Prevalence of Vitamin D and Folate Deficiency among People of Different Age Groups in Bangladesh

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Abbreviations

- **GAIN** – Global Alliance for Improved Nutrition
- **MOHFW** – Ministry of Health and Family Welfare
- **IPHN** – Institute of Public Health Nutrition
- **25(OH)D** – 25-hydroxyvitamin D
- **SPSS** – Statistical Package for Social Sciences
- **BMI** – Body Mass Index
- **ANC** – Antenatal Care
- **NTD** – Neural Tube Defects
- **RBC** – Red Blood Cell
- **UVB** – Ultraviolet B
- **WHO** – World Health Organization
- **ECL** – Electrochemiluminescence
- **CV** – Coefficient of Variation
- **SOP** – Standard Operating Procedures
- **NPMBL** – Non-Pregnant, Non-Lactating
- **VDD** – Vitamin D Deficiency
- **BMD** – Bone Mineral Density
- **NGO** – Non-Governmental Organization
- **GDP** – Gross Domestic Product

Acknowledgements

We would like to express our heartfelt gratitude to all those who contributed to the successful completion of this research study.

First and foremost, we acknowledge the invaluable support of GAIN (Global Alliance for Improved Nutrition) for providing the necessary financial and technical assistance, which made this study possible. We also extend our sincere thanks to the Ministry of Health and Family Welfare, Bangladesh, for their continuous guidance and support throughout the research process.

We are deeply grateful to all the investigators and research team members for their dedication, expertise, and hard work. Special thanks to Dr. Rawshan Zahan Akhter Alo, the Principal Investigator, for their leadership and vision in guiding the study. Our sincere appreciation also goes to Prof. Dr. Rubena Haque, Prof. Dr. Shah Golam Nabi, Dr. Joyasish Roy, Dr. Mahbub Arefin Rezanur, Dr. Mohammad Shoayeb, and Dr. Tanzir Anwar for their invaluable contributions and commitment to the success of the research. Special thanks to Dr. Md. Rifat Uddin, supervisor of research team, for his hard work and dedication.

We are immensely thankful to all the study participants for their time, cooperation, and willingness to contribute to the study. Without their participation, this research would not have been possible.

We also express our appreciation to the relevant authorities for granting permission and ensuring the smooth conduct of the study. Their support in facilitating the research process has been vital to our success.

Lastly, we offer our deepest gratitude to the Almighty for His blessings, guidance, and strength throughout the research journey.

Thank you all for your unwavering support and contributions.

Abstract

Background

Micronutrient deficiencies, particularly vitamin D and Folate, are global public health concerns, significantly affecting populations in developing regions such as South Asia. In Bangladesh, deficiencies in these micronutrients are prevalent across different age groups, leading to various health implications.

Objective

The primary objective of this study was to assess the prevalence of vitamin D and Folate deficiencies among people of different age groups in Bangladesh. The study also aimed to identify associated sociodemographic factors and propose public health interventions.

Methodology

This cross-sectional, descriptive study was conducted from January to July 2025. A multistage random sampling technique was employed to select participants from eight divisions of Bangladesh, covering urban and rural areas. A total of 2481 individuals from five age groups (children, adolescents, pregnant mothers, adults, and geriatric individuals) participated in the study. Data was collected through face-to-face interviews to gather sociodemographic information, and blood samples were analyzed for serum vitamin D (25-hydroxyvitamin D) and folate levels using Electrochemiluminescence Immunoassay.

Results

- **Vitamin D Deficiency:** A significant 67.0% of participants were found to be deficient in vitamin D. Deficiency rates were highest in adolescents (83.3%) and pregnant women (77.8%). Urban residents (71.1%) exhibited higher deficiency rates compared to rural residents (63.1%).

- **Folate Deficiency:** Folate deficiency was less widespread, affecting 16.7% of the study population. The highest deficiency rates were observed in Sylhet (28.3%) and Chattogram (23.0%). Gender disparities were noted, with women exhibiting higher rates of deficiency.

Conclusion

Vitamin D deficiency remains a critical public health issue across all age groups, especially among adolescents, pregnant women, and females. Folate deficiency, while less prevalent, is still concerning in specific regions and demographics. The findings underscore the need for comprehensive public health interventions, including vitamin D supplementation programs and awareness campaigns, particularly targeting the most vulnerable groups. Further studies are needed to identify the causes of deficiencies and evaluate the effectiveness of potential interventions.

Contents

1. Introduction

Micronutrients, including vitamins and minerals, are critical to maintaining human health, supporting metabolic functions, and ensuring proper physiological development. Among them, vitamin D and Folate (vitamin B9) are two essential nutrients that play crucial roles in bone health, immune function, cell growth, and DNA synthesis. Despite their importance, deficiencies in these vitamins remain significant global public health concerns, affecting populations in both developing and developed countries. The prevalence of vitamin D and Folate deficiency varies geographically and demographically, influenced by factors such as diet, sunlight exposure, socioeconomic status, and health education. This study seeks to determine the prevalence of vitamin D and Folate deficiency among people, with a view to highlighting the scope of the problem and identifying possible public health interventions.

Vitamin D is a fat-soluble vitamin that is either synthesized in the skin upon exposure to sunlight (specifically UVB radiation) or obtained from dietary sources such as fatty fish, fortified dairy products, and supplements. It is converted in the liver to 25-hydroxyvitamin D [25(OH)D], the major circulating form, and then in the kidneys to its active form, calcitriol (1,25-dihydroxyvitamin D) [1]. Vitamin D is essential for calcium absorption in the gut and for maintaining adequate serum calcium and phosphate levels to enable normal bone mineralization [2]. It also plays roles in modulating cell growth, neuromuscular and immune function, and reducing inflammation [3].

Folate, on the other hand, is the synthetic form of folate, a water-soluble B-vitamin that is naturally present in many foods such as leafy greens, legumes, and citrus fruits. Folate functions as a coenzyme in the synthesis of nucleic acids and the metabolism of amino acids, making it essential for DNA synthesis, repair, and methylation [4]. It is particularly important during periods of rapid growth such as pregnancy and infancy. Folate deficiency can lead to megaloblastic anemia and, during pregnancy, to neural tube defects in the fetus [5].

Vitamin D deficiency is now recognized as a pandemic affecting over a billion people worldwide [6]. The prevalence is alarmingly high in regions with limited sun exposure, either due to high latitudes, indoor lifestyles, or cultural practices involving sun avoidance. A global study indicated that 37.3% of the population had serum 25(OH)D concentrations below 50 nmol/L, the threshold often used to define vitamin D deficiency [7]. In the United States, approximately 41.6% of adults are vitamin D deficient, with higher prevalence among African-Americans and Hispanics [8]. In Europe, deficiency rates vary widely, with some northern countries reporting levels as high as 50% during winter months [9]. In South Asia, including Bangladesh and India, studies have found deficiency rates exceeding 70% among women and children [10,11].

Folate deficiency, while less discussed in public discourse than vitamin D deficiency, remains a pressing issue in many low- and middle-income countries. The global burden is compounded by dietary inadequacies, malabsorption syndromes, alcoholism, and increased physiological needs during pregnancy and lactation [12]. In the United States and several developed countries where Folate fortification of food is mandatory, the prevalence of deficiency has markedly decreased [13]. However, in countries without mandatory fortification, particularly in South Asia and Sub-Saharan Africa, folate deficiency remains prevalent, with rates ranging between 20% to 40% in reproductive-aged women [14,15].

The clinical consequences of vitamin D deficiency extend beyond bone diseases such as rickets, osteomalacia, and osteoporosis. Vitamin D has been implicated in several non-skeletal conditions, including cardiovascular diseases, diabetes, autoimmune diseases, and certain cancers [16]. Deficiency has also been associated with poor immune response, increasing susceptibility to infections, including respiratory tract infections and COVID-19 [17,18].

Similarly, Folate deficiency has far-reaching health implications. In adults, it can cause megaloblastic anemia, characterized by large, immature red blood cells, fatigue, and irritability [19]. In pregnant women, low folate levels can lead to serious fetal abnormalities, including neural tube defects such as spina bifida and anencephaly [20]. There is also growing evidence linking folate deficiency to cognitive decline, depression, and an increased risk of certain cancers [21,22].

The risk factors for vitamin D deficiency include inadequate sunlight exposure, darker skin pigmentation, aging, obesity, malabsorption syndromes, chronic kidney disease, and certain medications such as anticonvulsants and glucocorticoids [23]. Cultural practices, such as wearing full-body coverings, and geographical factors, such as living in high-latitude regions, also contribute to lower vitamin D synthesis [24].

Risk factors for Folate deficiency are primarily nutritional inadequacy, increased physiological needs, malabsorption, and alcoholism. Pregnant women, individuals with gastrointestinal diseases like celiac disease or inflammatory bowel disease, and those on certain medications like methotrexate and anticonvulsants are at increased risk [25].

2. Justification

Vitamin D deficiency has been reported in over 1 billion people globally, with 37.3% of the world's population having levels below 50 nmol/L [7]. This deficiency is particularly prevalent in regions with limited sun exposure and among populations with darker skin, higher body fat percentage, or chronic illnesses [6,8,9]. The situation is equally concerning in South Asia, where over 70% of women and children are deficient in vitamin D, due to poor dietary intake and lifestyle factors limiting sun exposure [10,11]. In Bangladesh, although the National Micronutrient Survey 2022 provides valuable data on vitamin D status, it is limited to children under five years of age and non-pregnant, non-lactating (NPBL) mothers. Consequently, there remains a substantial knowledge gap regarding the vitamin D status of other age groups within the general population, justifying the need for this broader investigation. with 37.3% of the world's population having levels below 50 nmol/L [7]. This deficiency is particularly prevalent in regions with limited sun exposure and among populations with darker skin, higher body fat percentage, or chronic illnesses [6,8,9]. The situation is equally concerning in South Asia, where over 70% of women and children are deficient in vitamin D, due to poor dietary intake and lifestyle factors limiting sun exposure [10,11].

While Folate deficiency has been significantly reduced in countries with mandatory food fortification, it remains a major concern in many developing regions. In reproductive-aged women in South Asia and Africa, folate deficiency ranges from 20% to 40%, contributing to a high incidence of neural tube defects, anemia, and poor maternal and child health outcomes [14,15,20]. The persistence of folate deficiency in low-resource settings is exacerbated by limited dietary diversity, gastrointestinal disorders, and poor health literacy [12,25].

Given the preventable nature of these micronutrient deficiencies and their significant health implications—including skeletal disorders, immune dysfunction, congenital abnormalities, and chronic diseases—this study is critical for assessing the current prevalence of vitamin D and Folate deficiencies. Understanding the extent and associated factors of these deficiencies will provide a scientific basis for public health planning, including targeted nutrition interventions, awareness programs, and policy development for supplementation or food fortification initiatives.

The Technical Advisory committee for Vitamin D Fortification, MOHFW recommended to generate evidence on these deficiencies. Thus this study aims to identify the prevalence and factors contributing to vitamin D and folate deficiencies in Bangladesh.

3. Research question

What are the proportion of vitamin D and Folate deficiency among people of different age groups in Bangladesh?

4. Objectives

General objective

To Assess the proportion of vitamin D and Folate deficiency among people of different age groups in Bangladesh

Specific Objectives

- To identify the socio-demographic characteristics of the respondents
- To find out the prevalence of vitamin D deficiency among the respondents
- To assess the prevalence of Folate deficiency among the respondents

5. Literature review

Micronutrients are essential for maintaining physiological health and preventing disease. Among them, Vitamin D and Folate (Vitamin B9) have garnered substantial attention due to their multifaceted roles in bone metabolism, immune regulation, pregnancy outcomes, neurodevelopment, and cellular function. In developing countries like Bangladesh, the deficiency of these micronutrients is an under-recognized yet prevalent public health concern, transcending age, gender, and socioeconomic barriers. This review critically explores the prevalence, risk factors, and implications of Vitamin D and Folate deficiencies across various age groups in Bangladesh, supported by both national and international literature.

Vitamin D Deficiency: A Widespread Public Health Concern

Vitamin D, a fat-soluble vitamin primarily synthesized in the skin upon exposure to ultraviolet B (UVB) rays, is essential for calcium and phosphorus absorption, bone health, and immune modulation. Despite Bangladesh's favorable latitude for solar exposure, the prevalence of Vitamin D deficiency (VDD) is surprisingly high.

A study conducted in outpatient departments across urban hospitals in Bangladesh revealed that over 65% of adult patients exhibited Vitamin D deficiency, with many reporting musculoskeletal pain, generalized fatigue, and low immunity [26]. Surprisingly, even in outdoor workers and males, deficiency rates remained substantial, pointing toward other contributing factors such as pollution, melanin content, urbanization, and changing lifestyle patterns.

Among pregnant rural women, the scenario is even more alarming. One study reported a prevalence of 85% of Vitamin D deficiency or insufficiency, with risk factors including limited sunlight exposure, modest clothing, low socioeconomic status, and poor antenatal nutrition [27]. Vitamin D plays a critical role during pregnancy, influencing fetal bone formation, immune development, and reducing the risk of gestational diabetes, preeclampsia, and preterm birth. These findings underscore the importance of incorporating Vitamin D supplementation into antenatal care packages in Bangladesh.

Children are another vulnerable group. A 2021 study termed the situation as a "silent epidemic", with nearly 70% of children in selected rural regions of Bangladesh having Vitamin D levels below the sufficiency threshold [31]. Clinical manifestations included rickets-like symptoms, muscle weakness, and frequent respiratory infections. Similarly, Rahman et al. [29] conducted a multicentric study on urban and peri-urban children and found that over 60% were deficient, citing lack of outdoor play, dietary insufficiencies, and unfortified staple diets as core issues.

The National Micronutrient Survey 2019–2020 offers comprehensive insights into the Vitamin D status of specific population segments. It revealed that 39% of children under five and 31% of non-pregnant, non-lactating women were Vitamin D deficient [32]. However, the absence of data on adolescents, elderly populations, and adult men indicates significant knowledge gaps. This has implications for public health policy, as interventions remain fragmented and focused mostly on women of reproductive age and young children.

An often-overlooked demographic is female industrial workers, particularly garment workers. A study conducted among female garment workers found extremely low Vitamin D levels correlated with reduced bone mineral density and increased risk of fractures, exacerbated by long indoor work hours and inadequate diets [30]. The economic and productivity implications of such deficiencies in a labor-intensive sector like garments are profound.

Comparative International Perspectives

Globally, Vitamin D deficiency is also prevalent, albeit with different risk factors. In the United States, the NHANES (2001–2006) reported that 41.6% of adults were deficient, with the highest prevalence among African-Americans (82.1%) and Hispanics (69.2%) [28]. While supplementation and food fortification (milk, cereals, juices) are standard practices in the US, lifestyle and genetic factors still contribute to VDD.

A comparative study in India revealed that up to 80% of adults in urban settings are Vitamin D deficient despite abundant sunlight [33]. This "Asian paradox" suggests that sun exposure alone is insufficient if not accompanied by adequate duration, skin exposure, and

UVB intensity—conditions often hindered by pollution, clothing, and indoor living. These factors are equally present in Bangladesh.

In Europe, the prevalence varies with latitude and season, but 15–40% of adults experience deficiency during winter months [34]. Fortification and public health policies, such as cod liver oil supplementation in Nordic countries, have shown positive impacts in reducing VDD prevalence.

These global findings highlight that Bangladesh lags behind in preventive measures, with no established fortification policies or standardized national supplementation programs beyond targeted maternal health initiatives.

Biological and Clinical Implications of Vitamin D Deficiency

Vitamin D deficiency is associated with a multitude of health conditions:

- **In children**: Rickets, impaired growth, increased infection risk.
- **In adults**: Osteomalacia, myopathy, fatigue, depression.
- **In pregnant women**: Preterm birth, low birth weight, gestational complications.
- In the elderly: Increased fracture risk, impaired immunity, falls, and sarcopenia.

Furthermore, emerging evidence links Vitamin D insufficiency to chronic non-communicable diseases such as diabetes, hypertension, cardiovascular disease, and autoimmune conditions [35]. This broadens the scope of its public health importance beyond bone health alone.

Folate Deficiency: The Unseen Epidemic

Folate (Vitamin B9) is essential for DNA synthesis, cell division, and red blood cell formation. Its role in neural tube development makes it particularly critical for women of childbearing age. However, data on Folate deficiency in Bangladesh is scarce, especially across different age groups.

A cross-sectional study among rural pregnant women in northern Bangladesh revealed that 35–40% were folate deficient, increasing their risk of neural tube defects (NTDs), anemia, and pregnancy complications [32]. Despite WHO recommendations for daily Folate supplementation during pregnancy, compliance remains low, and supplementation often begins late into the first trimester, reducing its protective effect.

According to the National Nutrition Services, Folate supplementation is part of the ANC protocol; however, implementation gaps exist. A study by Nasreen et al. found that less than 60% of rural pregnant women received Folate supplementation consistently throughout pregnancy [37].

Among adolescents and non-pregnant women, data is even more limited. One study in Dhaka found that 28% of adolescent girls exhibited folate-deficient serum levels, raising concerns about their future reproductive health [38]. The elderly, too, are at risk due to reduced dietary intake, malabsorption, and polypharmacy, but national-level surveys have not yet captured this demographic.

Globally, countries such as Canada, the U.S., Chile, and South Africa have mandated Folate fortification of wheat flour and staple grains, resulting in significant reductions in NTD incidence (up to 50%) and improvement in population folate levels [33]. Bangladesh, despite a high NTD burden and folate deficiency prevalence, has not yet implemented mandatory folate fortification, representing a critical missed opportunity.

Age-Specific Considerations in Micronutrient Deficiencies

The needs and risks of Vitamin D and Folate vary widely across age groups:

- **Infants and children**: Require adequate Vitamin D for skeletal growth and brain development. Exclusive breastfeeding without Vitamin D supplementation often leads to deficiency.

- **Adolescents**: Experience rapid growth, hormonal changes, and nutritional vulnerabilities, particularly in girls due to menstruation.

- **Adults**: Face occupational and lifestyle risks, particularly those working indoors, in industrial settings, or with limited dietary diversity.

- **Women of reproductive age**: Require Folate for reproductive and maternal health.

- **Elderly**: Are at increased risk of both deficiencies due to lower absorption, comorbidities, and often solitary lifestyles with poor nutrition.

Bangladesh's current health surveillance and interventions mostly focus on under-five children and pregnant women, leaving adolescents, men, and the elderly largely neglected. A life-course approach to micronutrient monitoring and intervention is needed.

The reviewed literature paints a compelling picture: Vitamin D and Folate deficiencies are widespread, multifactorial, and inadequately addressed in Bangladesh. While studies highlight high prevalence across children, women, and industrial workers, large-scale national data across all age groups is lacking. There is also no mandatory food fortification policy, limited public awareness, and inconsistent supplementation efforts.

The present study seeks to bridge this gap by assessing the prevalence of both deficiencies across diverse age groups, generating evidence to inform future national nutrition strategies, supplementation programs, and food policy reforms in Bangladesh.

6. Methodology

Key Variables:

Dependent variable:

- Prevalence of vitamin D deficiency (25-hydroxyvitamin D)
- Folate deficiency (Serum Folate level)

Independent variables

Sociodemographic Variables:

- Age
- Sex
- Religion
- Marital status
- Gestational age
- Educational status
- Employment status
- Monthly family income

Type of the study: Descriptive types of cross-sectional survey were conducted followed quantitative approach.

Place of the study: The study was conducted in randomly selected households from each of the 8 divisions in Bangladesh using a multistage random sampling technique. From each division, a urban area and a rural area were chosen randomly. The list of study areas was given below:

Division	**Urban area**	**Rural Area**
1. Dhaka	Dhaka North city corporation	Singair Upazila, Manikganj
2. Rajshahi	Sirajganj municipalities	Gurudashpur Upazila, Natore
3. Sylhet	Sylhet city corporation	Biswamvarpur Upazila, Sunamganj
4. Mymensingh	Mymensingh city corporation	Islampur Upazila, Jamalpur
5. Chattogram	Chattogram city corporation	Dighinala Upazila, Khagrachari
6. Rangpur	Rangpur city corporation	Patgram Upazila, Lalmonirhat
7. Barisal	Barisal city corporation	Kalapara Upazila Potuakhali
8. Khulna	Khulna city corporation	Debhata Upazila, Satkhira

Duration of the study: The duration of the study will be 7 months from January 2025 to July 2025

Sample size:

Using the following formula (n=$\frac{Z^2 pq}{d^2}$), the calculated sample size is (Deign effect is considered 1.5):

Table 1: Total sample size

Study Subjects	**Population proportions from different study**	**Sample Size**
Children (6-9 years)	P= 35.2% [23]	351×1.5= 527
Adolescence (10-19 years)	P=37.2% [23]	359×1.5= 539
Pregnant mothers	P=17.3% [24]	220×1.5= 330
Adult people (Above 19 years to 60 years male and female)	P=67.2% [25]	339×1.5= 509
Geriatric people (Above 60 years male and female)	P=50%	384×1.5= 576
Total		**2481**

Division wise distribution: nh=(Nn×n)/N

- nh= Sample size in each division
- Nn= Total population in each division
- n= Total sample size
- N= Total population of Bangladesh

Table 2: Sample size of Dhaka division

Study Subjects	**Urban**	**Rural**
Children (6-9 years)	70	71
Adolescence (10-19 years)	72	72
Pregnant mothers	44	44
Adult people (Above 19 years to 60 years male and female)	68	68
Geriatric people (Above 60 years male and female)	77	77
Total	**331**	**332**

Table 3: Sample size of Barisal

Study Subjects	Urban	Rural
Children (6-9 years)	14	15
Adolescence (10-19 years)	15	15
Pregnant mothers	9	9
Adult people (Above 19 years to 60 years male and female)	15	15
Geriatric people (Above 60 years male and female)	16	16
Total	**69**	**70**

Table 4: Sample size of Chattogram

Study Subjects	Urban	Rural
Children (6-9 years)	53	53
Adolescence (10-19 years)	54	54
Pregnant mothers	33	33
Adult people (Above 19 years to 60 years male and female)	51	51
Geriatric people (Above 60 years male and female)	58	58
Total	**249**	**249**

Table 5: Sample size of Rajshahi

Study Subjects	Urban	Rural
Children (6-9 years)	33	32
Adolescence (10-19 years)	33	33
Pregnant mothers	21	20
Adult people (Above 19 years to 60 years male and female)	31	32
Geriatric people (Above 60 years male and female)	35	36
Total	**153**	**153**

Table 6: Sample size of Khulna

Study Subjects	Urban	Rural
Children (6-9 years)	28	28
Adolescence (10-19 years)	28	29
Pregnant mothers	17	18
Adult people (Above 19 years to 60 years male and female)	27	27
Geriatric people (Above 60 years male and female)	30	30
Total	**130**	**132**

Table 7: Sample size of Mymensingh

Study Subjects	**Urban**	**Rural**
Children (6-9 years)	20	19
Adolescence (10-19 years)	20	20
Pregnant mothers	12	12
Adult people (Above 19 years to 60 years male and female)	19	19
Geriatric people (Above 60 years male and female)	21	22
Total	**92**	**92**

Table 8: Sample size of Rangpur

Study Subjects	**Urban**	**Rural**
Children (6-9 years)	28	28
Adolescence (10-19 years)	28	29
Pregnant mothers	18	17
Adult people (Above 19 years to 60 years male and female)	27	27
Geriatric people (Above 60 years male and female)	31	30
Total	**132**	**131**

Table 9: Sample size of Sylhet

Study Subjects	Urban	Rural
Children (6-9 years)	18	17
Adolescence (10-19 years)	18	18
Pregnant mothers	11	11
Adult people (Above 19 years to 60 years male and female)	17	18
Geriatric people (Above 60 years male and female)	19	19
Total	**83**	**83**

Sampling technique:

Study place is selected by multistage probability sampling. To find the representative study sample, simple random sampling technique is administered. At first the data enumerators and field supervisors made a list of a hundred household as a sampling frame in each area of data collection. After that required sample size is selected randomly from the list of households.

Study Population:

The study population will be Children (6-9 years), Adolescence (10-19 years), Pregnant women, adult people (Above 19 years to 60 years male and female), and elderly people (Above 60 years male and female)

Inclusion criteria:

1. Children (6-9 years)
2. Adolescence (10-19 years)
3. Pregnant women, adult people (Above 19 years to 60 years male and female)
4. Elderly people (Above 60 years male and female)

Exclusion criteria:

1. Not willing to participate
2. Severely ill person
3. Patient with Kidney disease
4. Person taking Vitamin D supplement
5. Person taking folate supplement except pregnant women

Data collection methods:

Socio-demographic data will be collected through face-to-face interviews. Vitamin D level will be assessed by analyzing venous blood for 25-hydroxyvitamin D test and Folate level will be assessed by serum Folate test.

Data collection instruments:

- Semi-structured questionnaire
- Biochemical tests

Data collection procedure:

The study was conducted using a multistage probability sampling technique to ensure representation across different age groups and geographic areas. Initially, a sampling frame of 100 households was prepared in each selected area by trained data enumerators and field supervisors. From this frame, the required number of households was selected using simple random sampling to obtain a representative study sample.

Selected respondents were invited to attend a designated nearby health facility the following morning. Upon arrival, data enumerators provided a detailed explanation of the study's objectives and procedures, and written informed consent was obtained from each participant. Sociodemographic information was collected through face-to-face interviews using a structured questionnaire.

Following data collection, medical technologists performed venipuncture to collect blood samples from the participants using standard procedures. The blood samples were stored in appropriately labeled test tubes and immediately processed in the field. Using a portable centrifuge machine, the samples were centrifuged on the same day to separate serum from blood cells.

The separated serum and blood cells were stored in a portable cooling box capable of maintaining a temperature range of 2–4°C for at least 48 hours. These samples were then transported to the IPHN laboratory by trained porters within 12 hours of collection. On the next day of collection, designated laboratory technologists at the IPHN laboratory performed biochemical analyses of the samples to determine Vitamin D and folate levels.

Biochemical measures:

All laboratory analyses for this study were conducted at the IPHN Central Laboratory, a government-designated facility equipped for advanced biochemical testing. To ensure the reliability of laboratory results, a pre-study validation was carried out by testing 30 blood samples in parallel at the IPHN Laboratory and the National Institute of Laboratory Medicine & Referral Center (NILMRC), which serves as a national reference laboratory in Bangladesh. The comparison showed a **coefficient of variation (CV) of 2.32%**, which is within the acceptable limit of less than 5%, confirming good inter-laboratory reliability [42].

Vitamin D and folate levels were measured using the **Automated Electrochemiluminescence (ECL) Immunoassay Analyzer**, known for its high sensitivity and precision. The reagents used included 25-OH Vitamin D Total for Vitamin D and Serum Folate for folate estimation, both based on electrochemiluminescence immunoassay techniques. All laboratory procedures adhered strictly to validated Standard Operating Procedures (SOPs), ensuring consistency, accuracy, and quality assurance throughout the analytical process.

According Institute of medicine (IOM), the cut of point was considered for Vitamin D Normal, Insufficient and deficient level [40]:

Category	25(OH)D Level (ng/mL)
Deficiency	< 20
Insufficiency	20 – 29.9
Sufficiency	≥ 30

According to World Health Organization (WHO), the cut of point was considered for folate Normal, Insufficient and deficient level [41]:

Folate Status	Serum Folate Level (ng/mL)
Deficient	≤ 4.0 ng/mL
Sufficient (Normal)	> 4.0 ng/mL

Data management and analysis:

After collection of whole range of data, will be checked, rechecked, edited, coded and recoded simultaneously avoiding irrelevant and unreliable information for quality management. Data will be analyzed according to the study objective through IBM software - Statistical Package for Social Science (SPSS) version 25.

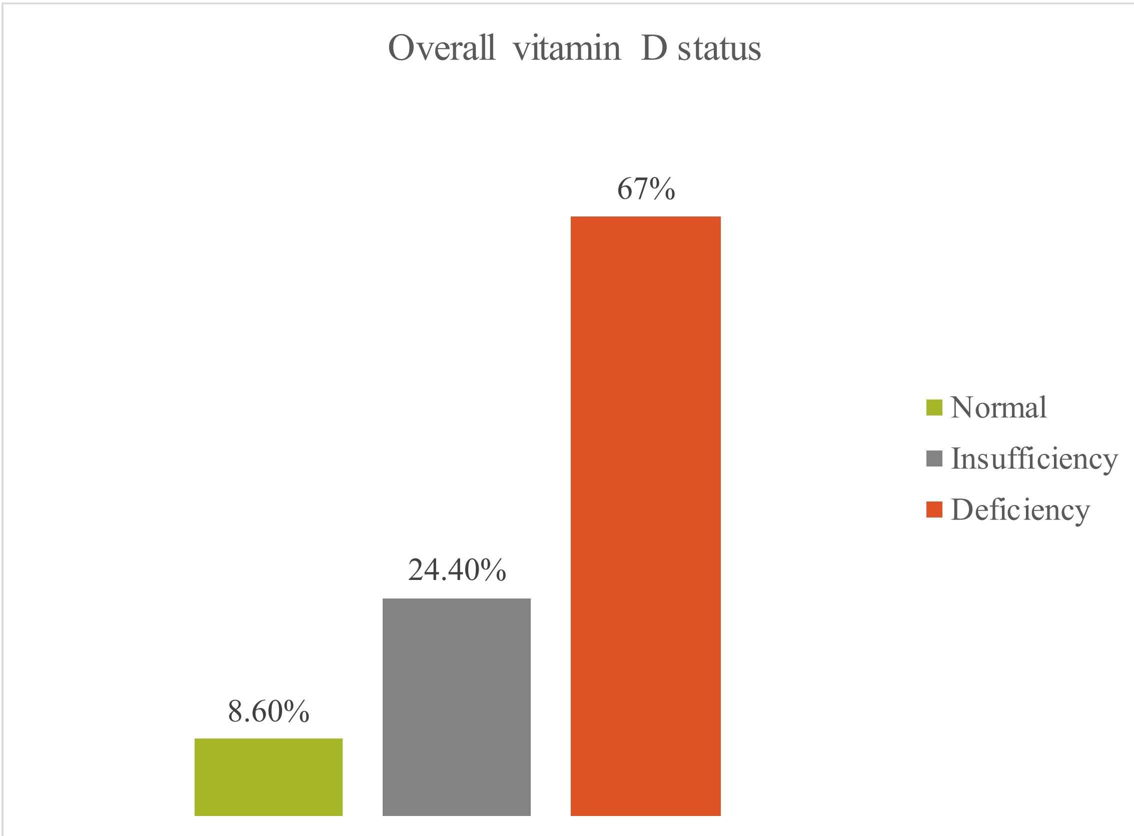
Ethical consideration:

- Ethical clearance of the study was obtained from the Ethical Review Committee of the Institute of Public Health Nutrition (IPHN)
- The respondents have explained the aim and objectives of the study, the reasons for inviting them to the study, the risks and benefits, and the confidentiality of the data
- Informed written consent was taken from each participant
- The respondents were given the complete freedom to withdraw their participation if they wanted at any point of time
- Data were collected from those only who gave their consent willingly

7. Results

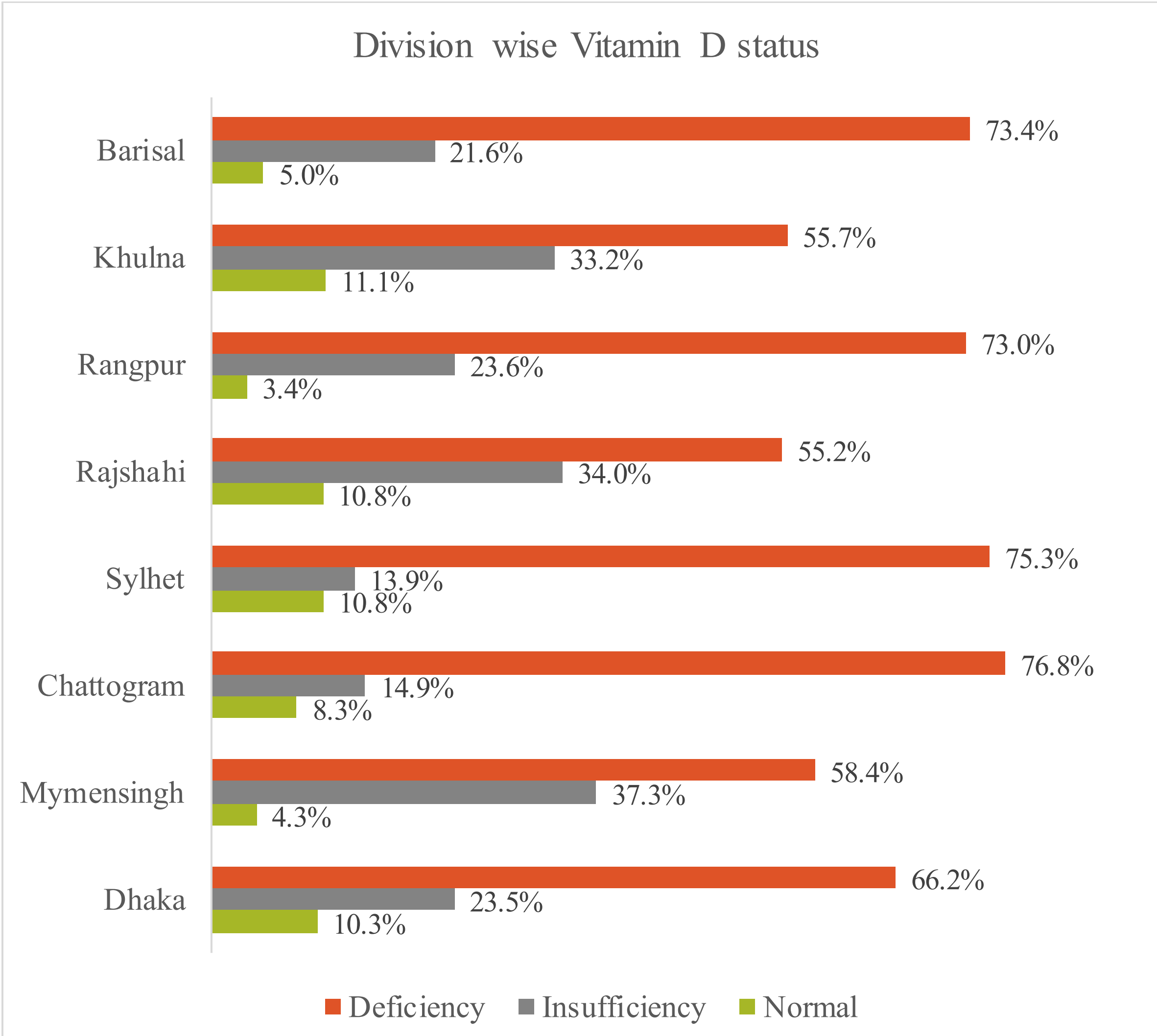
Vitamin D status

Figure 1: Overall Vitamin-D status (n=2480)



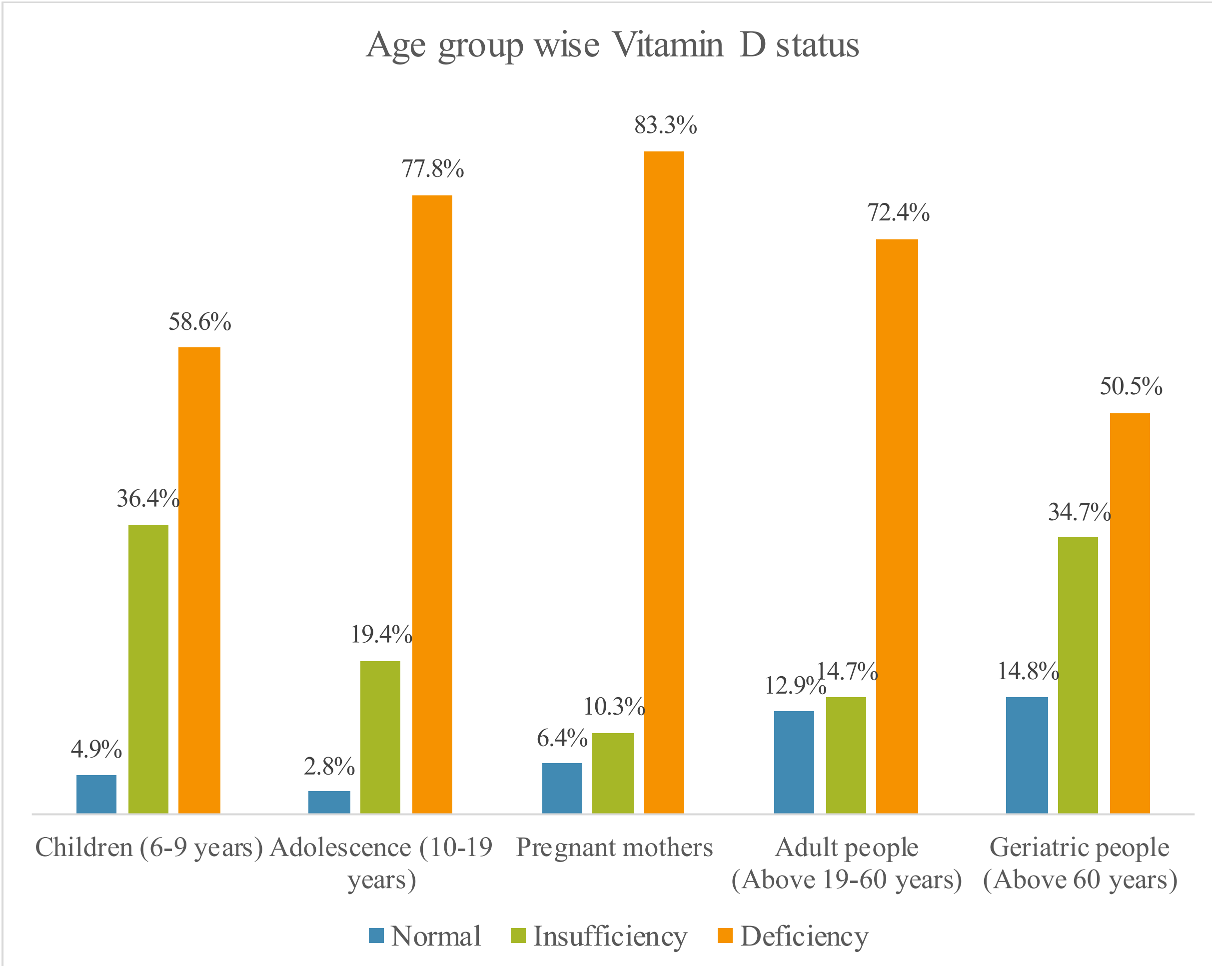
This figure shows the overall distribution of Vitamin D levels among the study population. A significant majority, 67.0%, were Vitamin D deficient, followed by 24.4% with insufficiency, and only 8.6% had sufficient Vitamin D levels. This highlights a widespread deficiency across the population.

Figure 2: Division wise Overall Vitamin-D status (n=2480)



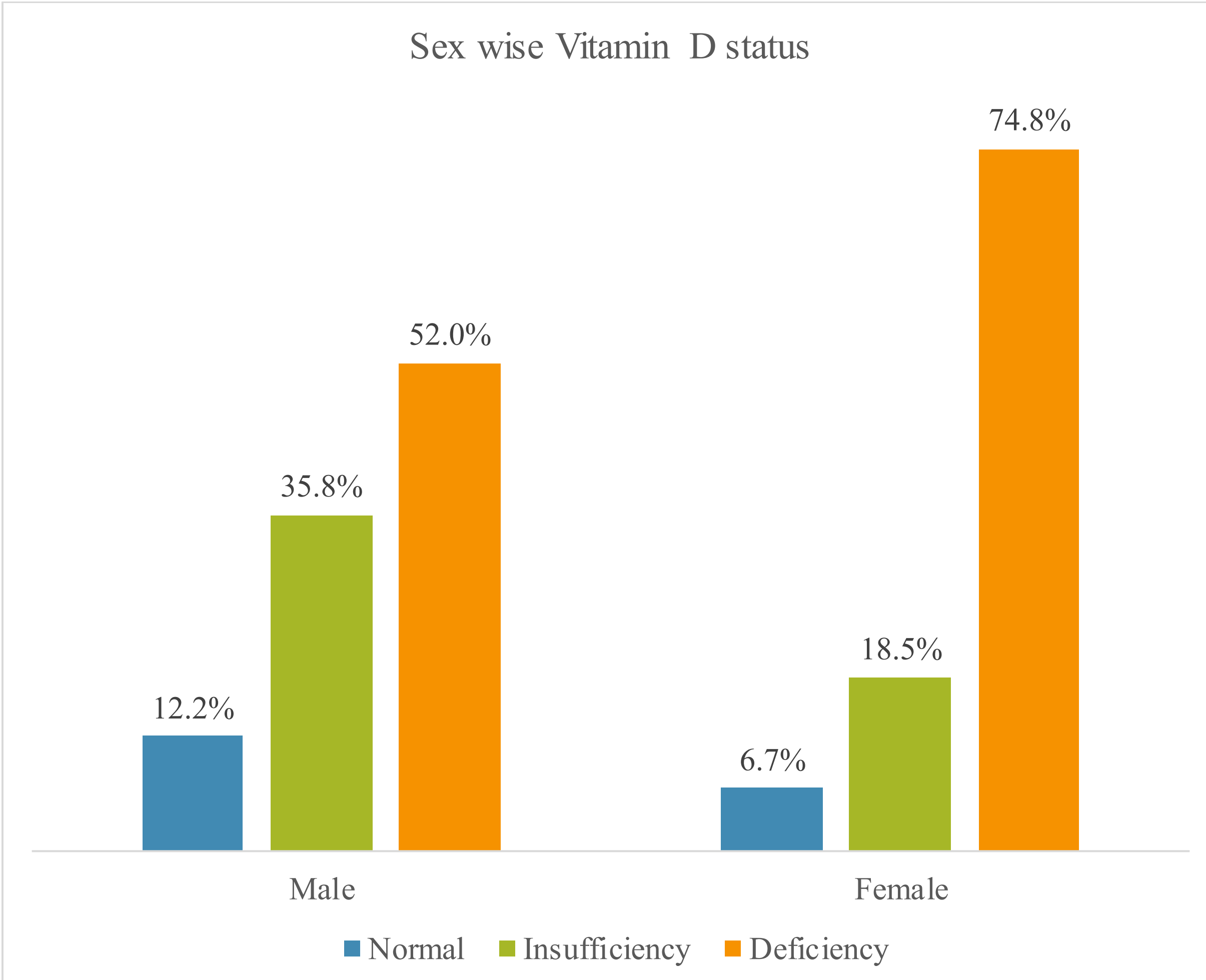
Vitamin D deficiency varied considerably by division. The highest deficiency was observed in Chattogram (76.8%) and Barisal (73.4%), while Rajshahi and Khulna had the lowest deficiency rates (55.2% and 55.7%, respectively). Normal Vitamin D levels were highest in Khulna (11.1%) and Dhaka (10.3%).

Figure 3: Age group wise Overall Vitamin-D status (n=2480)



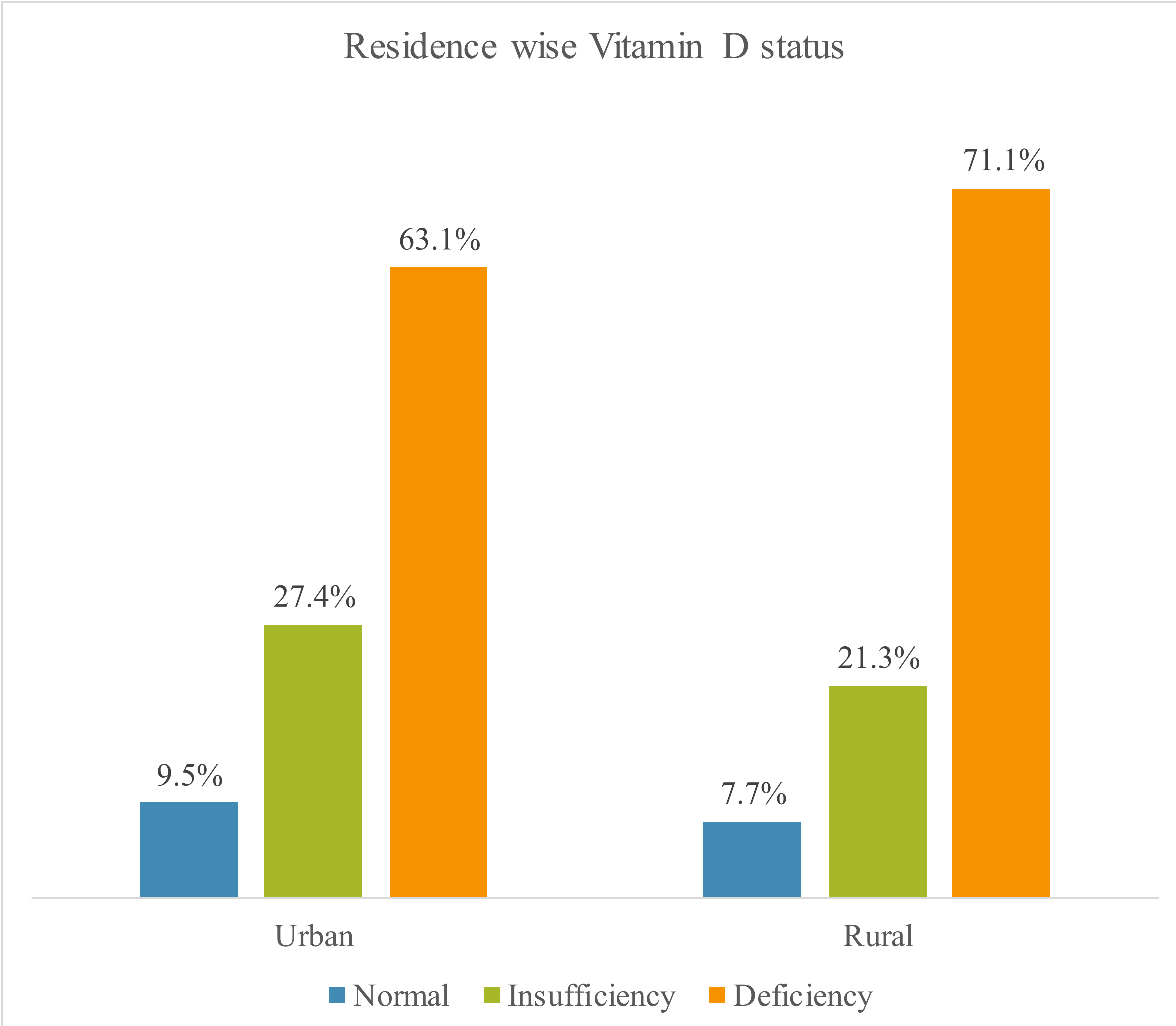
Among all age groups, adolescents (10–19 years) had the highest deficiency at 83.3%, followed by pregnant mothers (77.8%) and children aged 6–9 years (58.6%). Geriatric individuals (60+ years) had the lowest deficiency at 50.5%, but also the highest sufficiency rate (14.8%).

Figure 4: Sex wise Overall Vitamin-D status (n=2480)



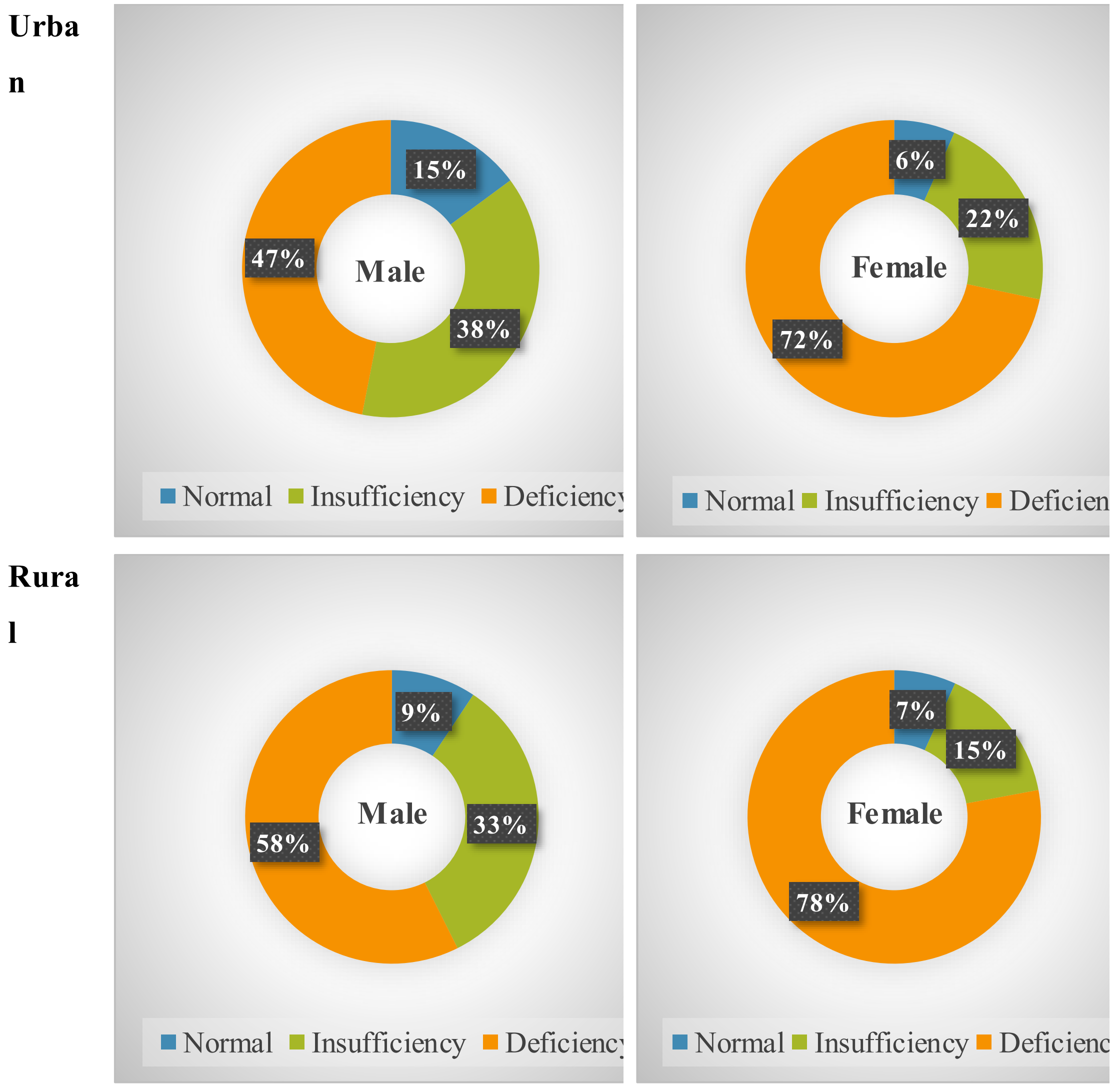
Female participants showed a higher prevalence of deficiency (74.8%) compared to males (52.0%). Normal Vitamin D levels were more common in males (12.2%) than in females (6.7%).

Figure 5: Residence wise Overall Vitamin-D status (n=2480)



Urban residents had a higher deficiency rate (71.1%) compared to rural residents (63.1%). Conversely, sufficiency was more common in rural areas (9.5%) than urban areas (7.7%).

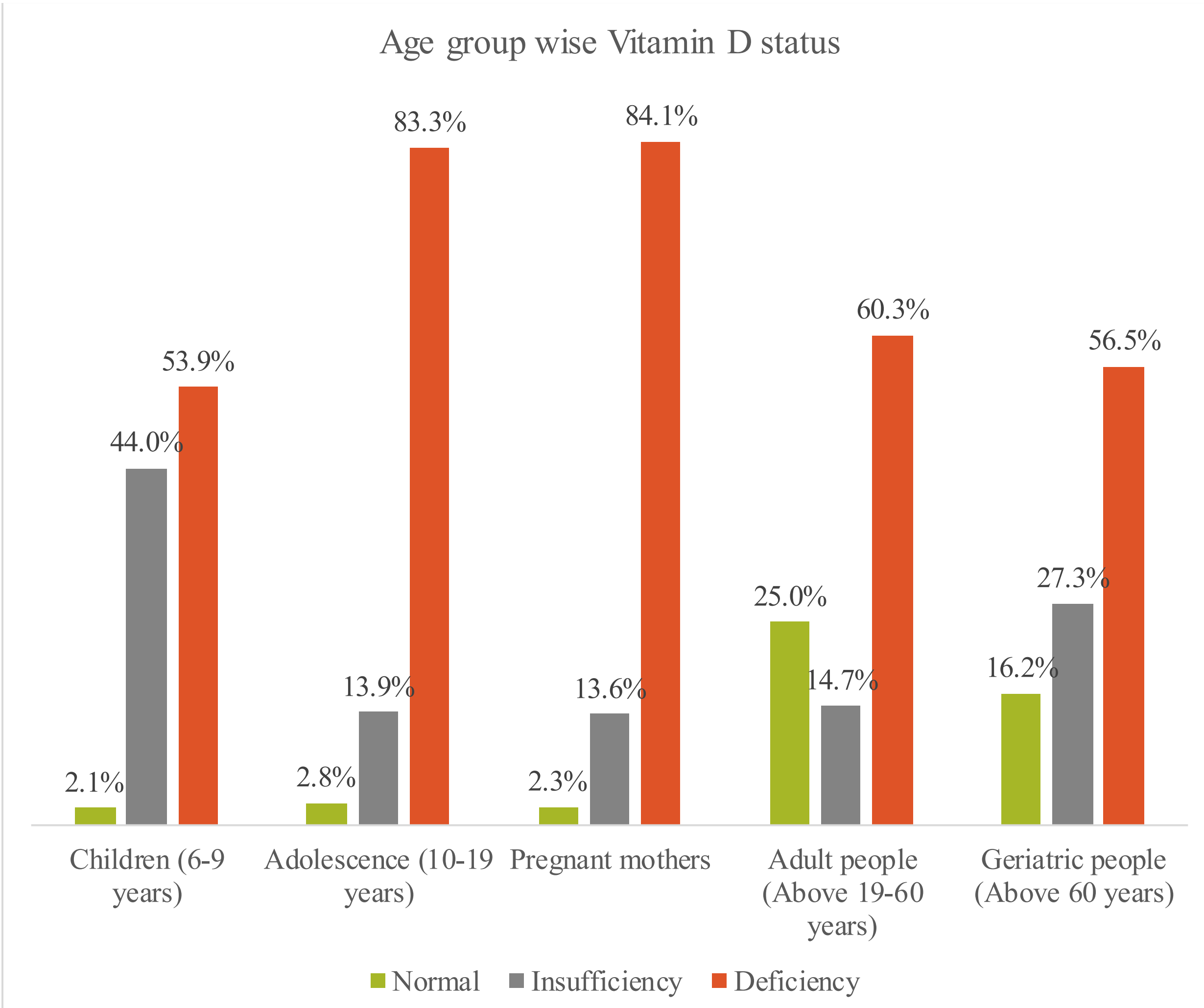
Figure 5: Residence wise Overall Vitamin-D status according to sex (n=2480)



Among urban males, 47% were deficient, while 72% of urban females were deficient. In rural settings, 58% of males and 78% of females were deficient. Normal levels were found more often in rural males (9%) and urban males (15%), whereas rural and urban females had the lowest sufficiency (6% and 7%, respectively).

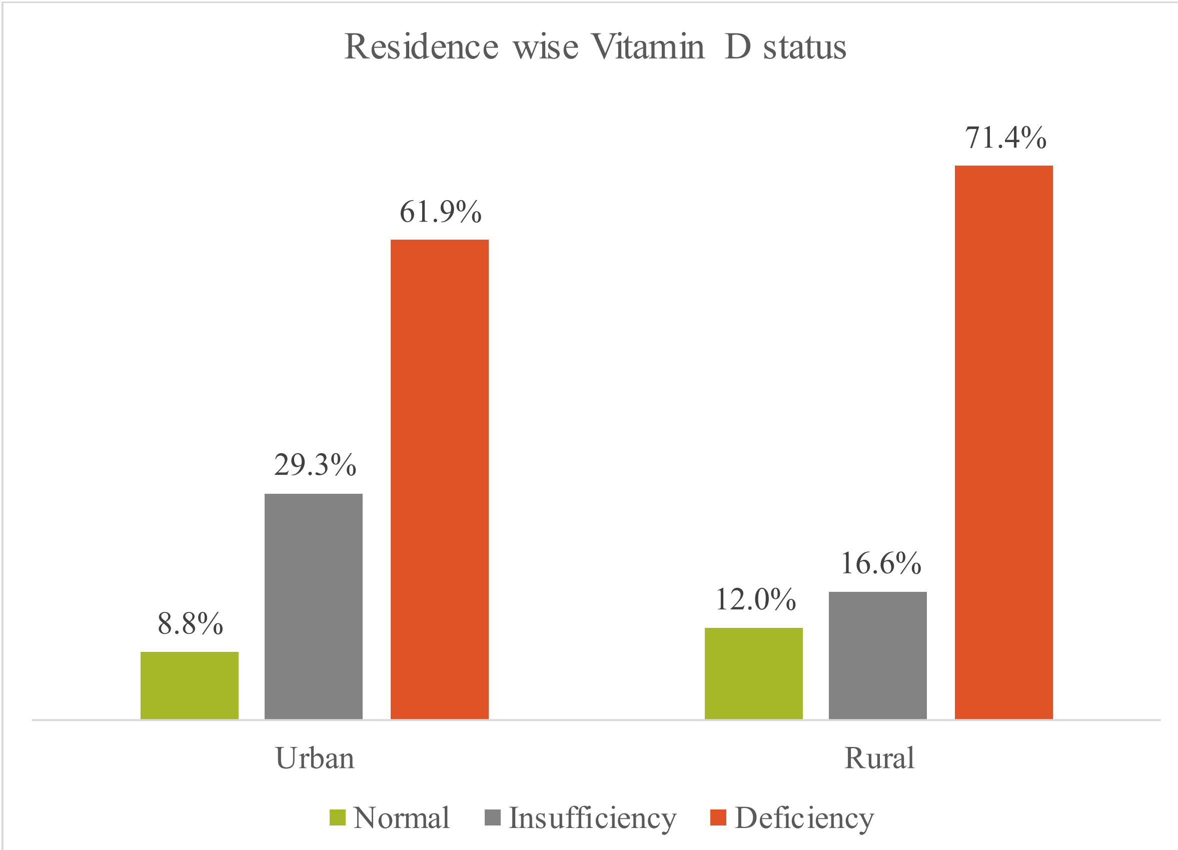
Dhaka Division

Figure 7: Age Group-wise Vitamin-D Status in Dhaka Division (n=663)



In Dhaka, adolescents (10–19 years) and pregnant mothers had the highest Vitamin D deficiency at 83.3% and 84.1%, respectively. Children (6–9 years) had a lower deficiency rate of 53.9%, and geriatric participants had the lowest at 56.5%. Normal levels were rare across all groups, peaking at only 14.7% among the elderly.

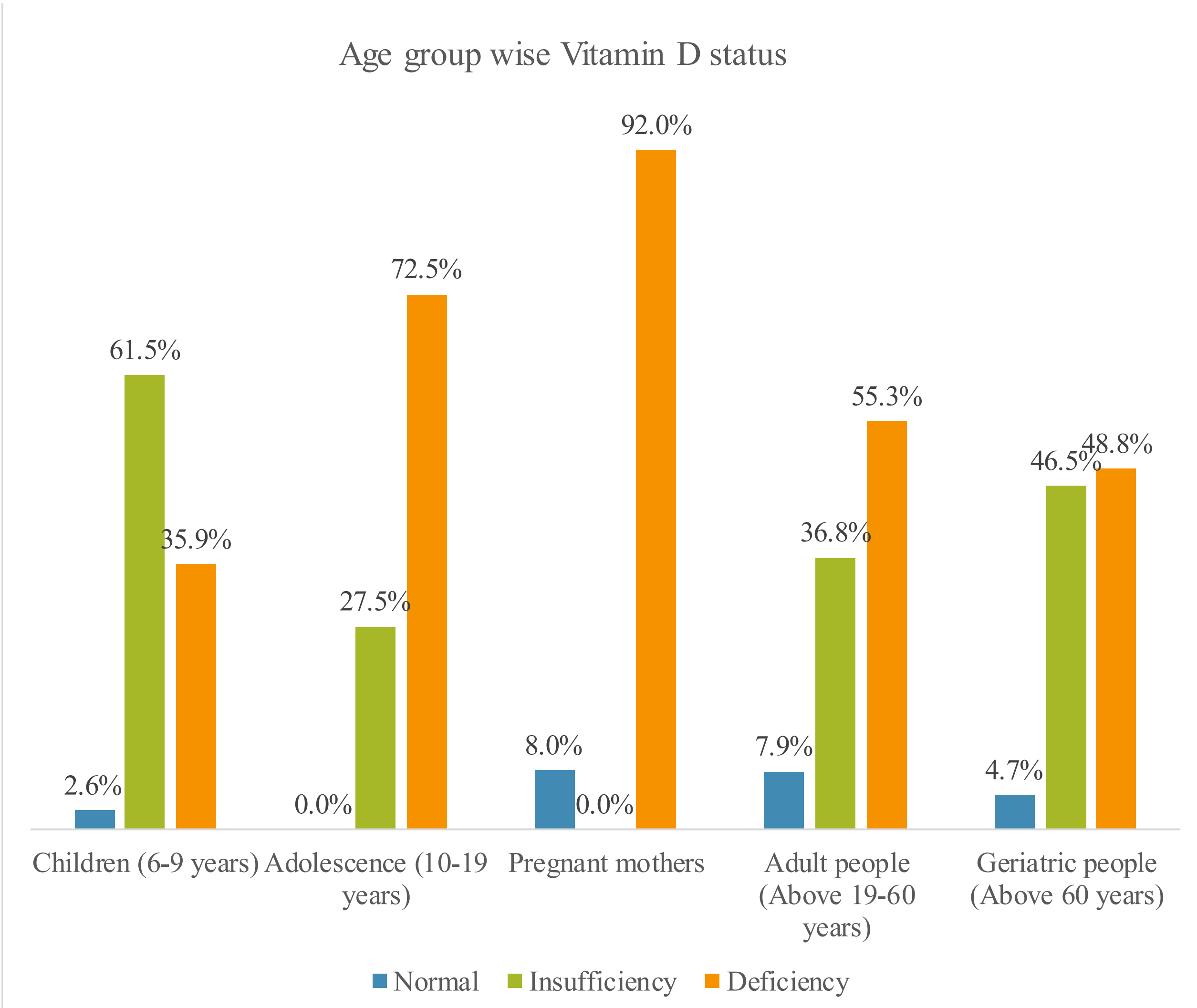
Figure 8: Residence wise Overall Vitamin-D status in Dhaka Division (n=663)



Urban residents in Dhaka had a deficiency rate of 61.9%, while rural residents showed a slightly higher rate of 71.4%. Vitamin D sufficiency was more common among urban dwellers (8.8%) compared to rural ones (6.3%).

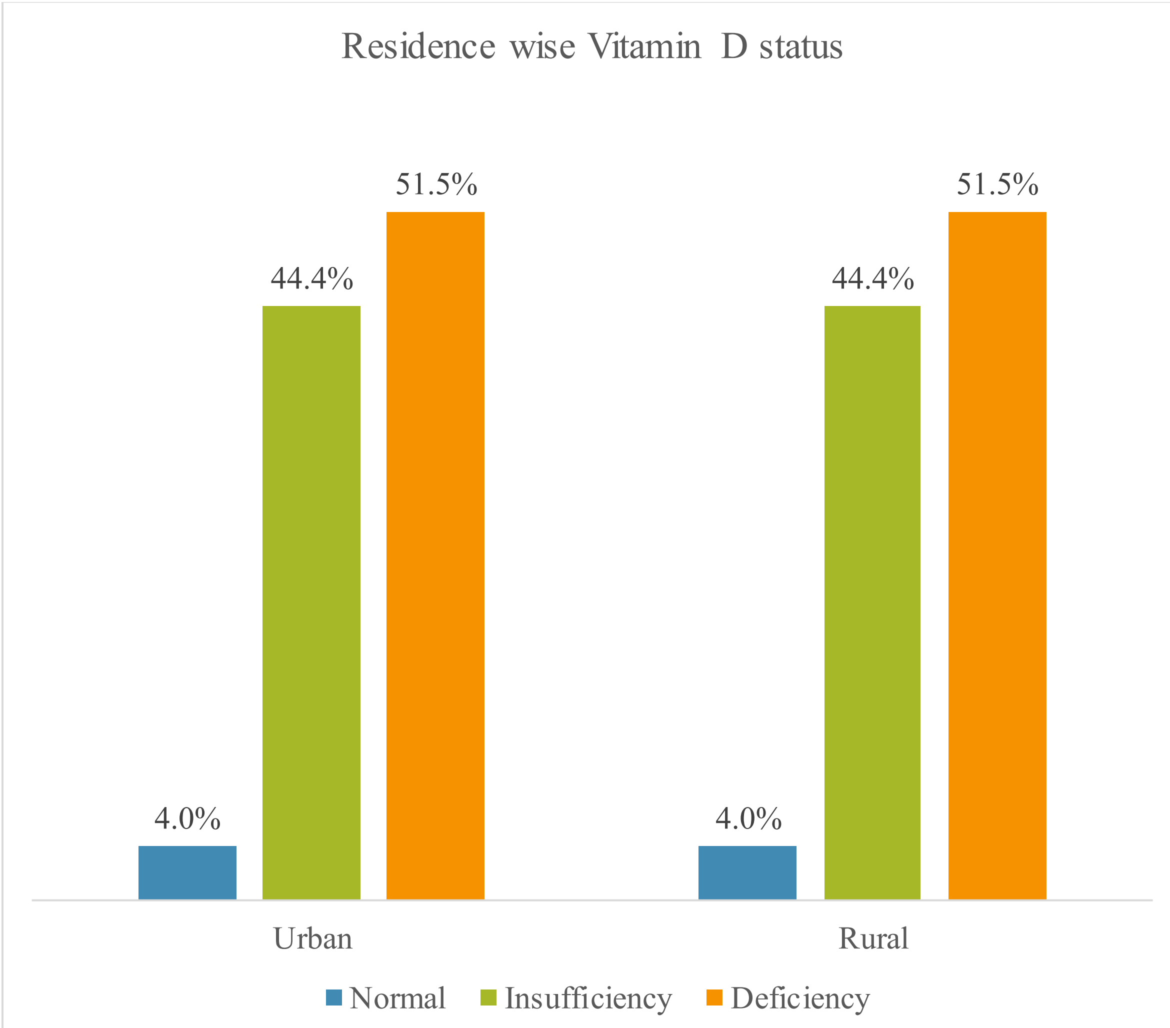
Mymensingh Division

Figure 9: Age group wise Overall Vitamin-D status in Mymensingh Division (n=185)



Vitamin D deficiency was extremely high across all age groups in Mymensingh. Adolescents had the highest rate at 92.0%, followed by children (61.5%) and adults (55.3%). Normal Vitamin D levels were negligible or absent in some groups, particularly among adolescents and pregnant women.

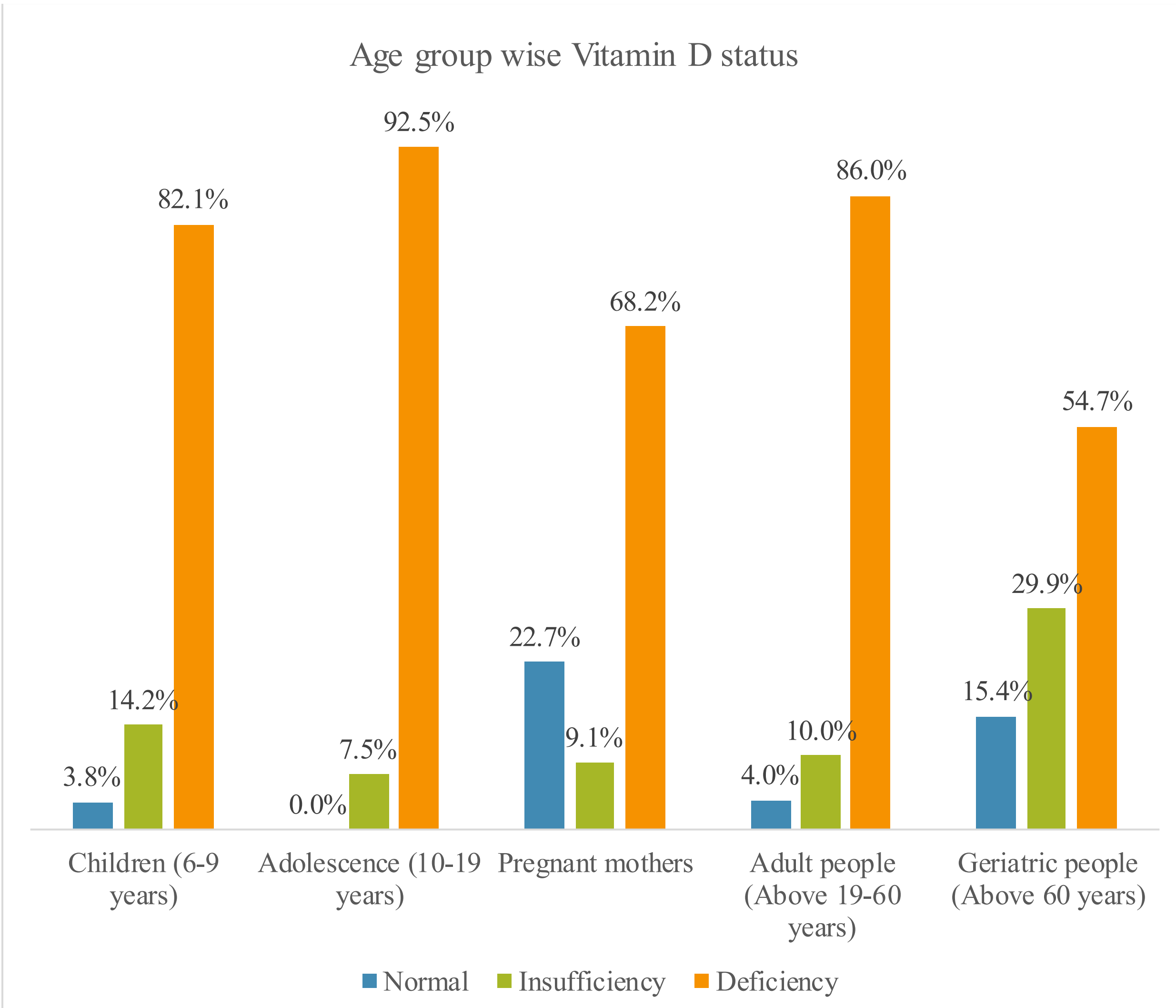
Figure 10: Residence wise Overall Vitamin-D status in Mymensingh Division (n=185)



Both urban and rural residents had equal Vitamin D deficiency at 51.5**%**. Normal and insufficient levels were equally distributed (4% and 44.4%) across both urban and rural populations.

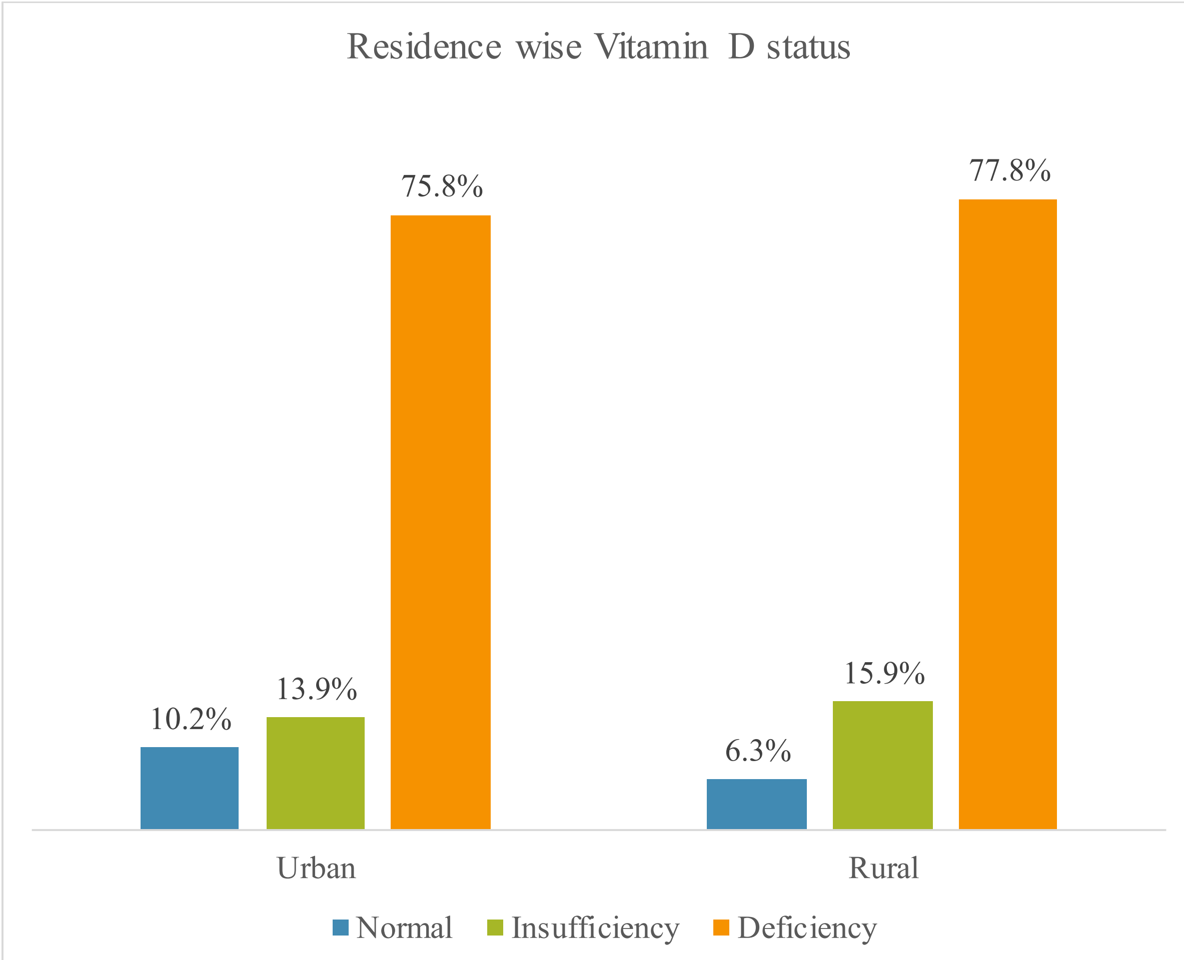
Chattogram Division

Figure 11: Age group wise Overall Vitamin-D status in Chattogram division (n=496)



Deficiency was highly prevalent among adolescents (92.5%) and pregnant mothers (82.1%). Children and older adults also showed concerning rates (68.2% and 54.7%, respectively). Sufficiency was generally low, especially among adolescents (0%).

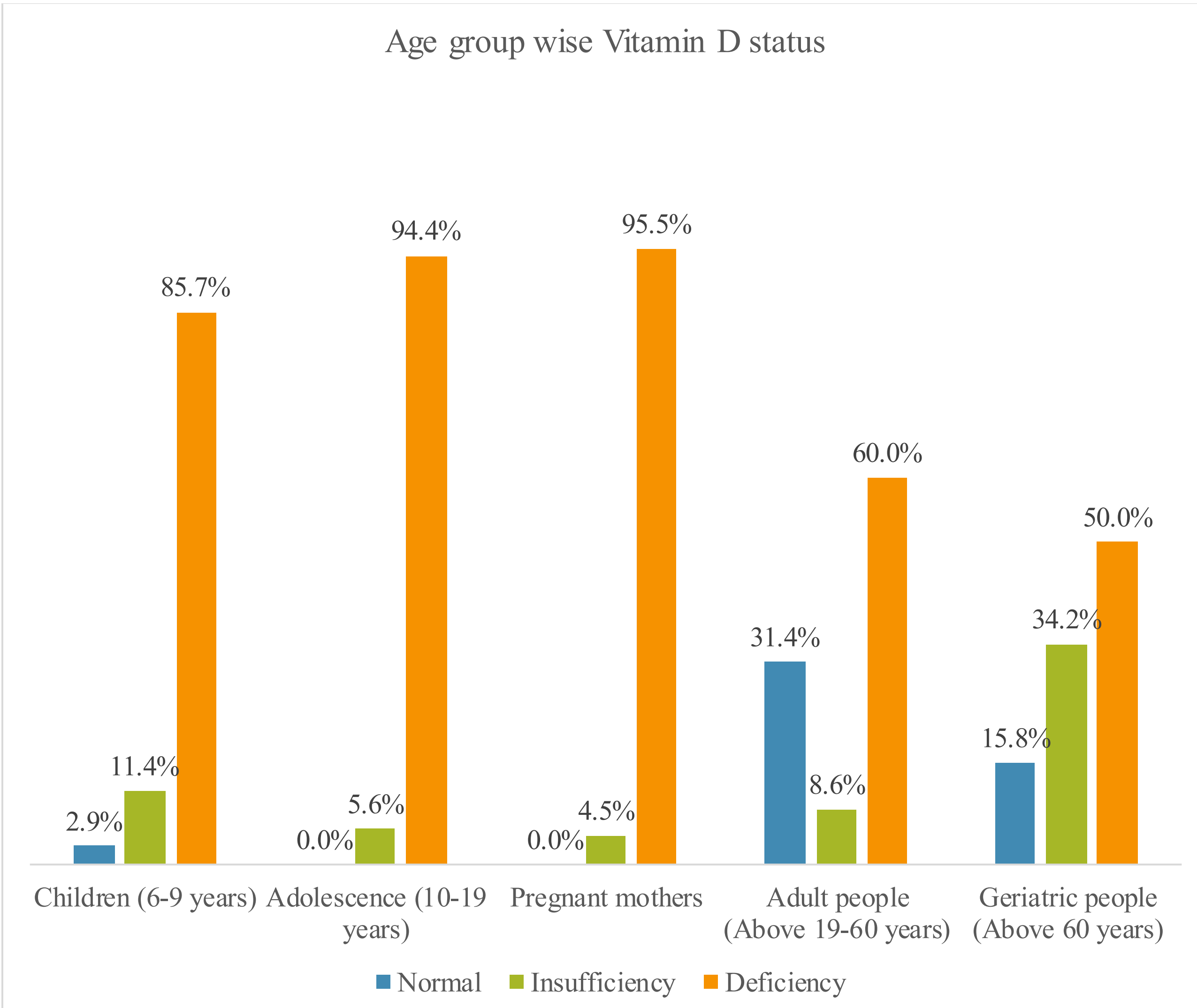
Figure 12: Residence wise Overall Vitamin-D status in Chattogram division (n=496)



Urban residents had a deficiency rate of **75.8%**, while rural residents showed a similar rate at **77.8%**. Sufficiency remained very low, with only 10.2% in urban and 6.3% in rural residents.

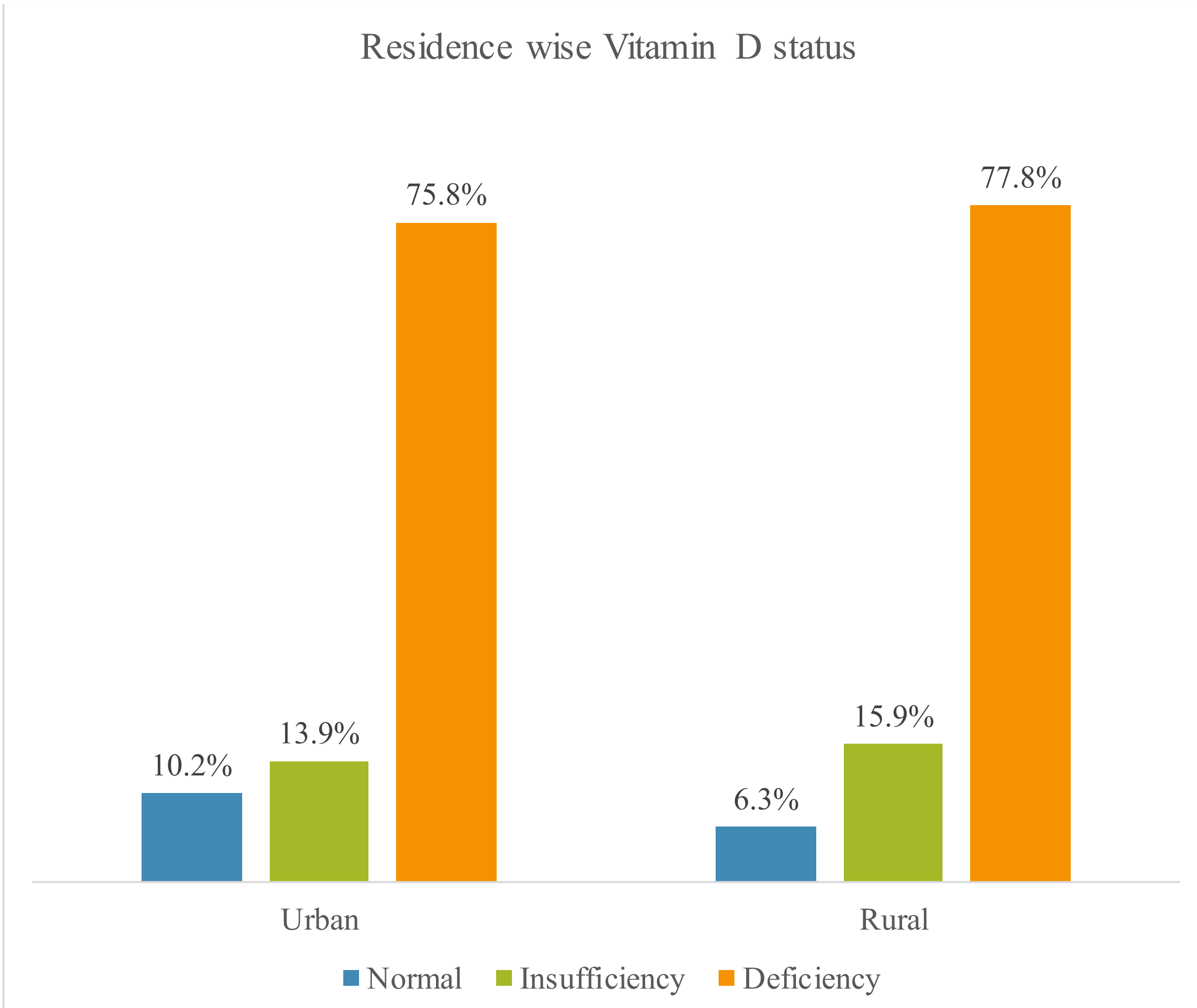
Sylhet Division

Figure 13: Age group wise Overall Vitamin-D status in Sylhet division (n=166)



Adolescents and pregnant mothers in Sylhet had deficiency rates of **94.4%** and **95.5%**, respectively—the highest in any division. Children also had a high deficiency at **85.7%**, with normal levels nearly absent in all groups.

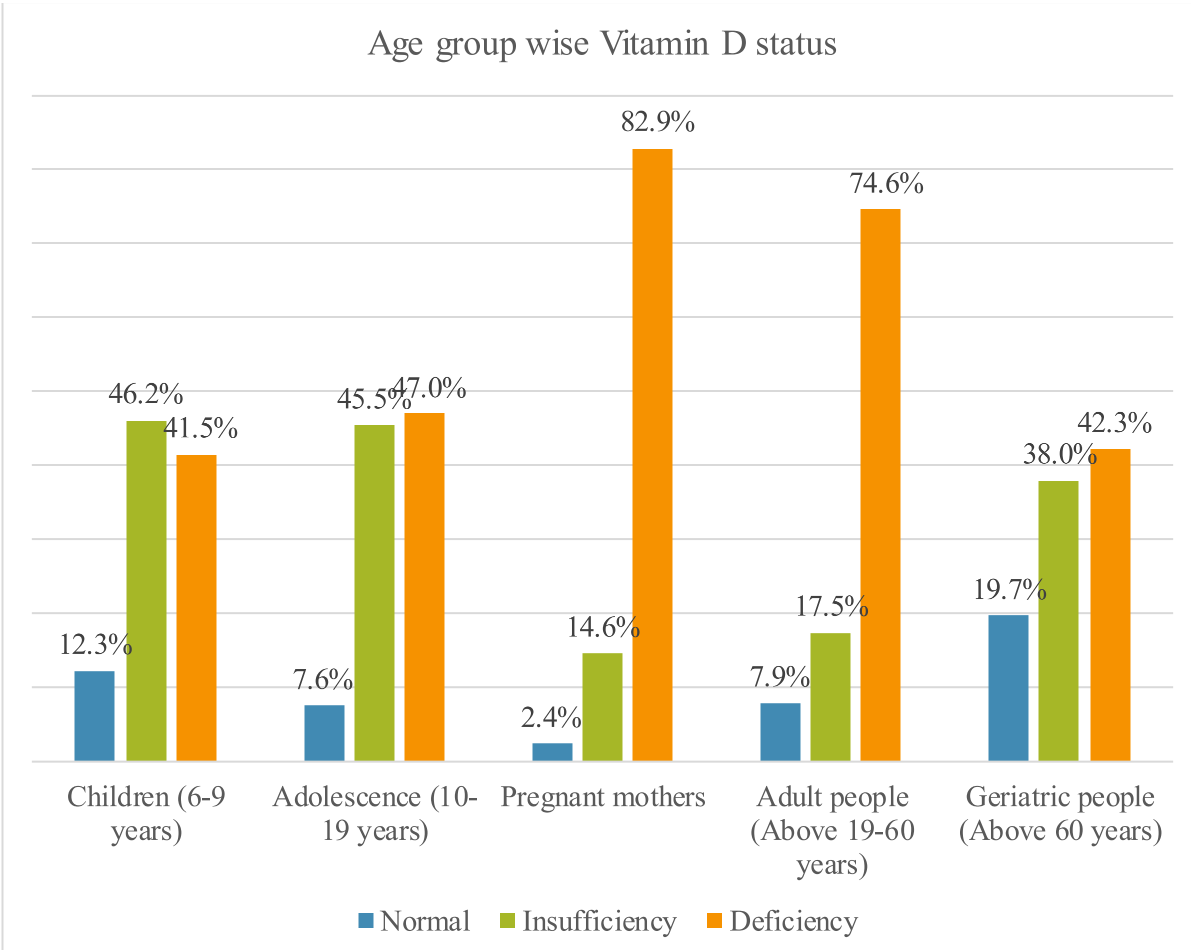
Figure 14: Residence wise Overall Vitamin-D status in Sylhet division (n=166)



Deficiency was widespread in both urban (75.8%) and rural (77.8%) areas. Normal levels were found in just 10.2% of urban and 6.3% of rural participants.

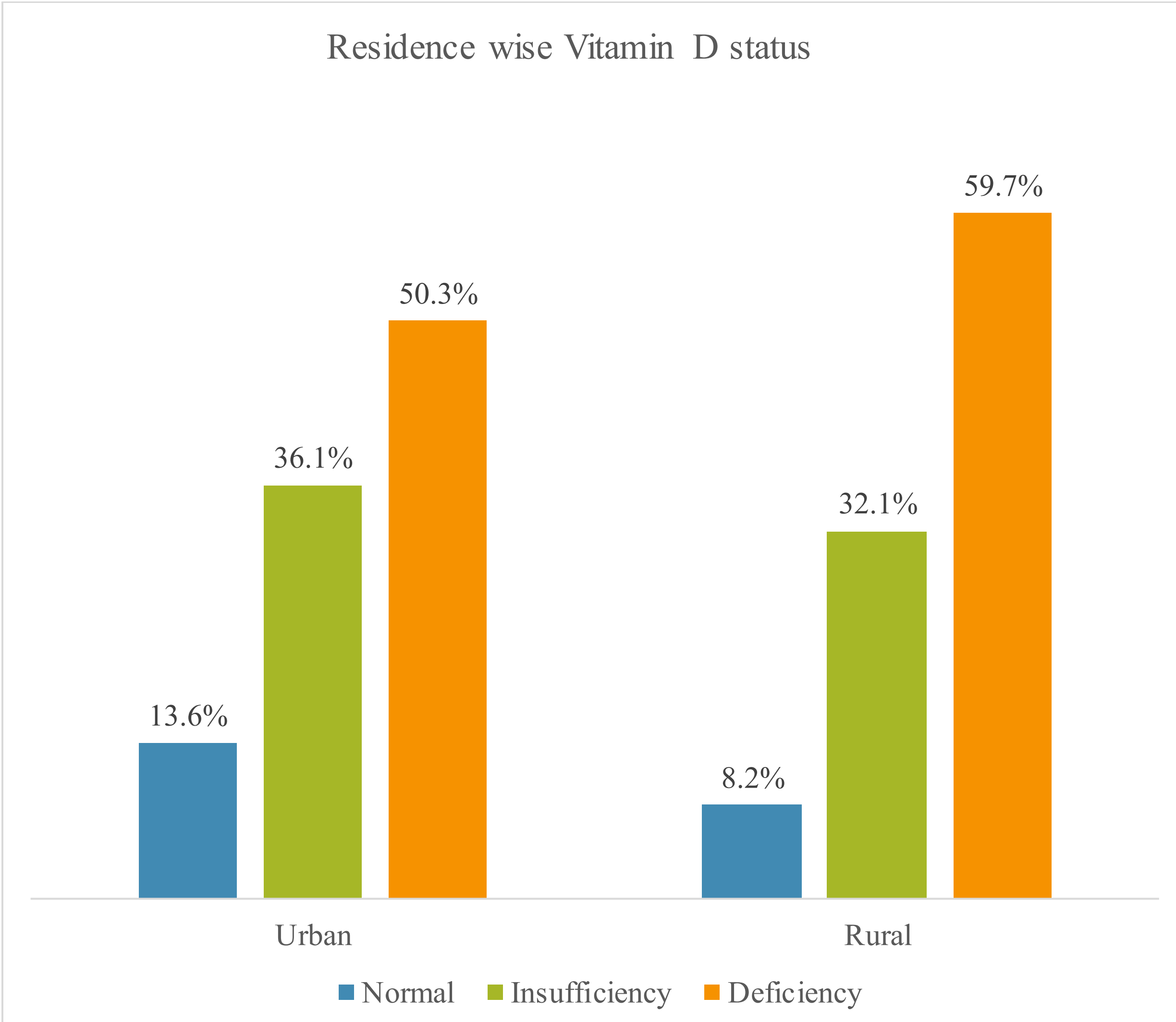
Rajshahi Division

Figure 15: Age group wise Overall Vitamin-D status in Rajshahi division (n=306)



Vitamin D status in Rajshahi was comparatively better. Geriatric individuals had the lowest deficiency (42.3%) and the highest normal level (19.7%). Adolescents had the highest deficiency (82.9%), though pregnant mothers and adults showed moderate deficiency.

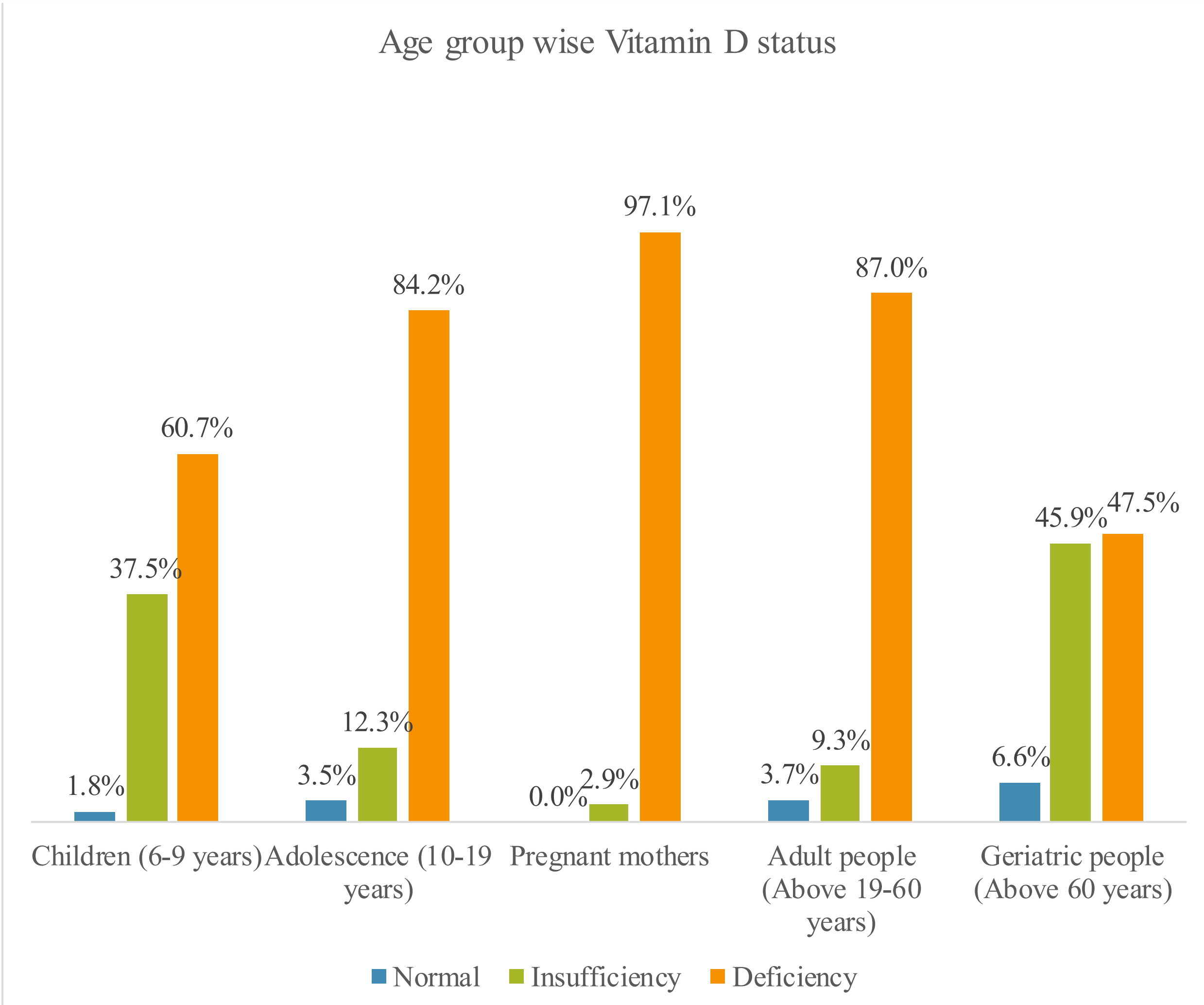
Figure 16: Residence wise Overall Vitamin-D status in Rajshahi division (n=306)



Rural residents fared slightly better, with 59.7% deficiency versus 50.3% among urban residents. Normal levels were higher in urban areas (13.6%) compared to rural (8.2%).

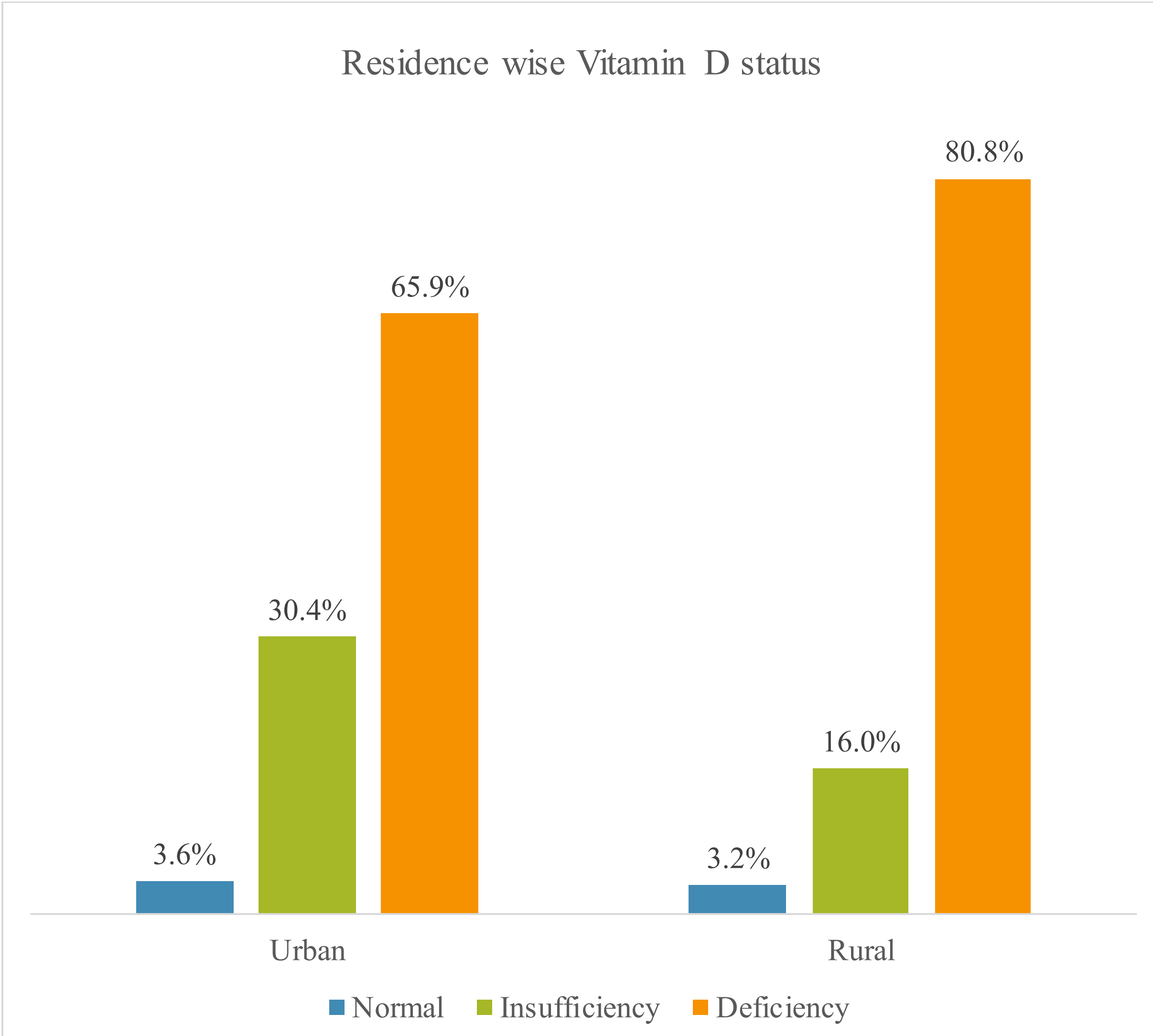
Rangpur Division

Figure 17: Age group wise Overall Vitamin-D status in Rangpur division (n=263)



Pregnant mothers and adolescents had extremely high deficiency rates at **97.1% and 84.2%**, respectively. Normal levels were minimal across all groups, particularly in pregnant women (0%).

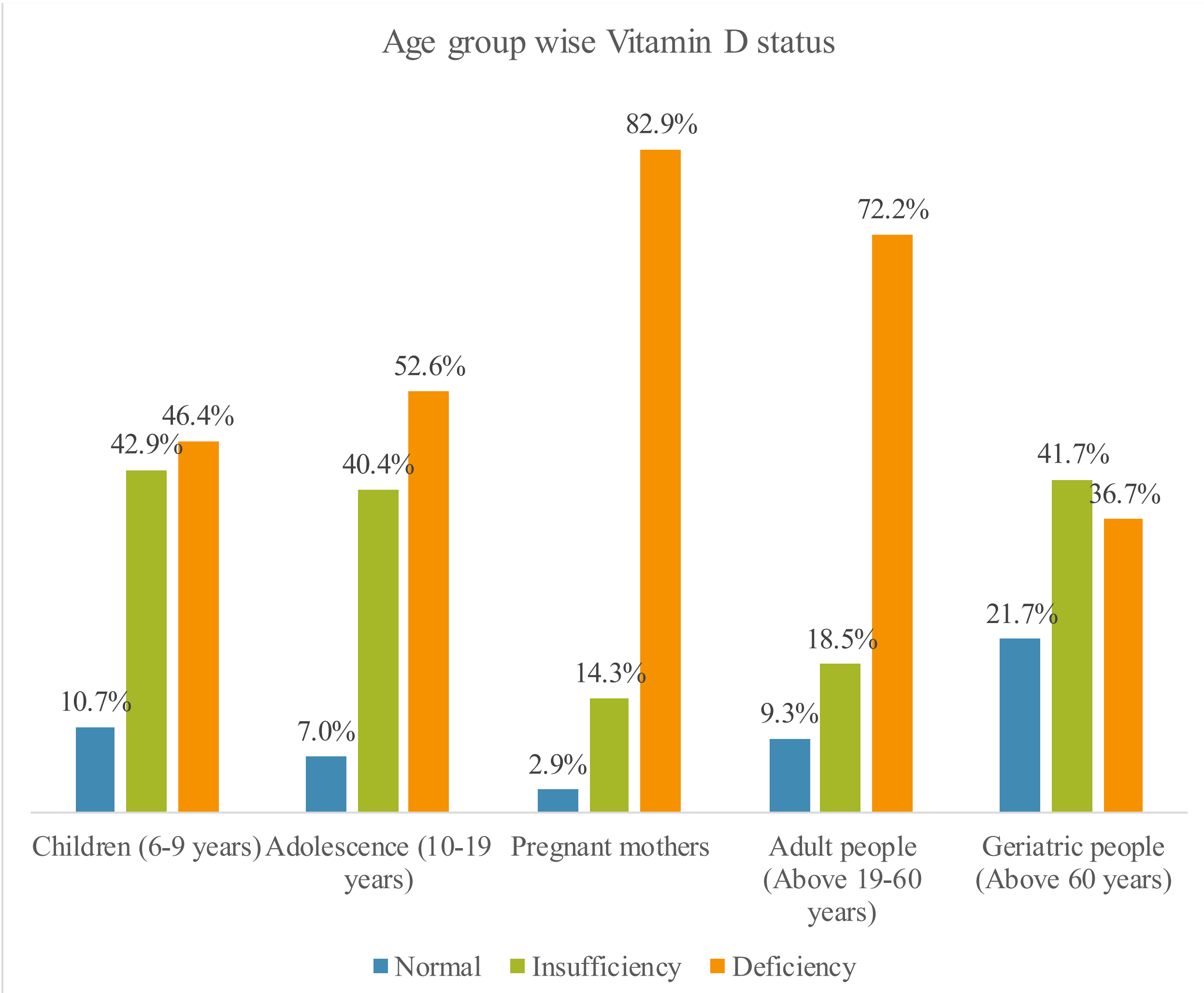
Figure 18: Residence wise Overall Vitamin-D status in Rangpur division (n=263)



Rural residents had a significantly higher deficiency rate (**80.8%**) compared to urban (65.9%). Normal Vitamin D levels were very low—just 3.2% in rural and 3.6% in urban areas.

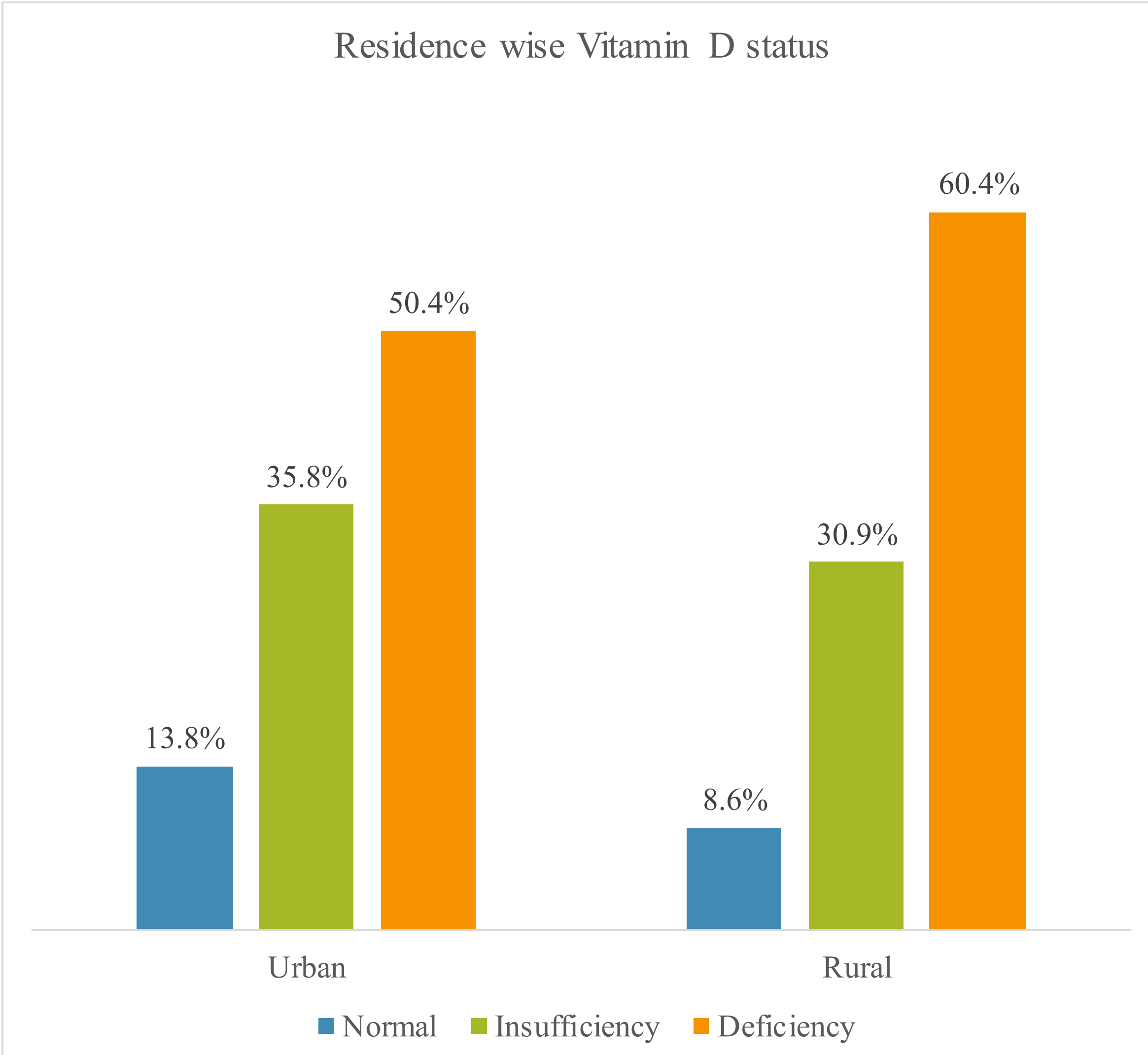
Khulna Division

Figure 19: Age group wise Overall Vitamin-D status in Khulna division (n=262)



Geriatric individuals had the lowest deficiency in this division at 36.7%, while adolescents showed the highest at 82.9%. Children and adults had moderate levels of deficiency. Sufficiency peaked at 21.7% among the elderly.

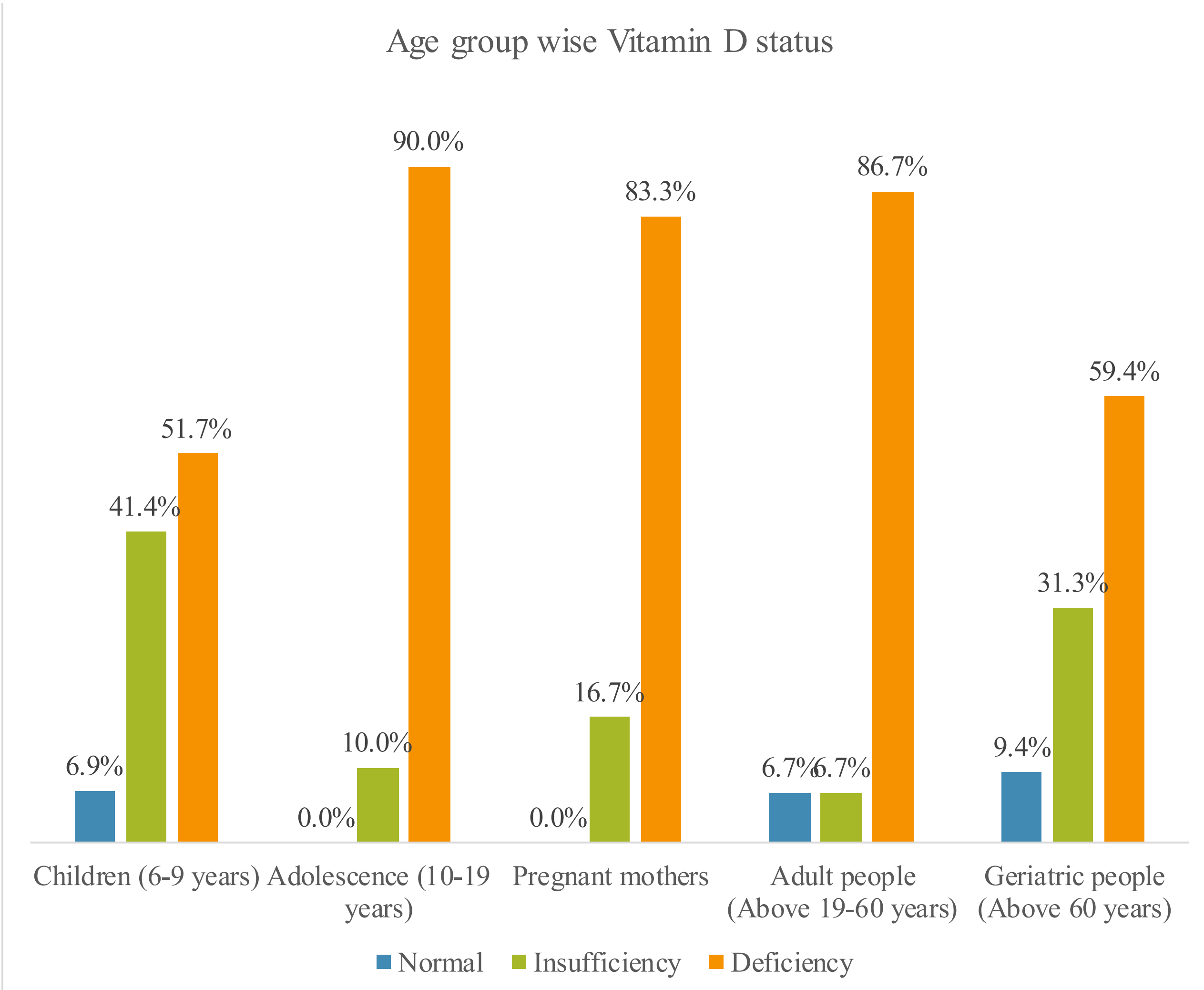
Figure 20: Residence wise Overall Vitamin-D status in Khulna division (n=262)



Deficiency was higher among rural participants (60.4%) than urban ones (50.4%). Normal levels were higher in urban areas (13.8%) compared to rural (8.6%).

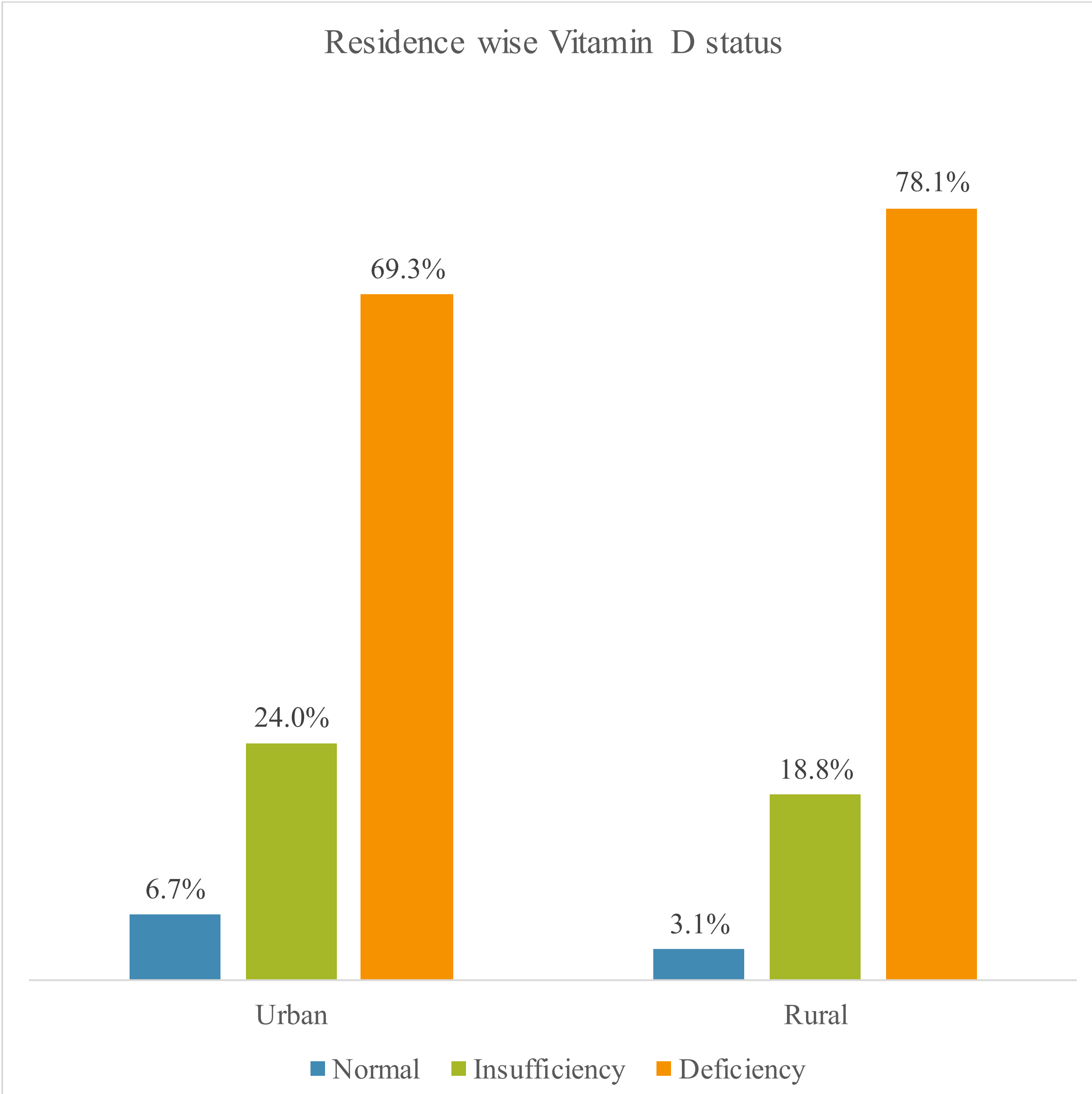
Barisal Division

Figure 21: Age group wise Overall Vitamin-D status in Barisal division (n=139)



Adolescents and pregnant mothers in Barisal had extremely high deficiency rates (90.0% and 83.3%, respectively). Geriatric individuals showed lower deficiency (59.4%), and normal levels were negligible or zero across most groups.

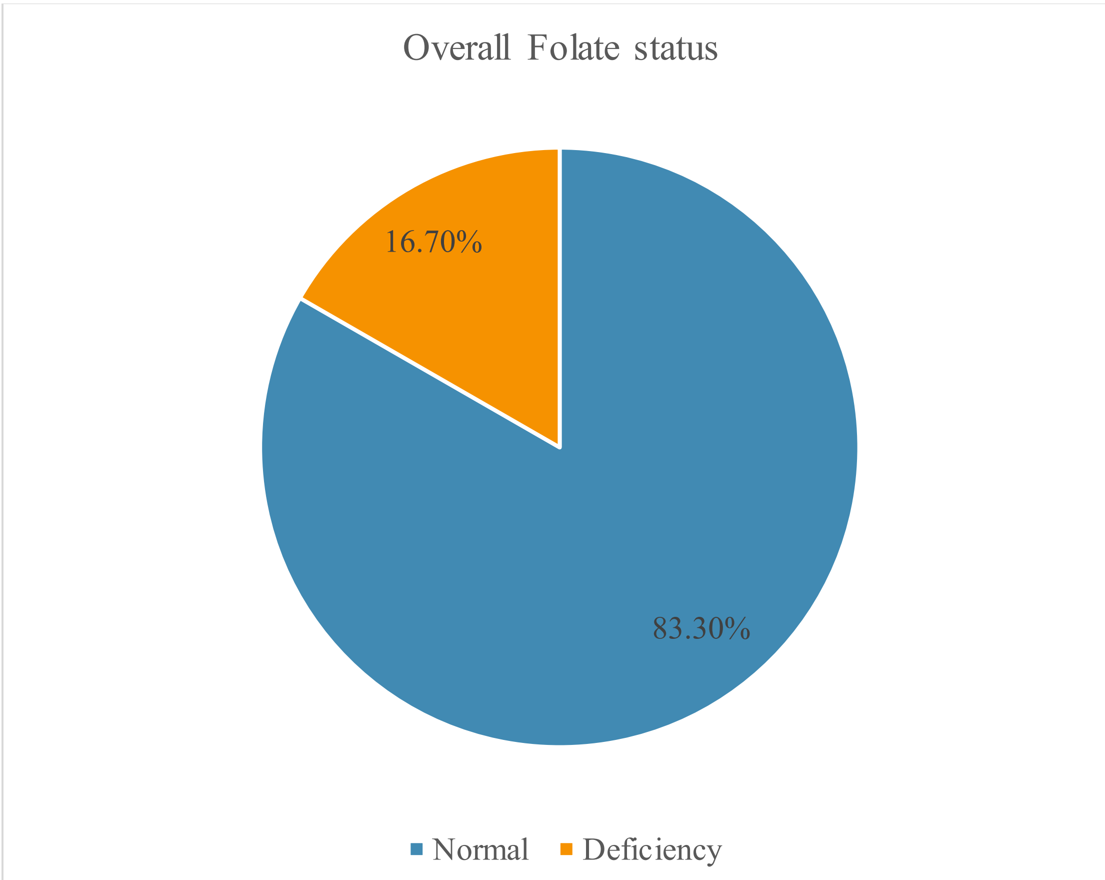
Figure 22: Residence wise Overall Vitamin-D status in Barisal division (n=139)



Rural residents had a slightly higher deficiency rate (78.1%) than urban residents (69.3%). Sufficiency was low in both groups, with only 6.7% of urban and 3.1% of rural participants being sufficient.

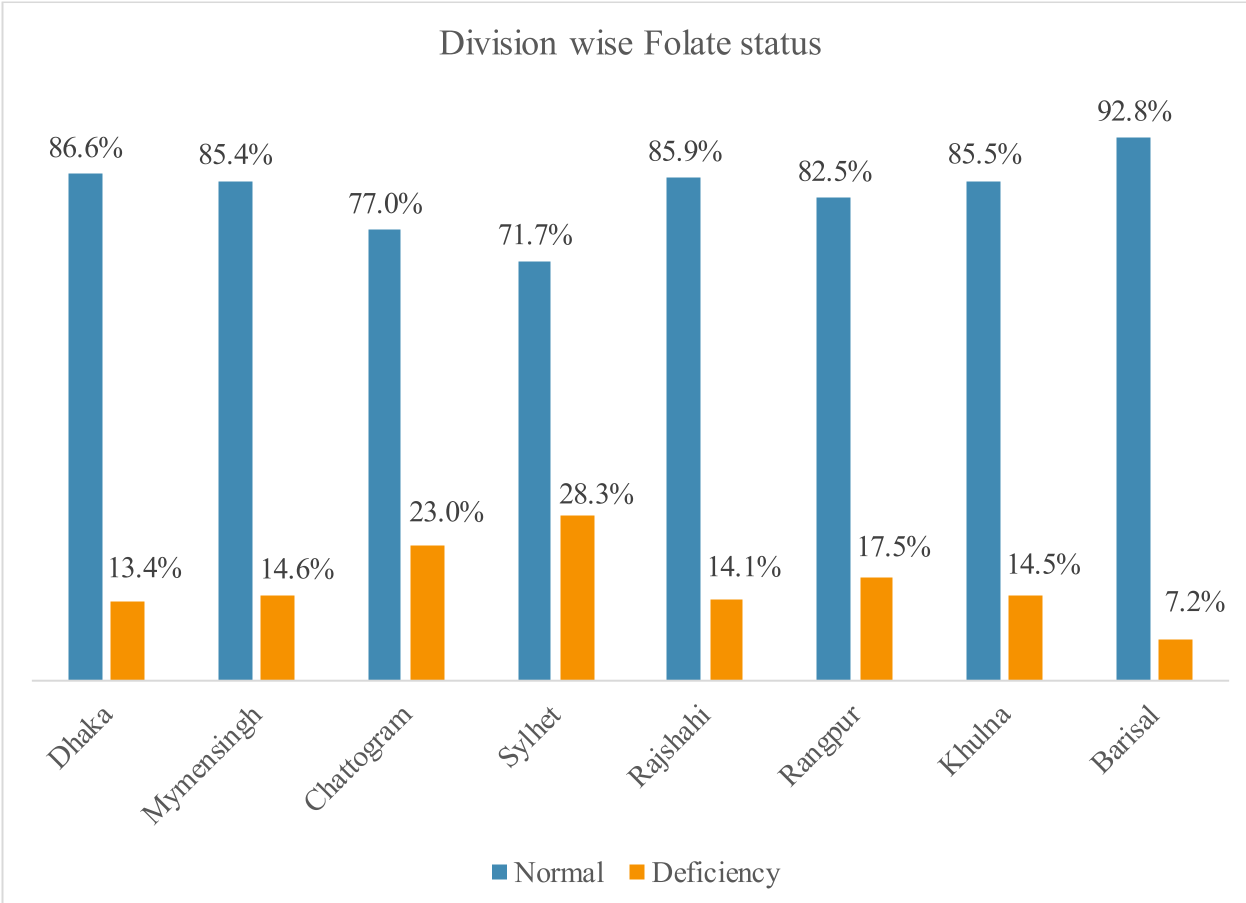
Folate status

Figure 23: Overall Folate status (n=2480)



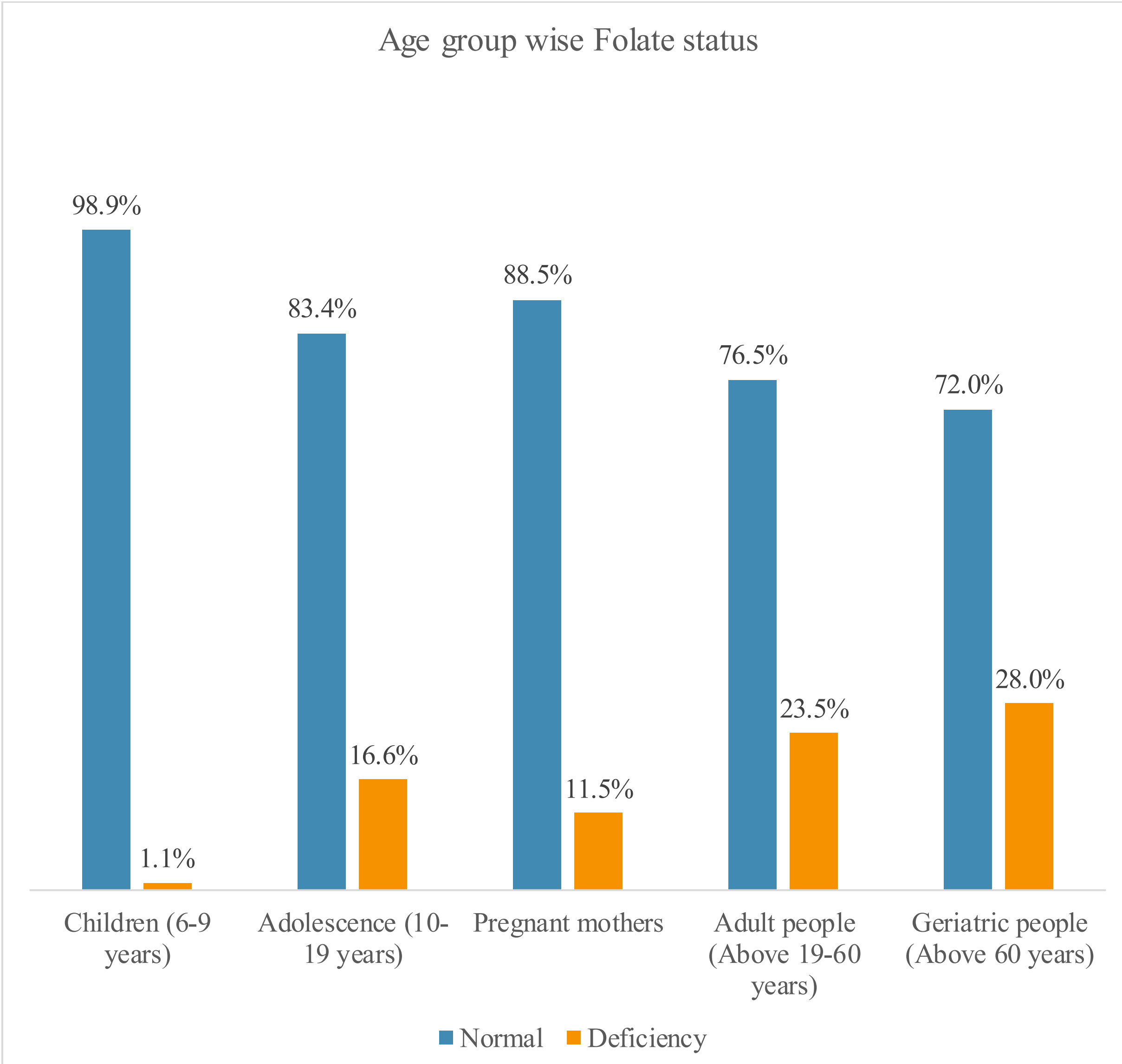
This figure shows the overall folate status of the study population. A large majority, **83.3%**, had normal folate levels, while **16.7%** were folate deficient—indicating that folate deficiency is less widespread than Vitamin D deficiency in the general population.

Figure 24: Division wise Overall Folate status (n=2480)



Folate deficiency varied across divisions. The highest deficiency was observed in Sylhet (28.3%) and Chattogram (23.0%), while Barisal (7.2%) and Rajshahi (14.1%) showed relatively lower deficiency. Most divisions had over 80% with normal folate levels.

Figure 25: Age group wise Overall Folate status (n=2480)

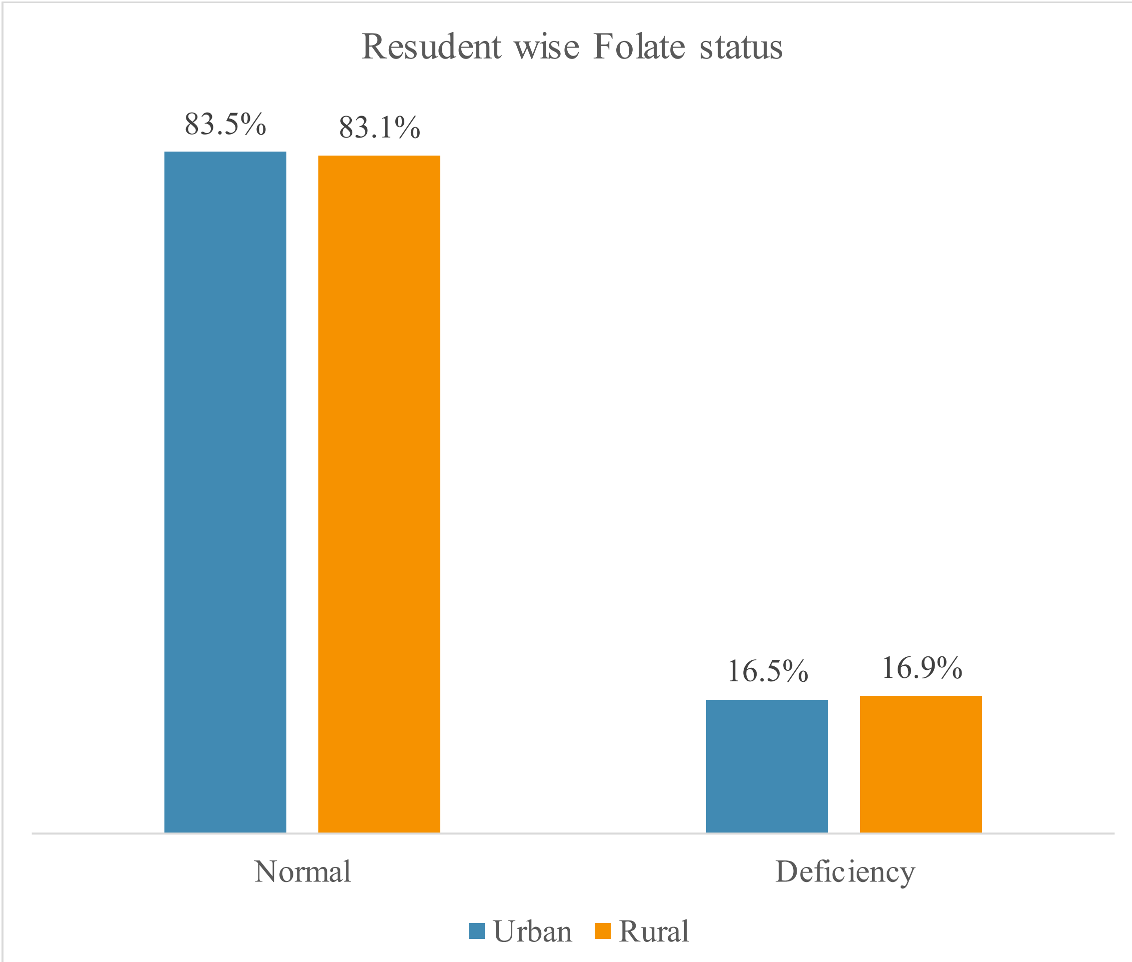


Children aged 6–9 years had the best folate status, with 98.9% having normal levels. Geriatric people (60+ years) had the highest deficiency rate at 28.0%, followed by adults (23.5%) and adolescents (16.6%).

Figure 26: Sex wise Overall Folate status (n=2480)

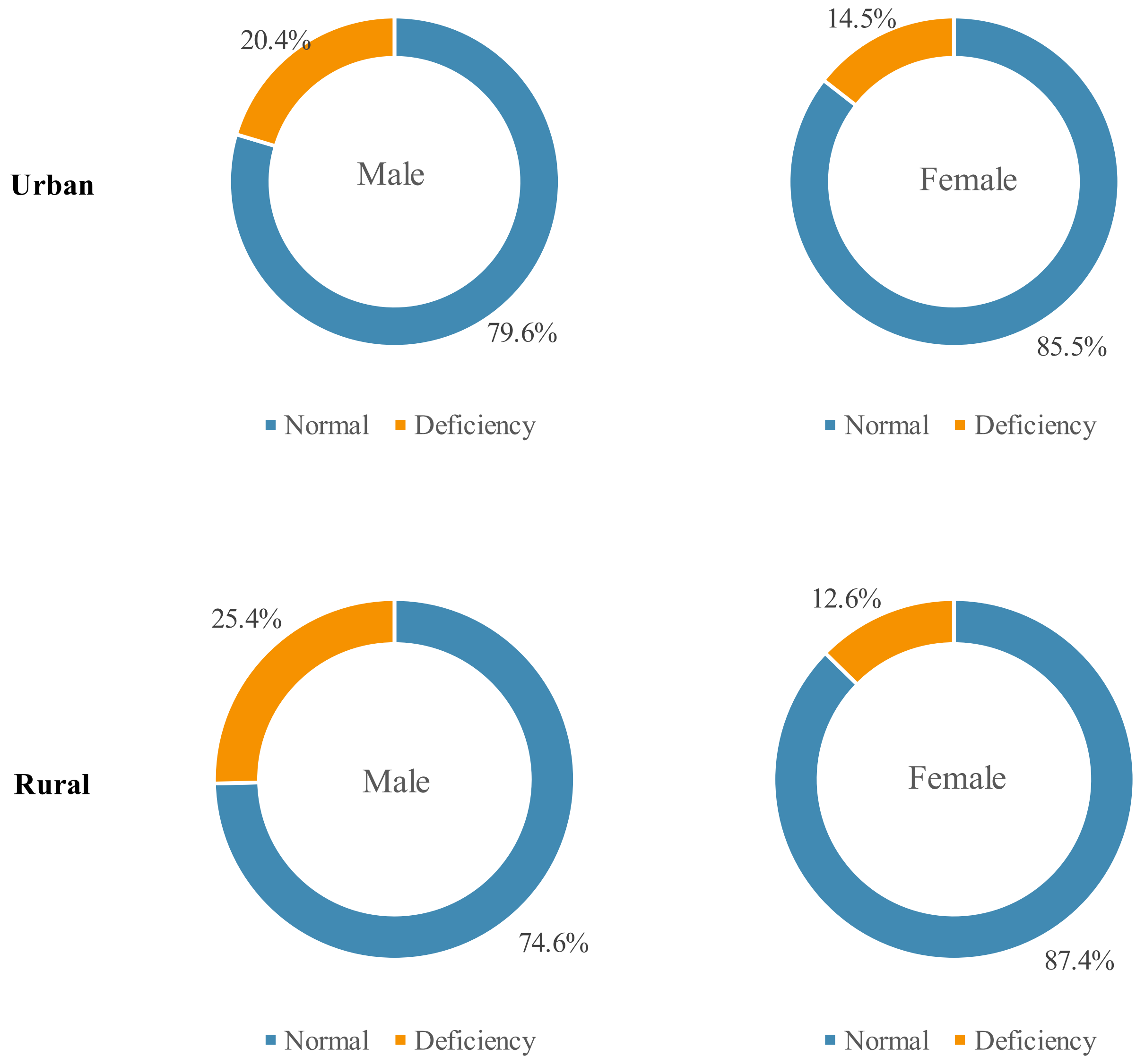
Female participants had a higher rate of normal folate levels (86%) compared to males (77%). Correspondingly, folate deficiency was more prevalent in males (23%) than in females (14%).

Figure 27: Residence wise Overall Folates tatus (n=2480)



There was minimal difference in folate status between urban and rural populations. Urban residents had 83.5% normal and 16.5% deficiency, while rural residents had 83.1% normal and 16.9% deficiency.

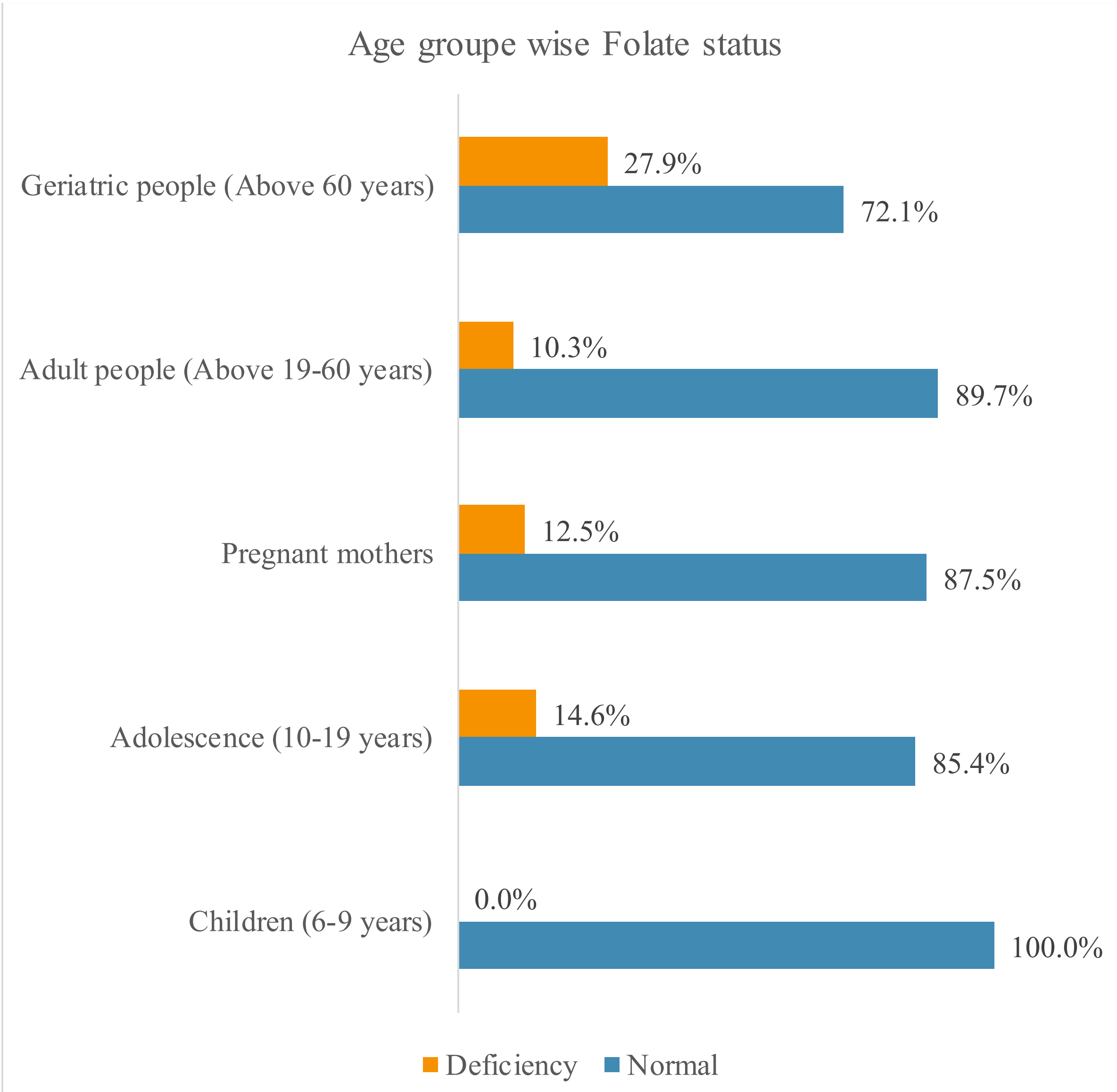
Figure 28: Residence wise Overall Folates tatus according to sex (n=2480)



Among urban males, 20.4% were folate deficient, compared to 14.5% of urban females. In rural areas, male deficiency rose to 25.4%, while rural females had the lowest deficiency at 12.6%, showing a consistent gender disparity favoring females.

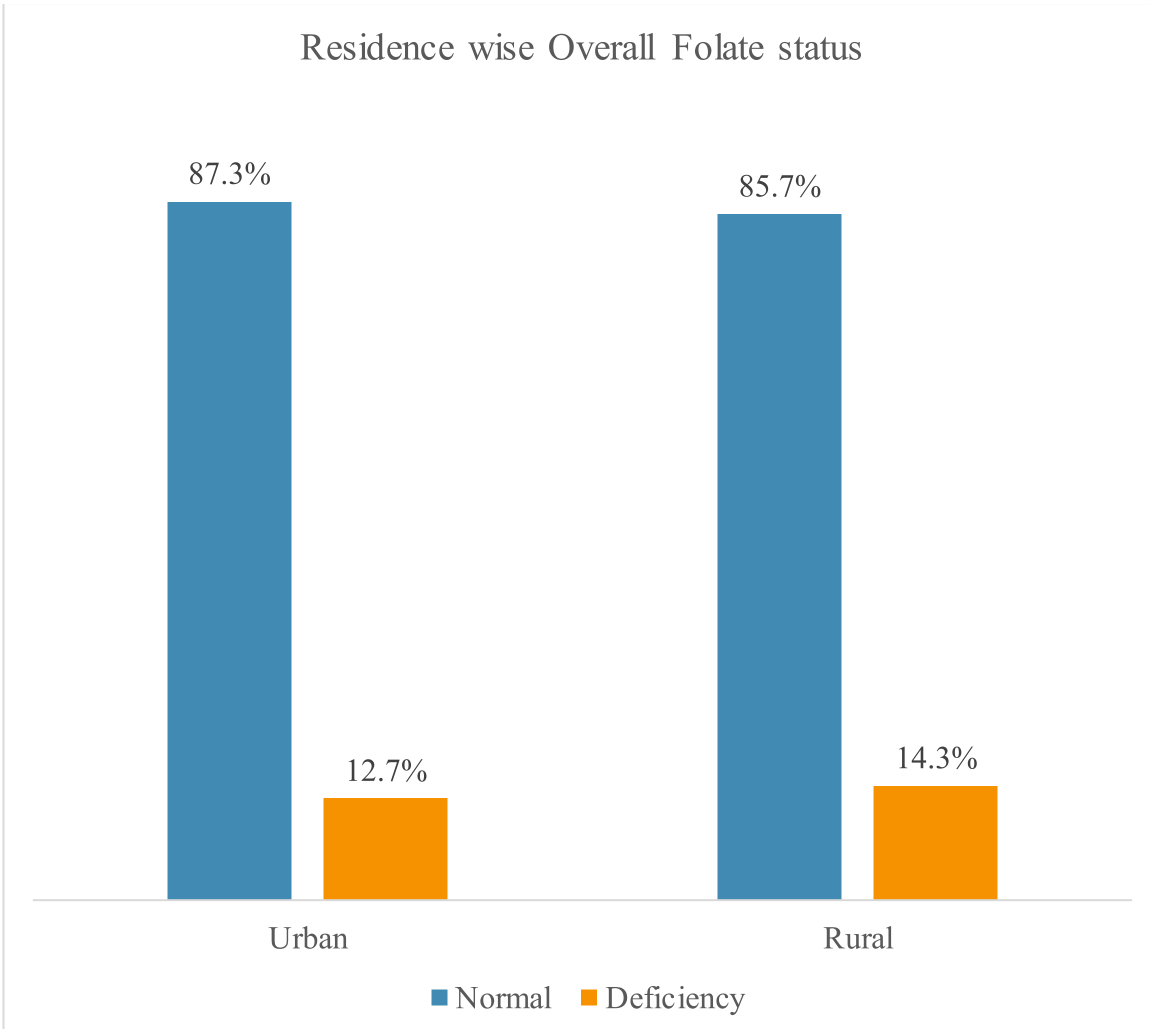
Dhaka Division

Figure 29: Age group wise Folate status in Dhaka division (n=663)



Children had 100% normal folate levels in Dhaka, while geriatric individuals had the highest deficiency (27.9%). Adolescents and adults showed relatively better status, with 85.4% and 89.7% normal levels, respectively.

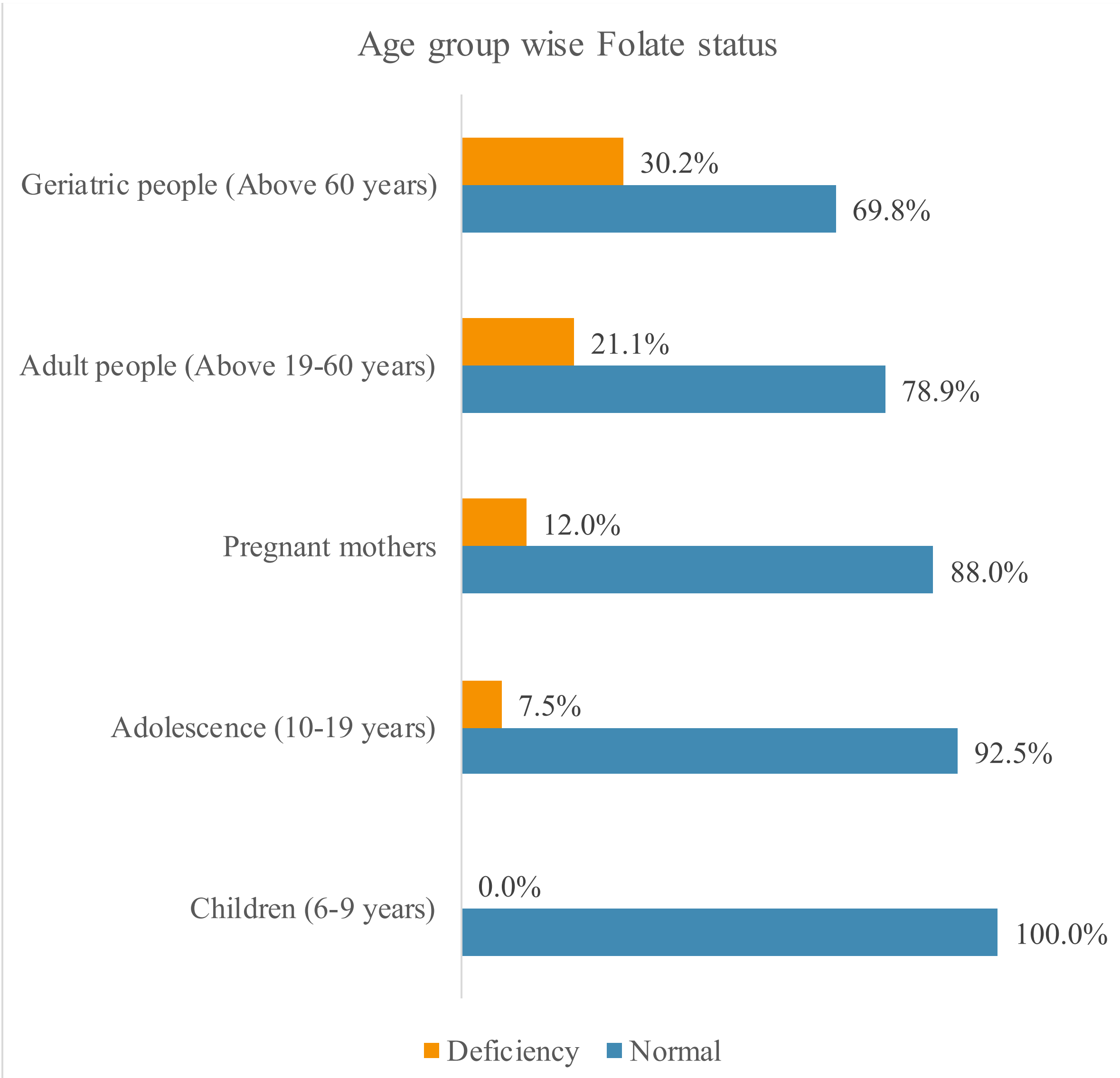
Figure 30: Residence wise Overall Folate status in Dhaka division (n=663)



Folate deficiency was slightly higher in rural residents (14.3%) than in urban ones (12.7%), with most residents in both groups showing normal levels above 85%.

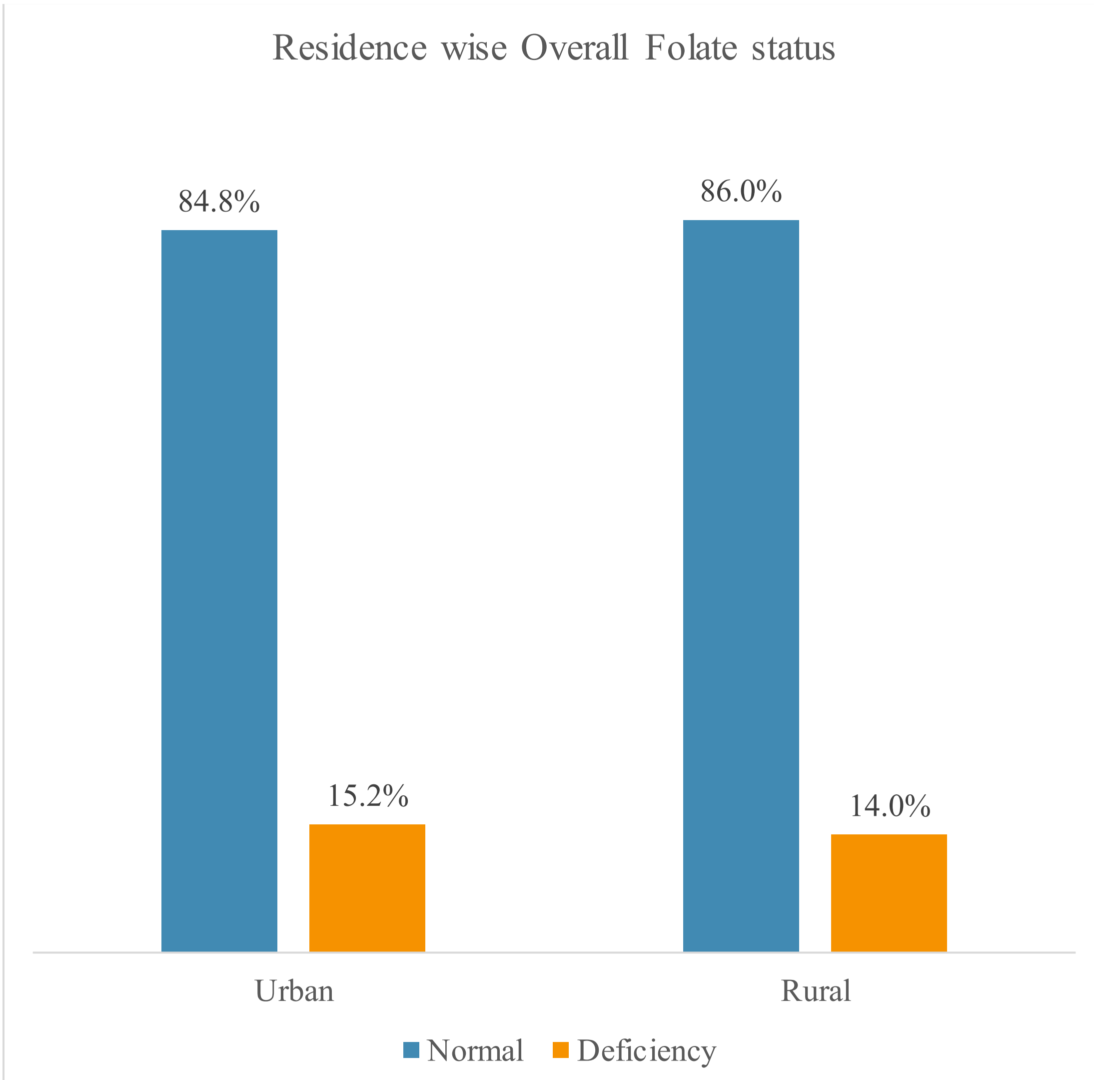
<u>Mymensingh Division</u>

Figure 31: Age group wise Overall Folate status in Mymensingh division (n=185)



Children again had 100% normal folate status. Geriatric individuals showed the highest deficiency at 30.2%, followed by adults (21.1%) and pregnant mothers (12.0%).

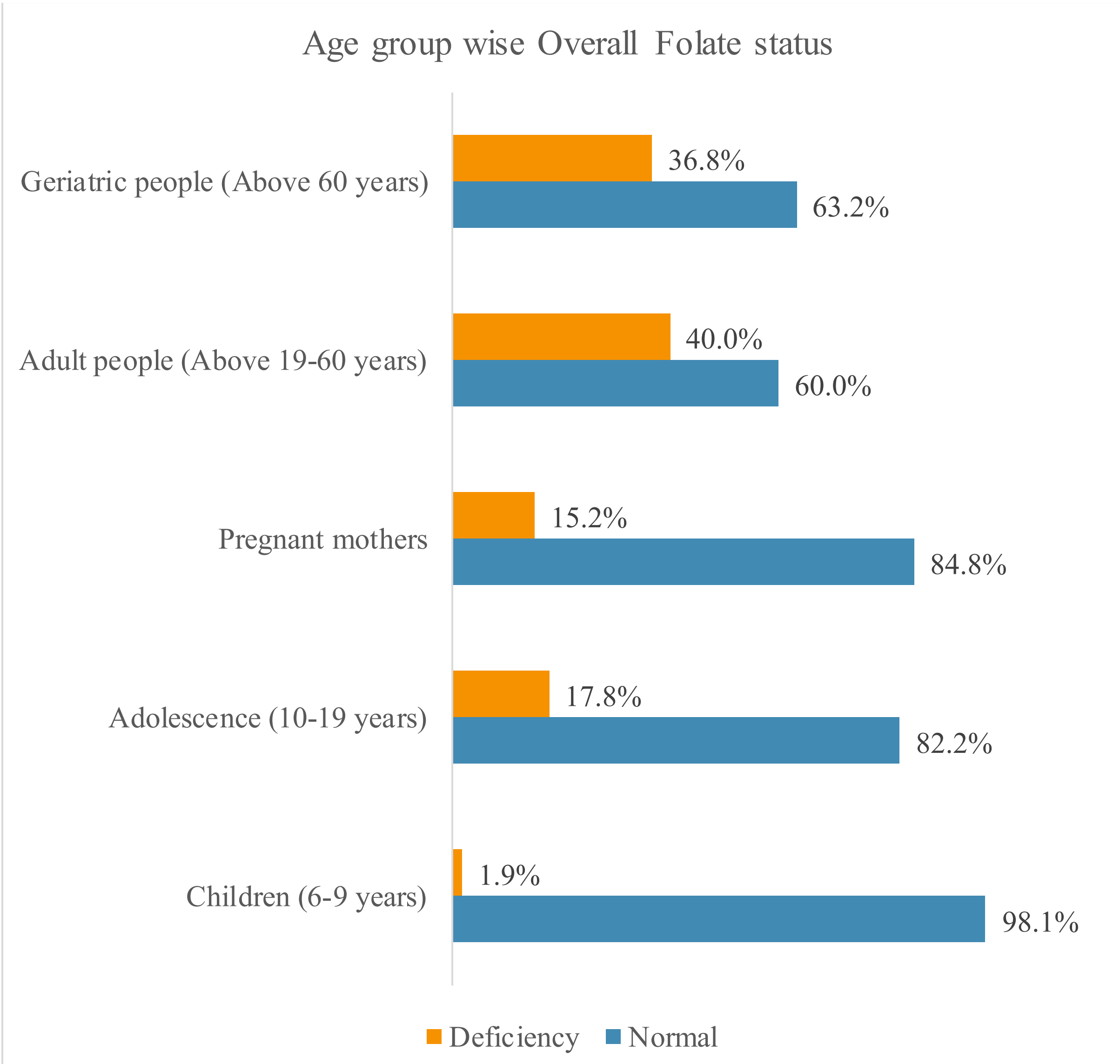
Figure 32: Residence wise Overall Folate status in Mymensingh division (n=185)



Urban and rural residents had very similar folate status, with normal levels at 84.8% and 86.0%, respectively, and deficiency around 15% in both groups.

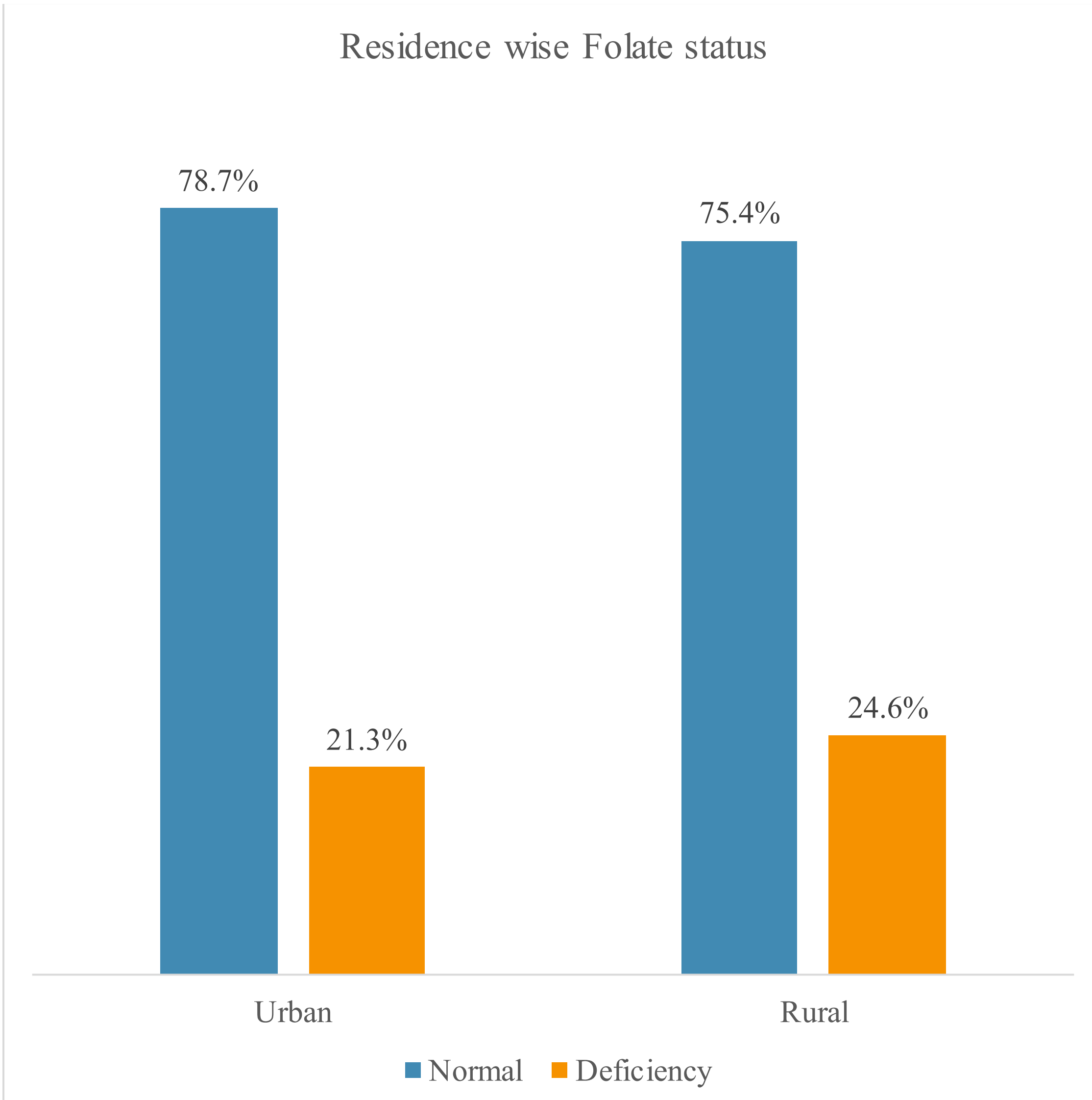
Chattogram Division

Figure 33: Age group wise Overall Folate status in Chattogram division (n=496)



Children had 98.1% normal folate levels. Deficiency was more pronounced in adults (40.0%) and geriatric people (36.8%), indicating an age-related decline in folate sufficiency.

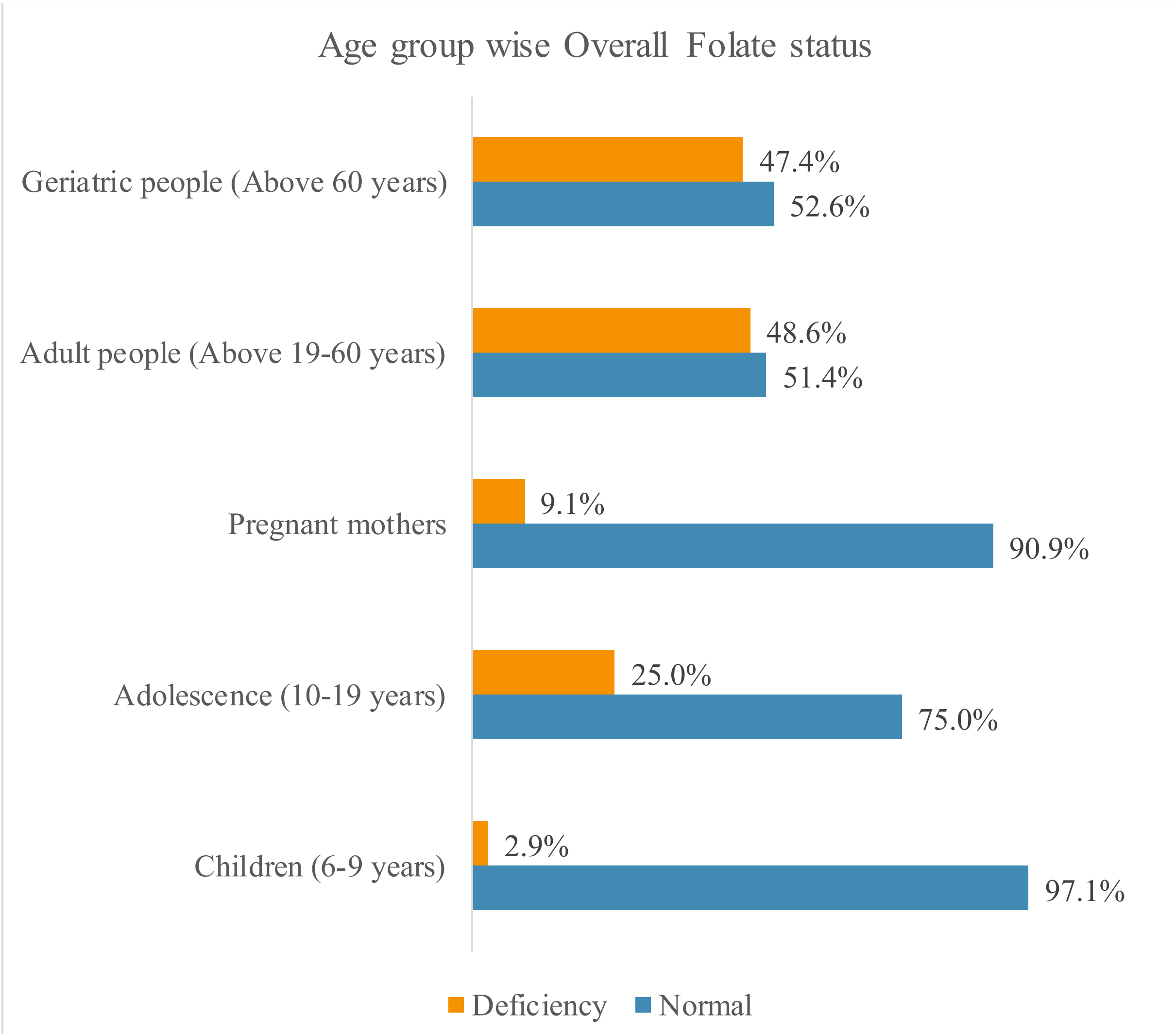
Figure 34: Residence wise Folate status in Chattogram division (n=496)



Folate deficiency was slightly higher in rural areas (24.6%) than in urban areas (21.3%), although both showed suboptimal levels compared to other divisions.

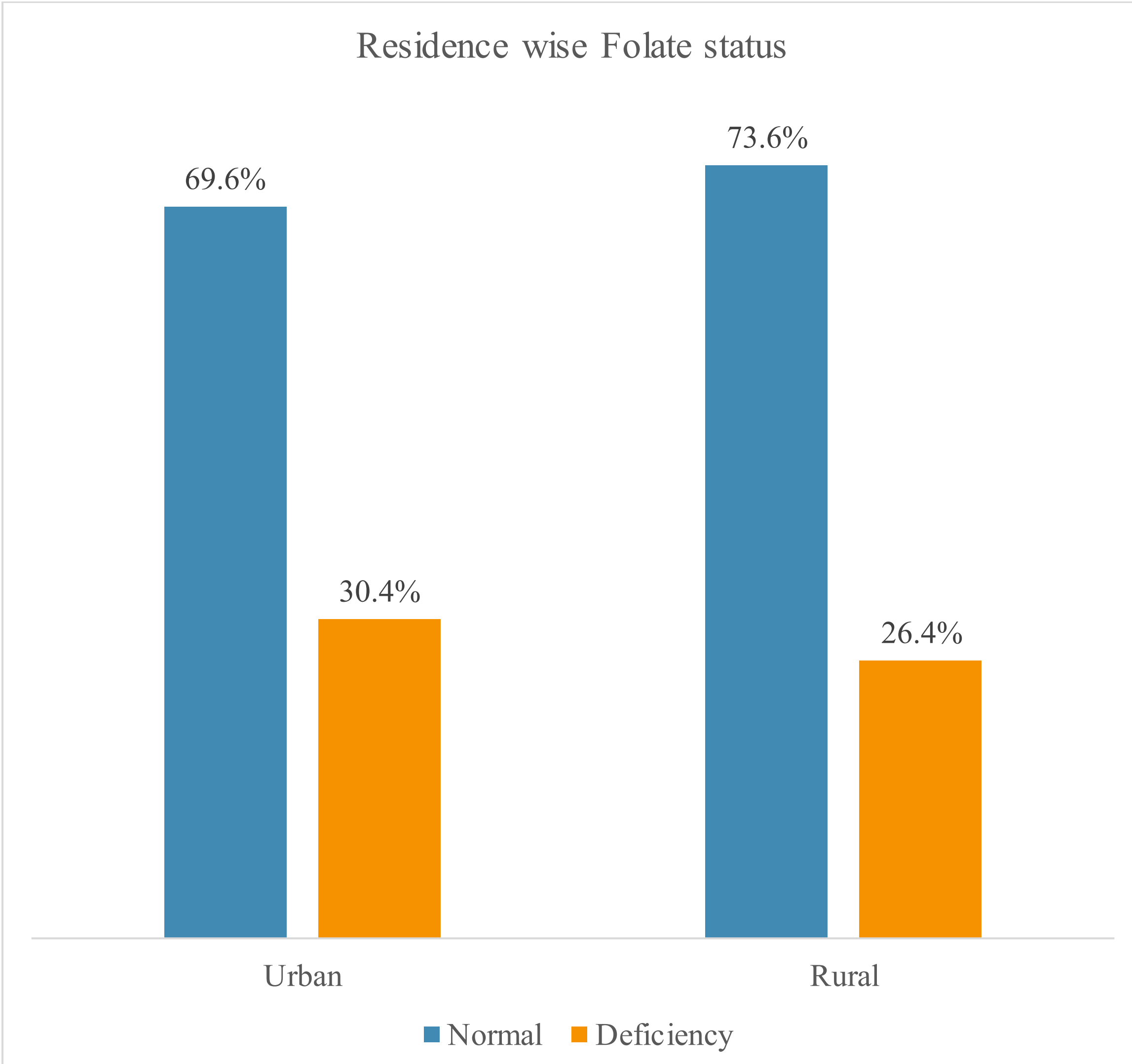
Sylhet Division

Figure 35: Age group wise Overall Folate status in Sylhet division (n=166)



Sylhet showed a worrying trend, with adults (48.6%) and geriatric individuals (47.4%) having the highest deficiency rates. Children and pregnant mothers fared much better, with normal levels above 90%.

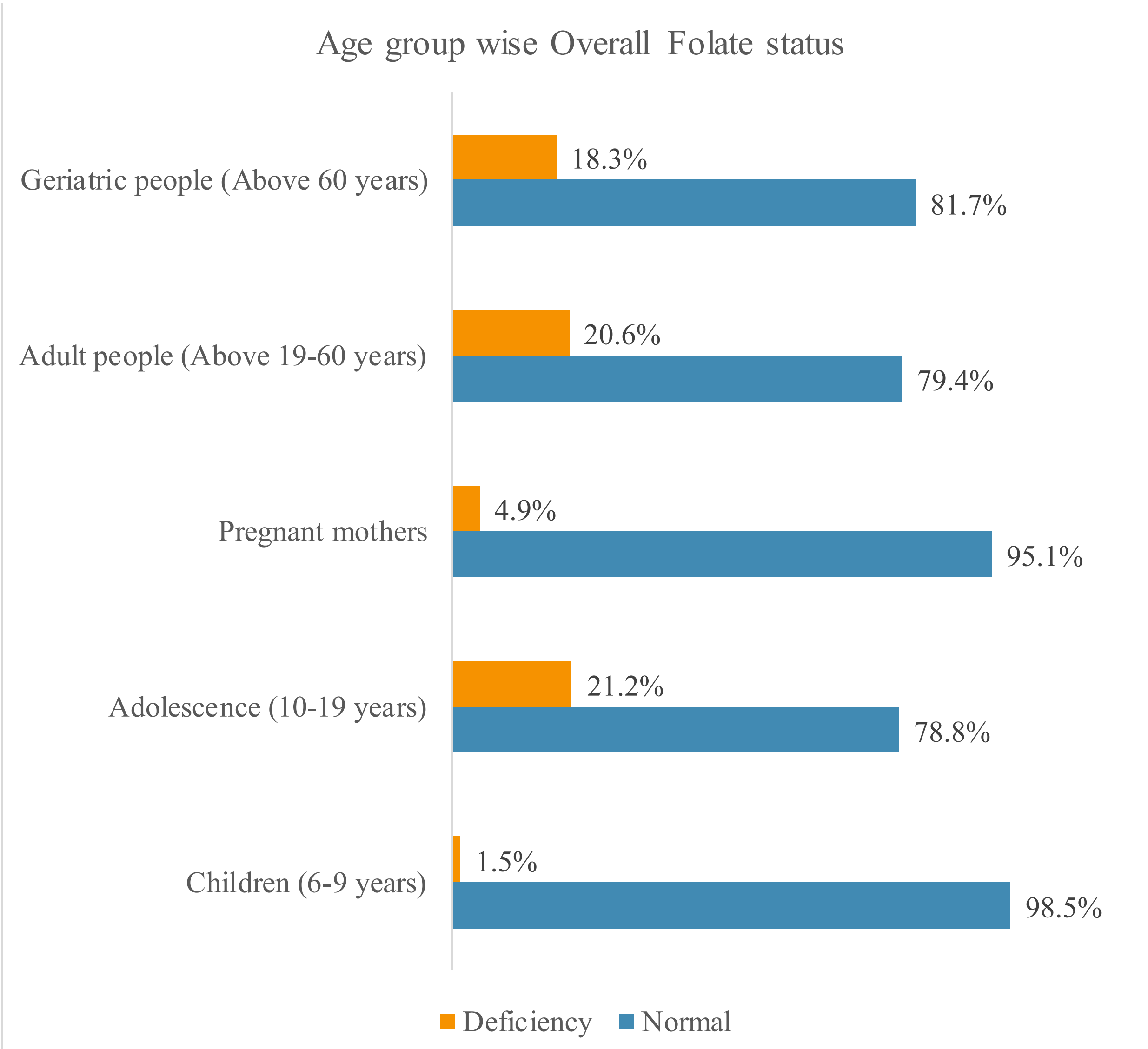
Figure 36: Residence wise Folate status in Sylhet division (n=166)



Deficiency was higher among urban residents (30.4%) compared to rural ones (26.4%), indicating Sylhet has one of the highest folate deficiency rates in the country.

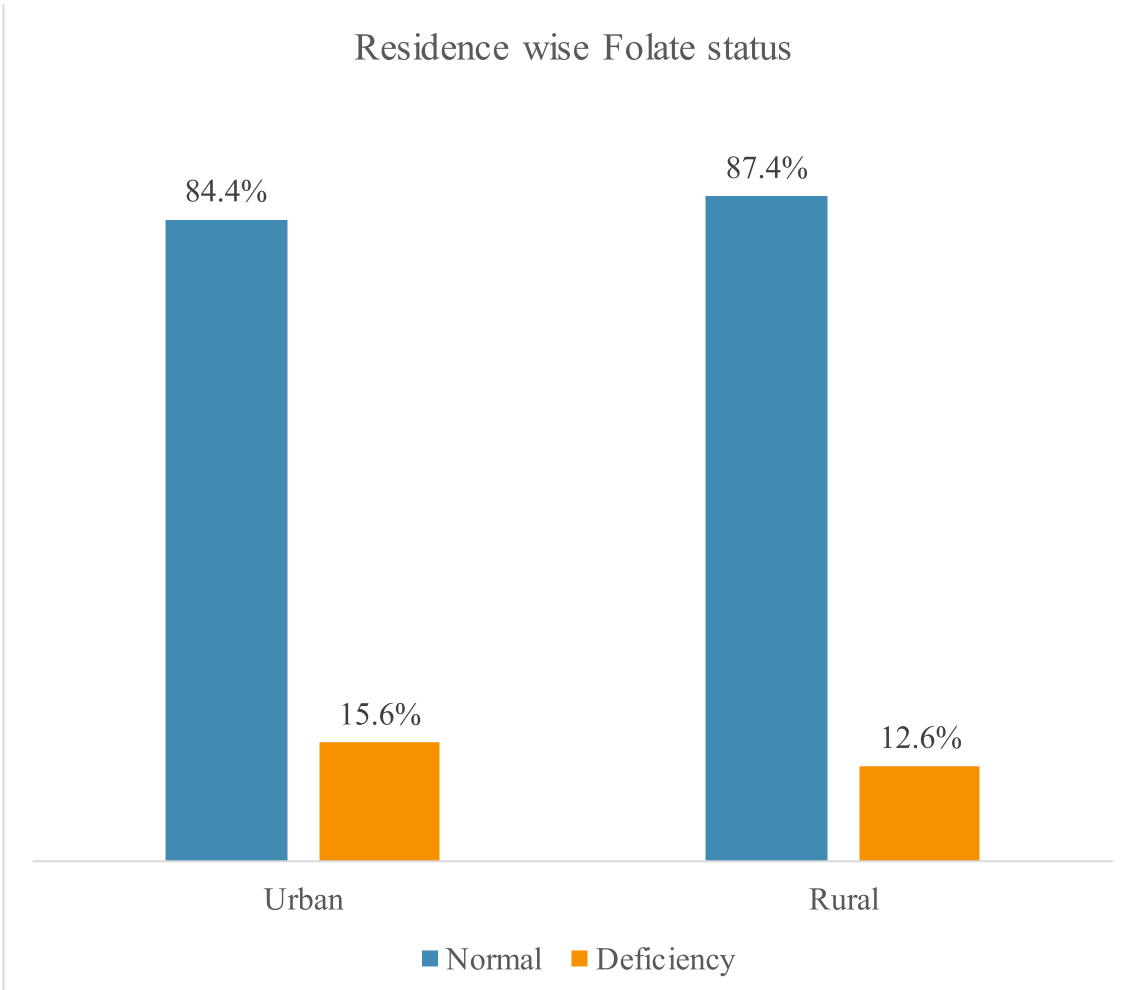
Rajshahi Division

Figure 37: Age group wise Overall Folate status in Rajshahi division (n=306)



Folate status in Rajshahi was quite good across all age groups. Children had 98.5% normal levels, and even geriatric individuals had 81.7% normal levels. The highest deficiency was in adolescents at 21.2%.

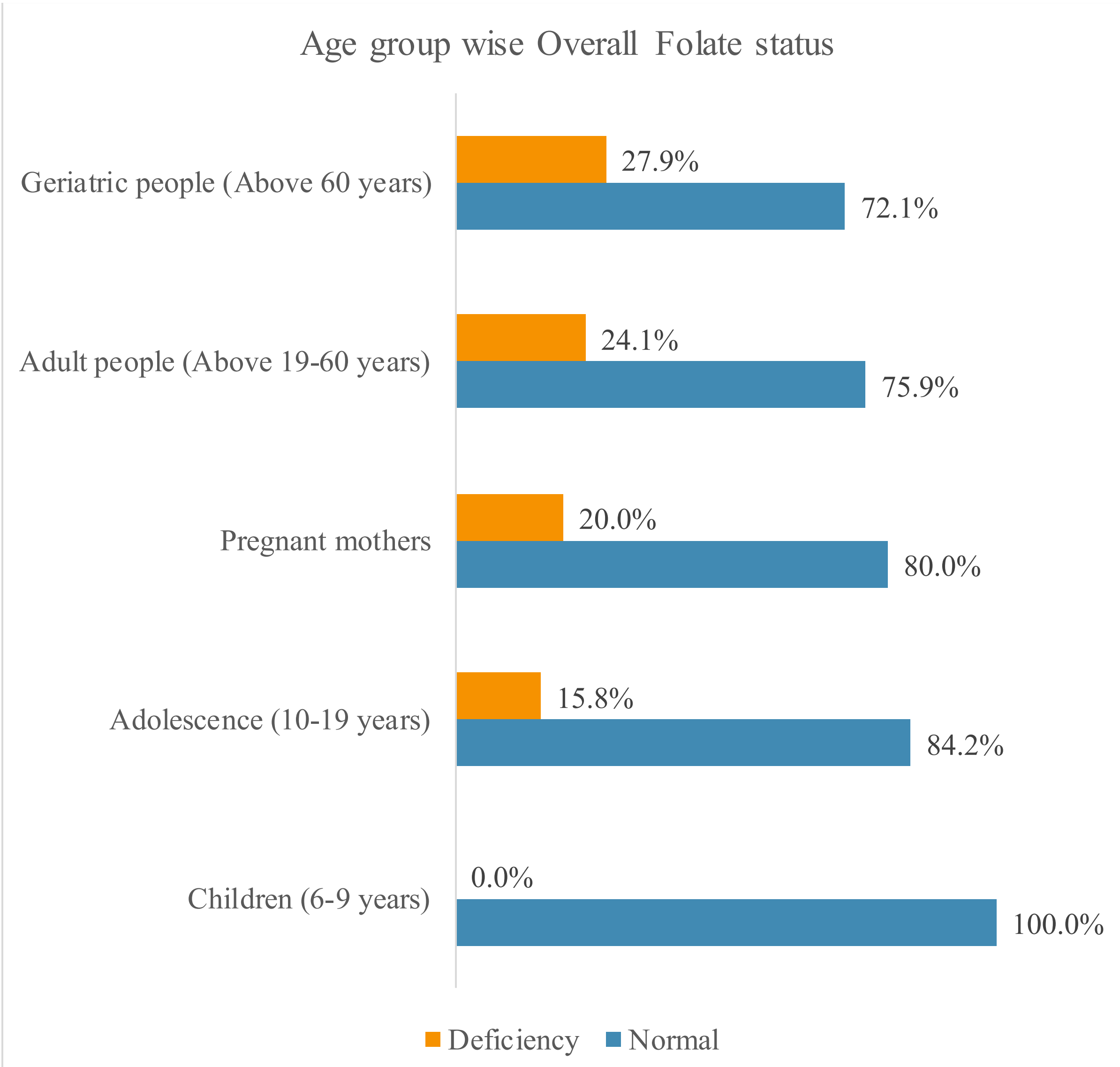
Figure 38: Residence wise Folate status in Rajshahi division (n=306)



Urban and rural residents had similarly high folate sufficiency rates at 84.4% and 87.4%, respectively, with only minor differences in deficiency (15.6% vs. 12.6%).

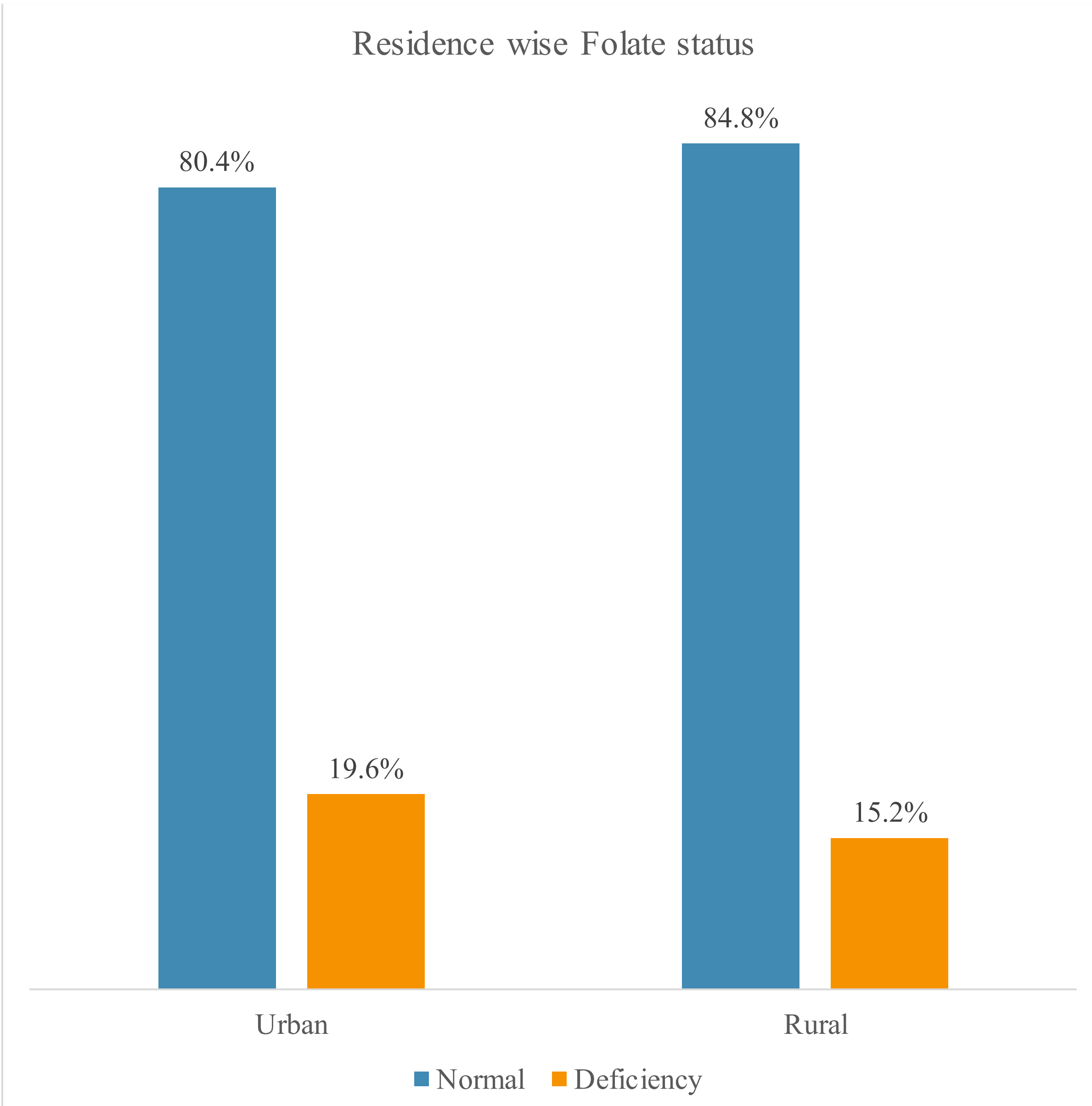
Rangpur Division

Figure 39: Age group wise Overall Folate status in Rangpur division (n=263)



Children had perfect folate status (100%), but adults and geriatric groups had notable deficiencies at 24.1% and 27.9%, respectively. Adolescents and pregnant mothers also had moderate deficiency levels.

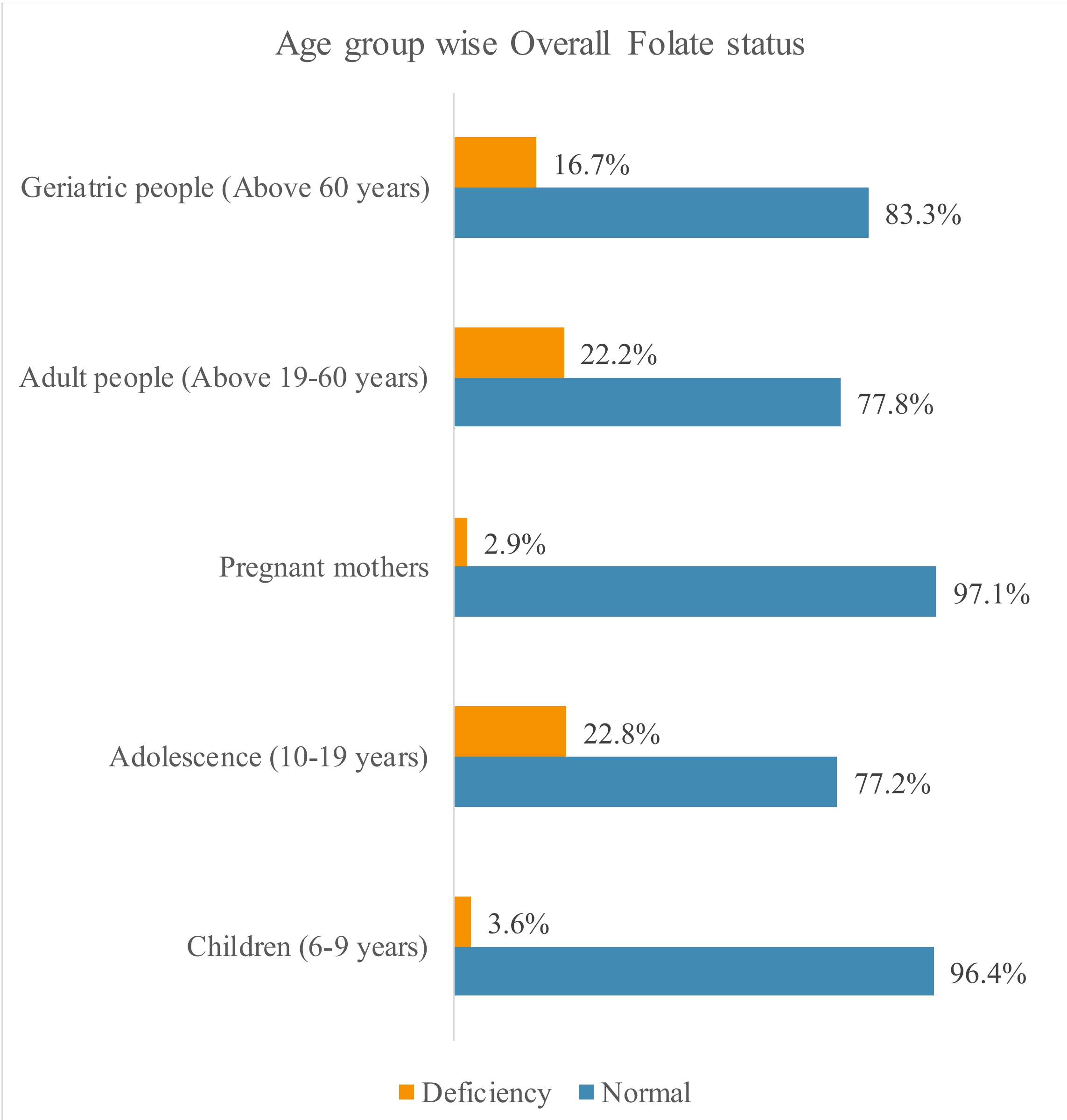
Figure 40: Residence wise Folate status in Rangpur division (n=263)



Urban residents had slightly more folate deficiency (19.6%) than rural ones (15.2%), but both groups showed generally acceptable levels of sufficiency.

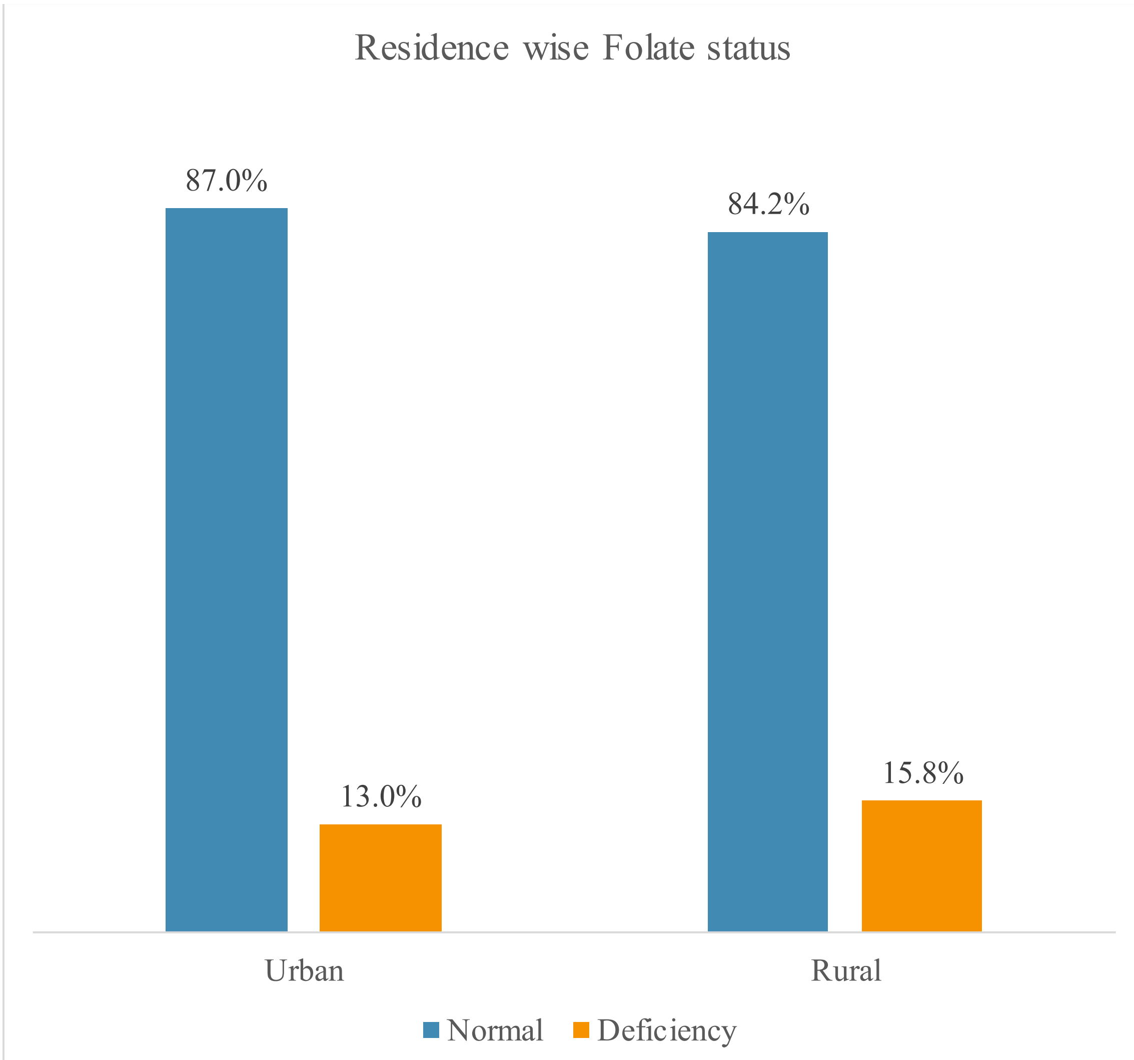
Khulna Division

Figure 41: Age group wise Overall Folate status in Khulna division (n=262)



Children and pregnant mothers had excellent folate status (over 96% normal), while adolescents and adults had slightly higher deficiency levels. Geriatric people had 16.7% deficiency.

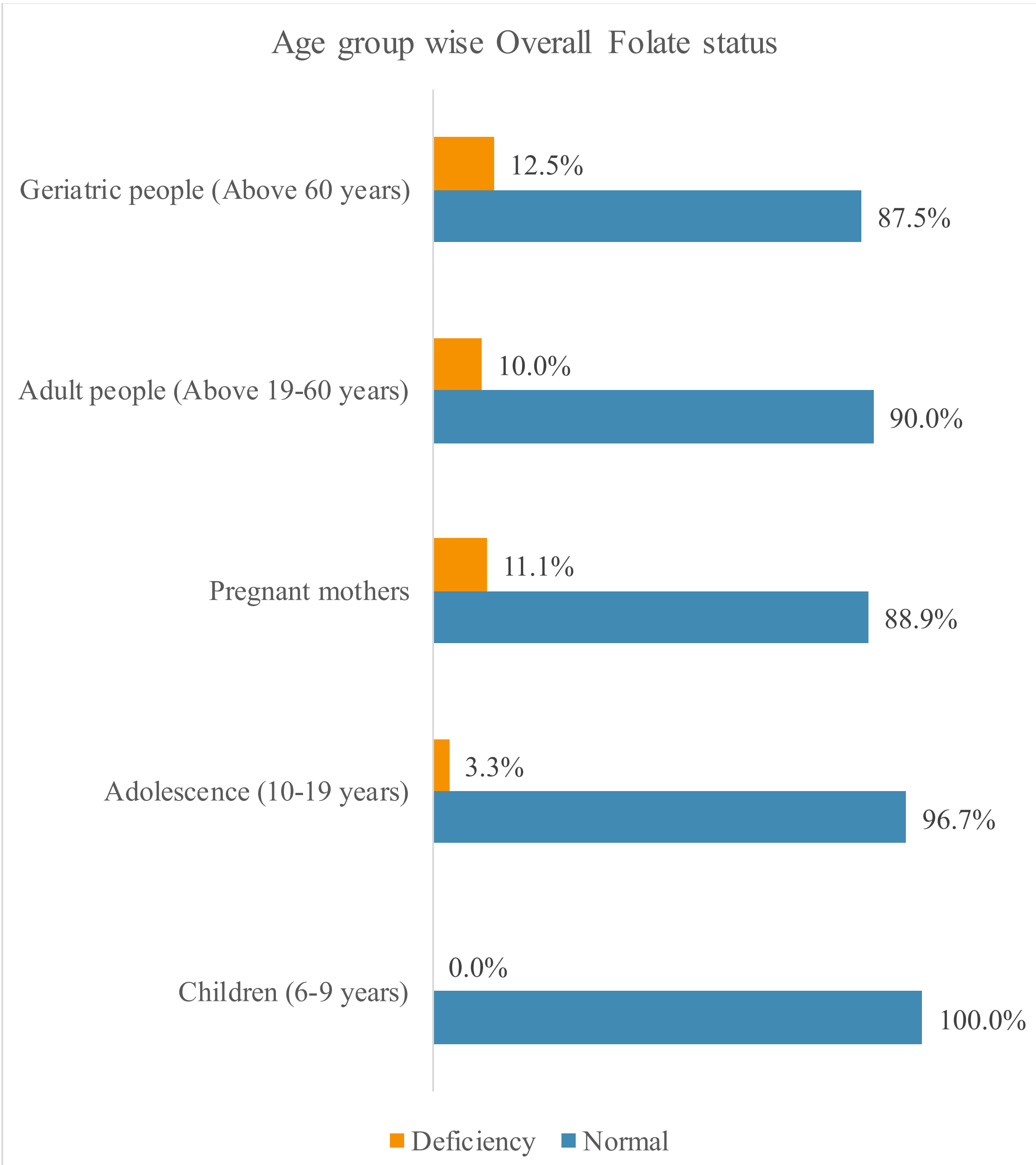
Figure 42: Residence wise Folate status in Khulna division (n=262)



Folate sufficiency was slightly higher in urban areas (87.0%) than rural ones (84.2%), with deficiency levels below 16% in both groups.

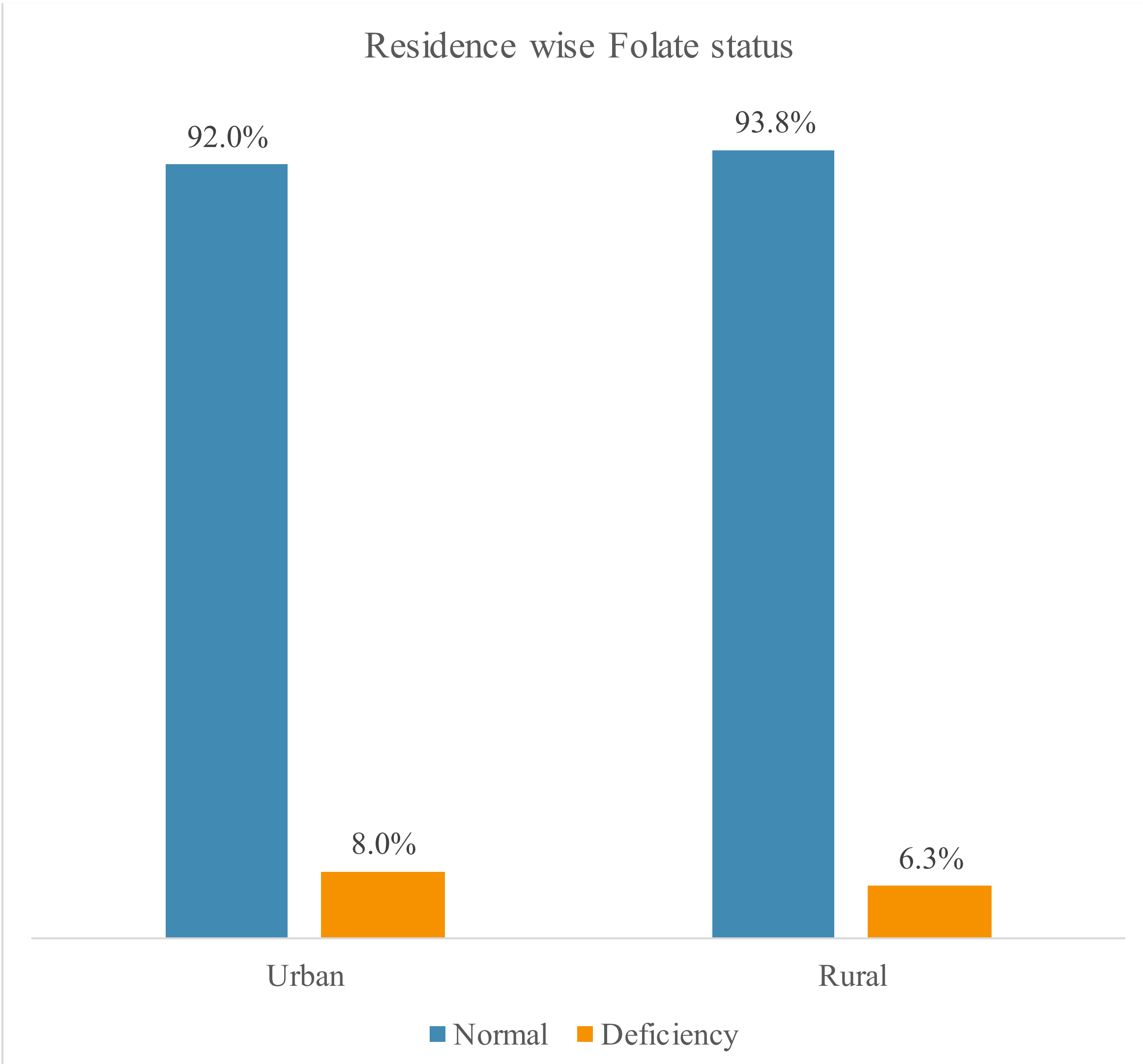
Barisal Division

Figure 43: Age group wise Overall Folate status in Barisal division (n=139)



Folate status was very good in Barisal. Children had 100% normal levels, and even the most vulnerable group—geriatric individuals—had 87.5% with normal folate.

Figure 44: Residence wise Folate status in Barisal division (n=139)



Folate deficiency was lowest among all divisions, with only 8.0% in urban and 6.3% in rural areas being deficient. Barisal had the most favorable folate status across residences.

8. Discussion

The prevalence of vitamin D deficiency in this study was found to be 67.0%, with significant variations observed across different age groups, regions, and genders. This finding is consistent with previous research conducted in Bangladesh, where vitamin D deficiency is highly prevalent. For example, Islam et al. (2002) found that 85% of pregnant rural women had insufficient levels of vitamin D, which is similar to the high deficiency rates of 77.8% observed in this study for pregnant women [11]. Additionally, adolescents in our study had the highest deficiency rate (83.3%), which aligns with other studies, such as Rahman et al. (2021), who reported 70% deficiency among children in rural regions of Bangladesh [30].

Our study also identified significant urban-rural disparities, with 71.1% of urban residents being vitamin D deficient compared to 63.1% in rural areas. This urban-rural gap is supported by global literature. For instance, a study conducted in the United States found that urban populations tend to have higher vitamin D deficiency rates due to factors such as air pollution and lifestyle changes, despite better access to healthcare [9]. In India, urban populations also experience high deficiency rates due to limited sunlight exposure, particularly in cities like Delhi and Mumbai, even though they are geographically located in sunny regions [8].

Furthermore, the gender disparity observed in our study (74.8% for females versus 52.0% for males) can be attributed to cultural and social practices, such as the wearing of full-body coverings among women, which restrict sunlight exposure. Similar findings were reported by Akhtaruzzaman et al. (2019), who found that female garment workers in Bangladesh had lower vitamin D levels due to long indoor work hours and inadequate sunlight exposure [13]. This finding is consistent with the broader trend observed globally, where women, especially in South Asian countries, face higher vitamin D deficiency due to sociocultural factors [7].

The clinical implications of vitamin D deficiency are significant. Vitamin D is essential for bone health, immune function, and the regulation of several chronic diseases. Our findings, which show high deficiency rates in pregnant women and adolescents, are concerning, as deficiencies in these groups are associated with adverse outcomes such as rickets, osteomalacia, and compromised fetal development [6]. The high deficiency in adolescents may also predispose them to long-term bone health issues, similar to the findings of Wacker and Holick (2013), who linked vitamin D deficiency to increased susceptibility to osteoporosis and fractures in adulthood [24].

Folate deficiency, while less widespread than vitamin D deficiency, is still a significant public health concern in Bangladesh. The overall deficiency rate of 16.7% in our study is in line with other studies in South Asia. For instance, a study by Akhtaruzzaman et al. (2020) found that 35-40% of pregnant women in rural Bangladesh were folate deficient [32]. Similarly, Taneja et al. (2007) reported folate deficiency in 27.6% of Indian children and adolescents, indicating a similar burden in the region [27].

Our study found that the highest folate deficiency rates were in the elderly (28.0%), followed by adults (23.5%). These findings align with other studies that show folate deficiency is more prevalent in older adults due to dietary inadequacies and decreased absorption capacity with age [19]. For instance, in a study by Pfeiffer et al. (2015), 27.1% of males in Korea had folate deficiency, which is comparable to the 23% deficiency rate observed in males in our study [22].

Regional variations in Folate deficiency were also significant, with Sylhet (28.3%) and Chattogram (23.0%) showing the highest rates, which is consistent with findings from other regions where dietary deficiencies are more pronounced due to limited access to diverse food sources and lower supplementation rates [26]. The study by Crider et al. (2011) also highlights the role of food fortification in reducing Folate deficiency, a strategy that has been successful in countries like the United States and Canada, where mandatory fortification has significantly reduced deficiency rates and neural tube defects [14].

The gender disparity in folate deficiency, with higher deficiency rates in males (23%) compared to females (14%), is another noteworthy finding. This trend mirrors the results of studies in other low- and middle-income countries, where adolescent boys and adult men are often neglected in folate supplementation programs [27]. It is crucial to recognize that Folate is vital not only for pregnant women but also for men and non-pregnant women to prevent anemia and promote overall health.

The findings of this study underline the urgent need for comprehensive public health strategies to address vitamin D and Folate deficiencies in Bangladesh. For vitamin D, the lack of a national supplementation program and food fortification policies is a critical gap. Countries like the United States and Nordic nations, where vitamin D fortification of foods such as milk and cereals is mandatory, have seen a significant reduction in deficiency rates [9]. Bangladesh should consider implementing similar fortification programs, especially in regions with the highest deficiency rates, to ensure widespread access to adequate vitamin D.

Moreover, public health campaigns promoting safe sun exposure and the importance of vitamin D-rich foods should be implemented nationwide. Additionally, screening programs for vitamin D deficiency should be incorporated into routine health check-ups for vulnerable populations such as pregnant women, adolescents, and the elderly. Similar recommendations were made by Holick (2007), who emphasized the need for systematic vitamin D screening and supplementation to prevent long-term health consequences [6].

For Folate, expanding supplementation programs and food fortification are key steps that need to be taken. As recommended by Crider et al. (2011), mandatory Folate fortification of staple foods could greatly reduce deficiency rates and prevent the occurrence of neural tube defects in newborns [14]. Furthermore, targeted health education programs should raise awareness of folate-rich foods and the importance of Folate supplementation, particularly among men, adolescents, and the elderly, who are often overlooked in existing interventions.

This study highlights the critical public health issue of micronutrient deficiencies in Bangladesh, with vitamin D deficiency affecting a large portion of the population, particularly adolescents and pregnant women, and Folate deficiency impacting the elderly and adult males. The findings underscore the need for targeted public health interventions, including vitamin D supplementation, food fortification, and increased awareness campaigns to address these deficiencies. Without immediate action, the long-term health consequences could further strain the healthcare system and negatively impact the well-being of the population.

9. Conclusion

This national survey highlights a critical public health concern—vitamin D deficiency is widespread across all age groups in Bangladesh. The problem is especially severe among adolescents, pregnant women, and females in general. Certain regions, particularly the southeastern and southern parts of the country, are more affected than others, pointing to geographic disparities.

In contrast, folate deficiency appears to be less prevalent, though still concerning in older adults, adult males, and in specific divisions like Sylhet and Chattogram. Urban-rural differences in folate status are relatively minor, but rural females tend to have slightly better folate levels compared to their male counterparts.

Overall, these findings suggest that micronutrient deficiencies remain inadequately addressed, especially for vitamin D. The lack of national food fortification policies and inconsistent access to supplementation contribute significantly to the current situation. Without timely and targeted public health interventions, the long-term consequences may include poor bone health, immune dysfunction, pregnancy complications, and cognitive decline across different segments of the population.

10. Recommendations

A. Recommendations for Vitamin D

1. A national strategy should be developed to address the vitamin D deficiency level among all age group of people (Dietary diversification, Supplementation or Fortification).
2. Research and policy discussions should be encouraged to identify culturally appropriate and sustainable strategies for improving vitamin D status nationwide.
3. Further analytical study should be conducted to identify the causes of vitamin D deficiency on people of different age group.

B. Recommendations for Folate

1. Health education initiatives should be undertaken to raise awareness of folate-rich dietary sources, targeting adult and the elderly people.
2. The consistent availability of folic acid supplements should be ensured at all primary healthcare facilities.
3. Surveillance should be established to ensure continuous monitoring of folate deficiency.

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12. Annexure

Annexure 1: Summery literature review on Vitamin D deficiency

S/N	Title	Year	Study Place	Study Subjects	Sample Size	Test Name	Major Findings	Cut-off Value Used
1	**Vitamin D Status of Adults in the Outpatient Department in Bangladesh**	2018	Outpatient clinics, Bangladesh	Adults 18–89 yrs	189	25(OH)D	Deficiency: 67.2%, Insufficiency: 20.6%	Deficiency: <20 ng/mL; Insufficiency: 20–30 ng/mL
2	**Prevalence and Predictors of Vitamin D Deficiency in Pregnant Rural Women in Bangladesh**	2021	Four Upazilas (rural)	Pregnant women	515	Serum 25(OH)D	Deficiency: 17.3%, Insufficiency: 47.2%	Deficiency: <30 nmol/L (~12 ng/mL); Insufficiency: 30–50 nmol/L
3	**Vitamin D Deficiency among US Adults**	2018	USA	Adults ≥18 years	26,010	Serum 25(OH)D	Deficiency: 28.9%, Insufficiency: 41.4%	Deficiency: <20 ng/mL; Insufficiency: 20–30 ng/mL
4	**High Prevalence of Vitamin D Deficiency among Bangladeshi Children**	2017	Dhaka city	Children 0–16 yrs	300	Serum 25(OH)D	Deficiency: 31.9–46.8%, Insufficiency: 50–52.6%	Deficiency: <20 ng/mL; Insufficiency: 20–30 ng/mL
5	**Vitamin D Deficiency & Bone Status in Female Garment Workers**	2008	Urban factories	Female workers 18–36 yrs	200	Serum 25(OH)D	Deficiency: 16%, Insufficiency: 84%	Deficiency: <25 nmol/L (~10 ng/mL); Insufficiency: 25–50 nmol/L
6	**Vitamin D Deficiency among Children in Rural Bangladesh**	2020	Kaliakoir Upazila, Gazipur	Children 0–<18 yrs	300	Serum 25(OH)D	Deficiency: 30.2%–37.2% by age	Deficiency: <20 ng/mL

7	**National Micronutrient Survey, Bangladesh**	2020	All 64 districts	i) Children (6–59 months)ii) NPNL women (15–49 yrs)	Children: 1027Women: 1000	Serum 25(OH)D	Children: Deficiency 1.9%, Insufficiency 20%;Women: Deficiency 15.4%, Insufficiency 54.5%	Deficiency: <30 nmol/L (~12 ng/mL); Insufficiency: 30–50 nmol/L
8	**Association of Vitamin D with Neurodevelopment in Slum Infants**	2021	Dhaka slums	Infants aged 6–8 months	265	Serum 25(OH)D	Deficiency: 28.3%; associated with poorer cognitive, language & motor outcomes	Deficiency: <50nmol/L (<20 ng/ml)

Annexure 2: Summery literature review on Folate deficiency

S/N	Title & Country	Year	Study Subjects	Sample Size	Biomarker & Cut-off	Major Findings
1	**Folate, Vitamin B12, and Homocysteine Status in Korean Population (KNHANES)** – *South Korea*	2013–15	Korean adolescents & adults (national)	~8000	Serum folate: <10 nmol/L (≤4 ng/mL)	Folate deficiency: Males (27.1%), Females (8.2%)
2	**Vitamin B12 and Folate Deficiencies in Indian Children and Adolescents (CNNS)** – *India*	2016–18	Children and adolescents (1–19 years)	33,880 (in 3 age groups)	RBC folate; deficiency <305 nmol/L	Preschool: 22.8%, School: 27.6%, Adolescents: 35.6%
4	**Anemia and Vitamin B12/Folate Status in WRA** – *Southern India*	2021	Non-pregnant women of reproductive age	979	RBC folate: <305 nmol/LNTD-risk: <748 nmol/L	7.6% deficient, 79.3% below NTD-risk threshold; estimated NTD risk: 21 per 10,000 births
5	**Folate Deficiency & Preterm Birth** – *Bangladesh (Sylhet)*	2024	Pregnant women (24–28 weeks)	893	Serum folate <3 ng/mL	38% deficient; increased risk of preterm birth (aOR = 1.73)

6	**Maternal BMI & Nutritional Status in Early Pregnancy** – *Bangladesh (Dhaka)*	2019	Pregnant women (6–14 weeks)	498	Serum folate <3 ng/mL	0.4% deficient; low cord blood folate linked with low birth weight
7	**Folate Deficiency in Pregnancy – Haramaya District** – *Eastern Ethiopia*	2023	Pregnant women in rural community	446	Serum folate ≤4 ng/mL	49.3% folate deficiency; associated with low dietary diversity and no supplement use

Annexure 3: Questionnaire

তথ্যদানকারীর জন্য প্রশ্ন

গবেষনার নাম: Prevalence of Vitamin D and Folate Deficiency among People of Different Age Groups in Bangladesh

কোড:	
তারিখ:	
তথ্যপ্রদানকারীর নাম:	
তথ্যপ্রদানকারীর ঠিকানা ও মোবাইল নং:	

সেকশান ১: তথ্যদানকারীর আর্থ-সামাজিক অবস্থা

ক্রঃ নং	প্রশ্ন	উত্তর	কোড
১.	বয়স (পূর্ণ বছরে)		
২.	লিঙ্গ	১. পুরুষ ২. মহিলা	
৩.	ধর্ম	১. ইসলাম ২. সনাতন ৩. খ্রিষ্টান ৪. বৌদ্ধ ৫. অন্যান্য	
৪.	বৈবাহিক অবস্থা	১. অবিবাহিত ২. বিবাহিত ৩. তালাকপ্রাপ্ত ৪. পৃথক ৫. বিধবা ৬. অন্যান্য	
৫.	শিক্ষাগত যোগ্যতা	১. নিরক্ষর ২. প্রাথমিক ৩. মাধ্যমিক ৪. উচ্চ মাধ্যমিক৫. স্নাতক ৬. অন্যান্য	
৬.	**পেশা**	১. সরকারি চাকুরি ২. বেসরকারি চাকুরি ৩. ব্যাবসা ৪. বেকার ৫. দিনমজুর ৬. গৃহিণী	

		৭. অন্যান্য	
৭.	**বিভাগীয় এলাকা**	১. ঢাকা ২. ময়মসিংহ ৩. চটগ্রাম ৪. সিলেট ৫. রাজশাহী ৬. রংপুর ৭. খুলনা ৮. বরিশাল	
৮.	**গর্ভকালীন বয়স (গর্ভবতীদের জন্য)**		
৯.	**পরিবারের মাসিক আয়(টাকায়)**		
১০.	**বসবাসের স্থান**	১. শহর ২. গ্রাম	

সেকশান ২: রক্ত পরীক্ষার ফলাফল

ক্রঃ নং	**প্রশ্ন**	**উত্তর**
১.	ভিটামিন ডি লেভেল	(ng/ml)
২.	ফলিক এসিড লেভেল	(ng/ml)

কোড

বিভাগের জন্য কোড

ঢাকা	**DH**
চট্টগ্রাম	**CH**
রাজশাহী	**RA**
বরিশাল	**BA**
সিলেট	**SY**
রংপুর	**RN**
ময়মনসিং	**MY**
খুলনা	**KH**

তথ্যপ্রদানকারীর কোড

শিশু (৬-৯ বছর)	**C**
কিশোর/কিশোরী (১০-১৯ বছর)	**Y**
গর্ভবতী মা	**P**
প্রাপ্তবয়স্ক (২০-৬৫ বছর)	**A**
বয়স্ক (৬৫ বছরের ঊর্ধ্বে)	**G**

উদাহরণঃ

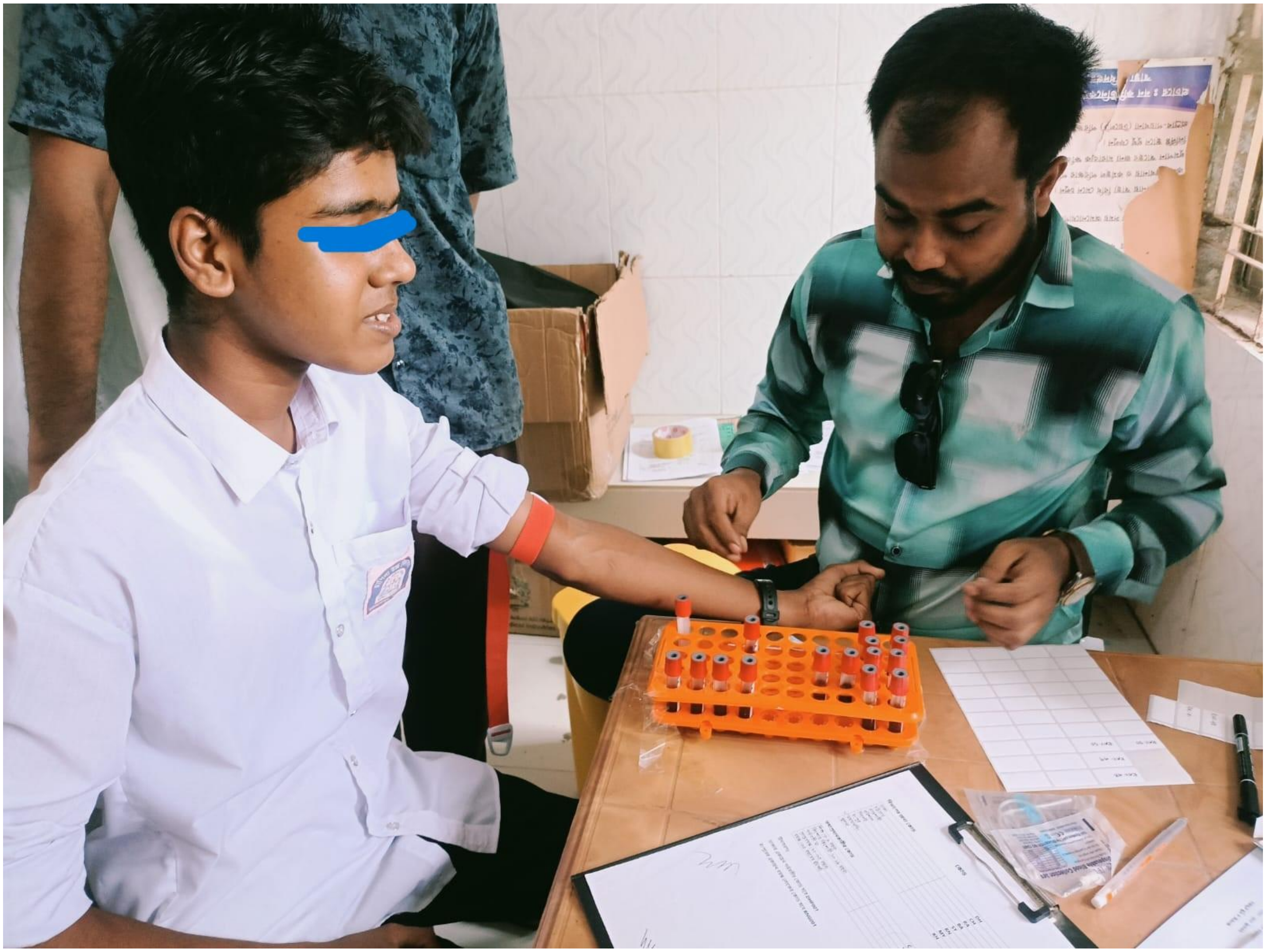
ঢাকার একজন গর্ভবতীর কোড হবে **DHP001**

রংপুরের একজন বয়স্ক লোকের কোড হবে **RNG001**

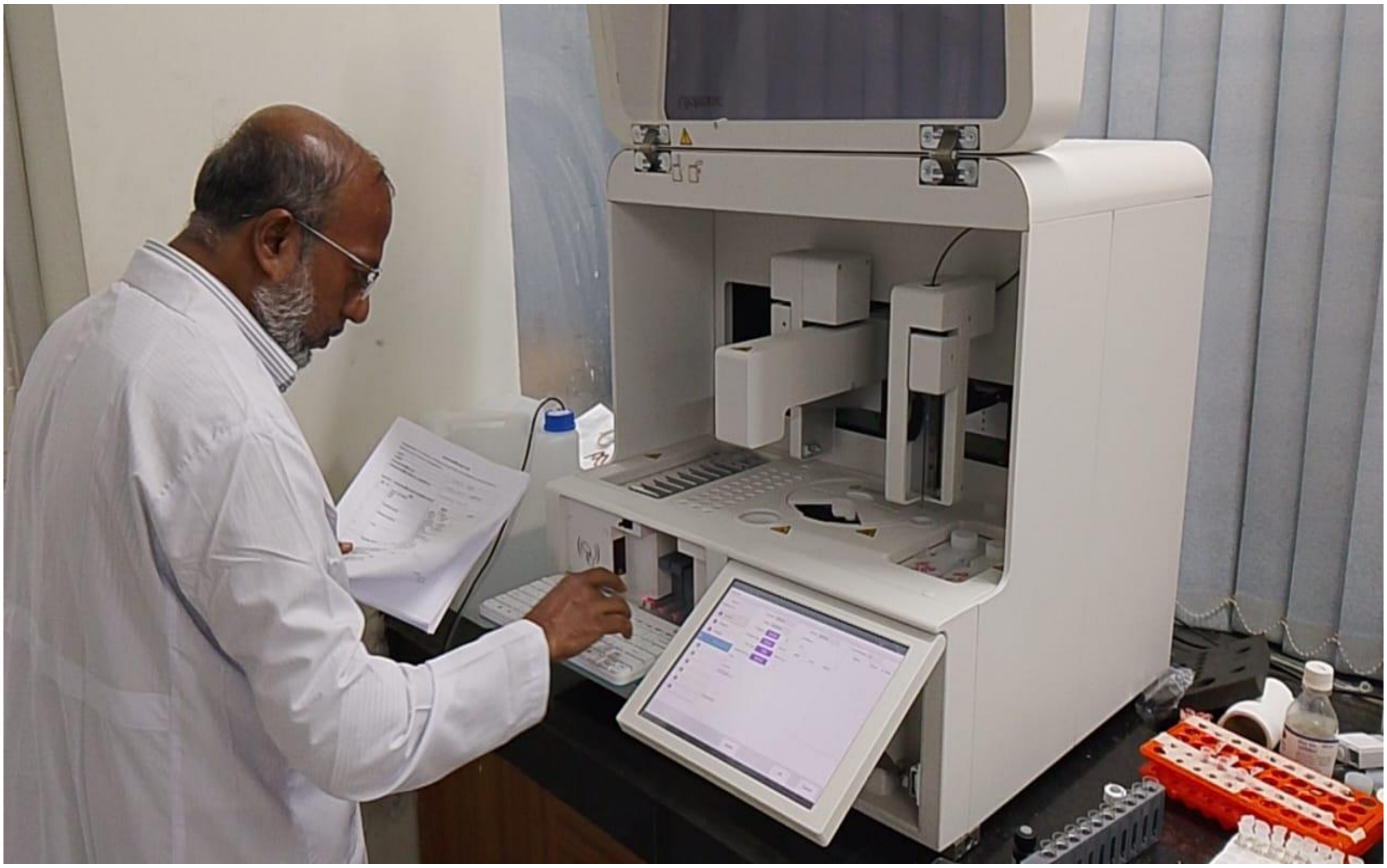
Annexure 4: Pictures of data collection


শিশুর স্বাভাবিক
৬ মাস বয়স


শিশুর স্বাভাবিক শারীরিক
৬ মাস বয়স পর্যন্ত



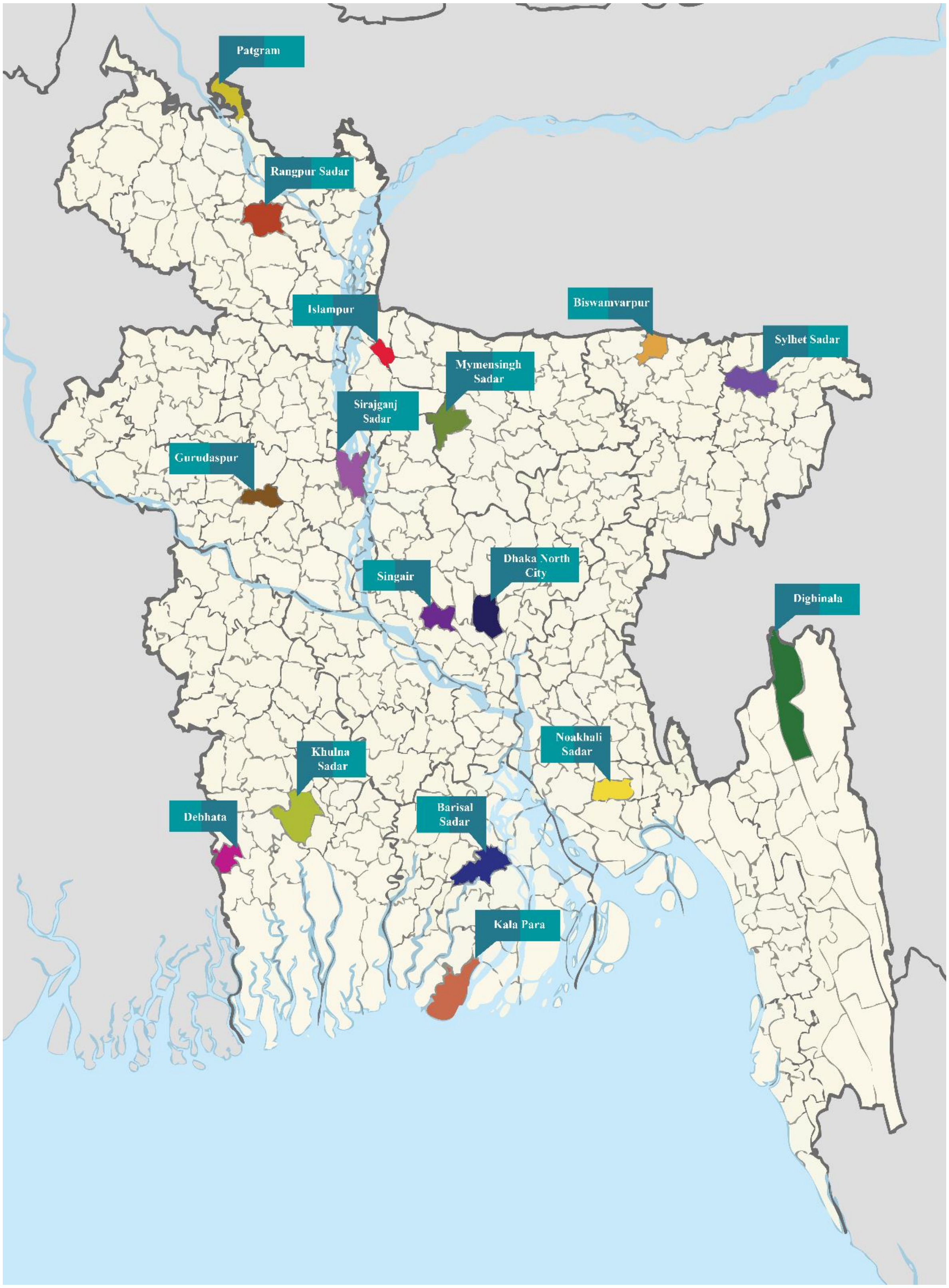

CL8000x




Lifotronic
eCL8000x


-20°C TO 50°C/-4°F TO 122°F
FRIDGE/FREEZER THERMOMETER

Annexure 5: Data collection places



THANK YOU