratory analysis. F.A. took the lead in the study planning and design, guided data collection and data analysis. O.I.S., F.A., A.K.R. and R.R. contributed to writing the manuscript. F.A. had the primary responsibility for the final content. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

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