

# VITAMIN D STATUS AND ASSOCIATED BIOCHEMICAL PROFILES AMONG RICKSHAW PULLERS IN DHAKA, BANGLADESH: A CROSS-SECTIONAL STUDY

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A thesis submitted to the Department of Biotechnology in partial fulfillment of the  
requirements for the degree of  
Master of Science in Biotechnology

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It is hereby declared that

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3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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## Approval

The thesis/project titled “Vitamin D Status and Associated Biochemical Profiles Among Rickshaw Pullers in Dhaka, Bangladesh: A Cross-Sectional Study” submitted by

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## **Ethics Statement**

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board. Prior to participation, all respondents were informed about the purpose, procedures, potential risks, and benefits of the study. Written informed consent was obtained from each participant before data and blood sample collection. Participation was entirely voluntary, and confidentiality and anonymity of participant information were strictly maintained throughout the study. Participants were informed of their right to withdraw from the study at any stage without any consequences.

## **Abstract**

Vitamin D deficiency is highly prevalent in South Asia despite abundant sunlight, and outdoor workers are often assumed to be protected due to prolonged sun exposure. This cross-sectional study assessed vitamin D status and associated biochemical parameters among 152 male rickshaw pullers in Dhaka, Bangladesh. Data were collected through interviews, anthropometric measurements, and biochemical analyses. Serum 25-hydroxyvitamin D was measured using chemiluminescence immunoassay. Vitamin D insufficiency was observed in 43.4% of participants, while 56.6% had sufficient levels; no participants were deficient. The mean serum vitamin D concentration was  $31.39 \pm 7.61$  ng/mL. Vitamin D levels were not significantly associated with socio-demographic, occupational, or lifestyle factors. Dyslipidemia was common, with elevated triglycerides and total cholesterol observed in 36.8% and 34.2% of participants, respectively. Smoking was significantly associated with higher triglyceride levels ( $p = 0.004$ ). Regarding electrolytes, potassium levels were within the normal range for all participants. However, calcium abnormalities were frequent, with 40.8% below and 23.7% above the normal range, while mild deviations were also observed for chloride and CO<sub>2</sub> levels. Despite extensive sun exposure, vitamin D insufficiency remains prevalent, highlighting the need for integrated occupational health interventions.

**Keywords:** Vitamin D status, Rickshaw pullers, Outdoor workers, Dyslipidemia, Bangladesh

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## Table of Contents

|                                                          |             |
|----------------------------------------------------------|-------------|
| <b>Declaration.....</b>                                  | <b>ii</b>   |
| <b>Approval .....</b>                                    | <b>iii</b>  |
| <b>Ethics Statement.....</b>                             | <b>iv</b>   |
| <b>Abstract.....</b>                                     | <b>v</b>    |
| <b>Acknowledgement .....</b>                             | <b>vi</b>   |
| <b>Table of Contents .....</b>                           | <b>vi</b>   |
| <b>List of Tables .....</b>                              | <b>viii</b> |
| <b>List of Figures.....</b>                              | <b>viii</b> |
| <b>List of Acronyms .....</b>                            | <b>ix</b>   |
| <b>Chapter 1: Introduction .....</b>                     | <b>1</b>    |
| <b>Chapter 2: Methods .....</b>                          | <b>4</b>    |
| 2.1 Study design and setting .....                       | 4           |
| 2.2 Study population and eligibility criteria.....       | 4           |
| 2.3 Sample size and sampling technique .....             | 4           |
| 2.4 Data collection procedure .....                      | 4           |
| 2.5 Blood sample collection and laboratory analysis..... | 5           |
| 2.6 Serum vitamin D measurement.....                     | 5           |
| 2.7 Biochemical parameters.....                          | 5           |
| 2.8 Statistical analysis.....                            | 6           |
| 2.9 Ethical considerations .....                         | 6           |

|                                                                             |           |
|-----------------------------------------------------------------------------|-----------|
| <b>Chapter 3: Results.....</b>                                              | <b>7</b>  |
| 3.1 Vitamin D status of the participants.....                               | 10        |
| 3.2 Biochemical parameters of the participants.....                         | 11        |
| 3.3 Association between vitamin D levels and biochemical parameters .....   | 13        |
| <b>Chapter 4: Discusssion .....</b>                                         | <b>13</b> |
| 4.1 Vitamin D status in outdoor occupational groups.....                    | 14        |
| 4.2 Non-association with socio-demographic and occupational variables ..... | 14        |
| 4.3 Biochemical profiles and cardiometabolic risk.....                      | 15        |
| 4.4 Smoking and triglycerides .....                                         | 15        |
| <b>Chapter 5: Conclusion.....</b>                                           | <b>18</b> |
| <b>Funding Information .....</b>                                            | <b>19</b> |
| <b>References.....</b>                                                      | <b>20</b> |

## List of Tables

|                                                                                    |    |
|------------------------------------------------------------------------------------|----|
| Table 1: Distribution of the study population by demographic characteristics ..... | 7  |
| Table 2: Distribution of biochemical parameters among participants .....           | 12 |
| Table 3: Comparison of serum triglyceride levels by smoking status.....            | 13 |

## List of Figures

|                                                                                        |    |
|----------------------------------------------------------------------------------------|----|
| Figure 1: Distribution of vitamin D status among rickshaw pullers .....                | 11 |
| Figure 2: Comparison of serum triglyceride levels between smokers and non-smokers..... | 14 |

## List of Acronyms

|                       |                                             |
|-----------------------|---------------------------------------------|
| <b>25(OH)D</b>        | 25-Hydroxyvitamin D                         |
| <b>UVB</b>            | Ultraviolet B                               |
| <b>CLIA</b>           | Chemiluminescence Immunoassay               |
| <b>BMI</b>            | Body Mass Index                             |
| <b>WHO</b>            | World Health Organization                   |
| <b>ALT</b>            | Alanine Aminotransferase                    |
| <b>AST</b>            | Aspartate Aminotransferase                  |
| <b>ALP</b>            | Alkaline Phosphatase                        |
| <b>HDL</b>            | High-Density Lipoprotein                    |
| <b>LDL</b>            | Low-Density Lipoprotein                     |
| <b>TG</b>             | Triglycerides                               |
| <b>CO<sub>2</sub></b> | Carbon Dioxide                              |
| <b>SD</b>             | Standard Deviation                          |
| <b>IQR</b>            | Interquartile Range                         |
| <b>CI</b>             | Confidence Interval                         |
| <b>ANOVA</b>          | Analysis of Variance                        |
| <b>IRB</b>            | Institutional Review Board                  |
| <b>SPSS</b>           | Statistical Package for the Social Sciences |
| <b>BDT</b>            | Bangladeshi Taka                            |
| <b>nmol/L</b>         | Nanomoles per Liter                         |
| <b>mg/dL</b>          | Milligrams per Deciliter                    |
| <b>U/L</b>            | Units per Liter                             |

# Chapter 1

## Introduction

Vitamin D is a fat-soluble, secosteroid hormone that plays a fundamental role in skeletal health by regulating calcium, phosphate, and magnesium homeostasis, thereby ensuring normal bone development and maintenance (Liu et al., 2025). Circulating serum 25-hydroxyvitamin D [25(OH)D] is the accepted biomarker of vitamin D status, with concentrations  $\geq 30$  ng/mL generally considered sufficient for optimal physiological function (Musarurwa et al., 2018). Beyond its classical role in preventing rickets and osteomalacia, accumulating evidence has established vitamin D as a pleiotropic hormone involved in cell proliferation, immune modulation, neuromuscular function, and inflammation, mediated through the widespread expression of vitamin D receptors in diverse tissues (Lai & Fang, 2013; Verstuyf et al., 2010). Consequently, vitamin D deficiency has been implicated in a broad range of non-skeletal conditions, including diabetes mellitus, hypertension, cardiovascular disease, autoimmune disorders, depression, and certain malignancies (Charoenngam et al., 2019; Visweswaran & Lekha, 2013; Holick, 2008).

Vitamin D is obtained primarily through endogenous synthesis in the skin following exposure to ultraviolet B (UVB) radiation, with additional contributions from dietary sources such as fatty fish, fish liver oils, egg yolks, and fortified foods. These sources provide ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>), which undergo sequential hydroxylation in the liver and kidneys to form the biologically active metabolite, 1,25-dihydroxyvitamin D. This active form enhances intestinal calcium absorption, promotes bone remodeling, and reduces renal excretion of calcium and phosphate (*Ergocalciferol - an Overview* | ScienceDirect Topics, n.d.; Nussey & Whitehead, 2001). Vitamin D deficiency can arise from inadequate dietary intake, insufficient sun exposure, impaired cutaneous synthesis, altered hepatic or renal metabolism, and resistance at target tissues (*Nutritional Assessment of Vitamin D Status*, n.d.).

Globally, vitamin D deficiency constitutes a major public health concern, affecting an estimated one billion individuals worldwide, with an additional 50% of the population experiencing insufficiency (Kaur et al., 2025). The burden is disproportionately high among older adults, individuals with obesity, institutionalized populations, and hospitalized patients, with obesity alone increasing deficiency risk by approximately 35% (Pereira-Santos et al., 2015). Marked regional disparities exist: while approximately 35% of adults in the United States are vitamin D deficient, prevalence estimates exceed 80% in several South Asian countries, including Pakistan, India, and Bangladesh (Liu et al., 2018; Siddiquee et al., 2021a). Among elderly populations, deficiency rates reach over 60% in high-income countries and are often even higher in low- and middle-income settings (Meshkin et al., 2024; Cui et al., 2023). Despite abundant sunlight in South Asia, vitamin D deficiency remains paradoxically widespread. A common assumption is that prolonged outdoor work confers protection against deficiency; however, growing evidence challenges this belief. Studies among outdoor workers in Tehran, India, and Saudi Arabia have reported high prevalence of vitamin D deficiency despite extensive sun exposure (Nimitphong & Holick, 2013; Saki et al., 2017; Harinarayan et al., 2013). Factors such as high atmospheric pollution which attenuates UVB penetration, darker skin pigmentation reducing cutaneous vitamin D synthesis, cultural clothing practices, and prolonged work during early morning or late afternoon hours may collectively limit effective UVB exposure (Rahman & Elmi, 2021; *Skin Pigmentation, Sun Exposure and Vitamin D Levels in Children of the Avon Longitudinal Study of Parents and Children* | *BMC Public Health* | *Springer Nature Link*, n.d.; Al-Yatama et al., 2019; Papadopoulou et al., 2025). Rickshaw pullers represent one of the largest and most vulnerable segments of the informal urban workforce in Dhaka, Bangladesh. They typically work long hours outdoors under physically demanding conditions and are often presumed to be protected from vitamin D deficiency due to continuous sun exposure (*How Dhaka's Rickshaw Pullers Bear a Hidden*

*Health Toll | The Daily Star*, n.d.). However, Dhaka is among the most polluted megacities globally, with dense particulate matter capable of significantly reducing UVB radiation at ground level. In addition, rickshaw pullers often belong to lower socioeconomic strata, with limited access to vitamin D-rich foods such as fatty fish, eggs, dairy products, and fortified foods. Poor dietary diversity, food insecurity, and lack of nutritional awareness may further exacerbate the risk of deficiency (undefined, undefined).

The health implications of vitamin D deficiency among rickshaw pullers are particularly concerning. Beyond skeletal manifestations such as bone pain and muscle weakness, deficiency has been associated with increased risks of cardiovascular disease, metabolic disorders, and malignancies conditions that can substantially impair physical capacity and productivity (Młynarska et al., 2025; GUPTA et al., 2011). For individuals whose livelihoods depend entirely on sustained physical labor, vitamin D deficiency may lead to reduced work efficiency, increased susceptibility to illness, loss of income, and perpetuation of poverty and health inequities (Plotnikoff et al., 2012).

Despite the public health relevance, data on vitamin D status among outdoor occupational groups in Bangladesh particularly rickshaw pullers remain scarce. Understanding the prevalence and determinants of vitamin D deficiency in this population is critical for informing targeted public health interventions, occupational health policies, and nutritional strategies. Such evidence is essential to challenge prevailing assumptions regarding sun exposure and vitamin D sufficiency and to guide context-specific interventions, including supplementation, dietary diversification, and health education programs.

Therefore, this study aims to assess the prevalence of vitamin D deficiency among rickshaw pullers in Dhaka city and to identify key demographic, dietary, and occupational factors associated with deficiency. By addressing an important evidence gap, the findings of this study have the potential to inform public health policy, improve occupational health outcomes, and

contribute to the broader understanding of vitamin D deficiency in sun-exposed populations living in highly polluted urban environments.

## **Chapter 2**

### **Methods**

#### **2.1 Study design and setting**

A cross-sectional study was conducted among rickshaw pullers in Dhaka, Bangladesh. Data collection was carried out between June-August 2025 at selected rickshaw stands across different areas of the city to ensure variability in socio-demographic and occupational characteristics.

#### **2.2 Study population and eligibility criteria**

Male rickshaw pullers aged 18 years and above, who had been actively working for at least 6 months, were eligible to participate. Participants were excluded if they had a known history of chronic liver disease, kidney disease, malabsorption disorders, endocrine disorders affecting vitamin D metabolism, or if they were currently receiving therapeutic vitamin D supplementation.

#### **2.3 Sample size and sampling technique**

The sample size was determined using the single population proportion formula, based on an assumed prevalence of vitamin D insufficiency of 50%. A 95% confidence level and a 5% margin of error were applied. To compensate for potential non-response or incomplete data, the final target sample size was increased, resulting in the enrollment of 152 participants. Participants were recruited using a convenience sampling approach from selected rickshaw stands in Dhaka, with recruitment conducted during working hours based on participant availability and willingness to participate.

## **2.4 Data collection procedures**

Data were collected through face-to-face interviews using a pre-tested structured questionnaire. The questionnaire captured information on: 1. Socio-demographic characteristics: age, marital status, education level, and monthly income. 2. Work-related factors: years of work experience, daily working hours, and working shift. 3. Lifestyle factors: smoking status, alcohol consumption, and multivitamin use

Anthropometric measurements were taken following standard procedures. Height was measured using a stadiometer and weight using a calibrated digital scale. Body mass index (BMI) was calculated as weight (kg) divided by height squared ( $m^2$ ) and categorized according to standard WHO criteria.

## **2.5 Blood sample collection and laboratory analysis**

Venous blood samples were collected under aseptic conditions by trained personnel. Samples were allowed to clot and were centrifuged to obtain serum, which was stored at appropriate temperatures until analysis.

## **2.6 Serum vitamin D measurement**

Serum 25-hydroxyvitamin D [25(OH)D] concentration was measured using a chemiluminescence immunoassay (CLIA) following the manufacturer's protocol. Vitamin D status was categorized based on predefined cut-off values as: Deficient:  $< 10$  ng/mL, Insufficient: 10–29.9 ng/mL, Sufficient:  $\geq 30$  ng/mL, Toxic:  $\geq 100$  ng/mL. Vitamin D concentrations were expressed in ng/mL.

## **2.7 Biochemical parameters**

Liver function tests (total bilirubin, ALT, AST, ALP), lipid profile (total cholesterol, triglycerides, HDL, LDL), and serum electrolytes (calcium, sodium, potassium, chloride, and

CO<sub>2</sub>) were analyzed using standard automated laboratory methods. Results were classified as below normal, normal, or above normal according to established laboratory reference ranges.

## **2.8 Statistical analysis**

Data were entered and analyzed using Microsoft Excel with statistical add-ins, including MetaXL and Excel-based statistical tools. Continuous variables were assessed for normality using the Shapiro–Wilk test and D’Agostino–Pearson test. Descriptive statistics were presented as mean  $\pm$  standard deviation (SD) for continuous variables and frequency with percentage for categorical variables.

Comparisons of mean vitamin D levels across socio-demographic, occupational, and lifestyle variables were performed using independent samples t-tests or one-way analysis of variance (ANOVA), as appropriate. For non-normally distributed variables, median and interquartile range (IQR) were reported.

Associations between biochemical parameters and lifestyle factors were examined using comparative analyses. Differences in serum triglyceride levels between smokers and non-smokers were assessed using an independent two-sample t-test assuming unequal variances. Effect estimates were reported with 95% confidence intervals (CI). A p-value of  $< 0.05$  was considered statistically significant.

## **2.9 Ethical considerations**

Ethical approval was obtained from the institutional review board. Written informed consent was obtained from all participants prior to enrollment. Confidentiality of participant information was maintained throughout the study, and all procedures were conducted in accordance with the principles of the Declaration of Helsinki.

## Chapter 3

### Results

A total of 152 participants were included in the study. The socio-demographic, work-related, and lifestyle characteristics of the respondents along with the mean outcome score are presented in Table 1.

**Table 1: Distribution of the study population by demographic characteristics (N=152)**

| Variables                                | n=152 | %     | Mean $\pm$ SD    | p value |
|------------------------------------------|-------|-------|------------------|---------|
| <b>Socio demographic characteristics</b> |       |       |                  |         |
| <b>Age (years)</b>                       |       |       |                  |         |
| 18-29                                    | 34    | 22.37 | 30.36 $\pm$ 6.54 | 0.683   |
| 30-39                                    | 51    | 33.55 | 30.71 $\pm$ 8    |         |
| 40-49                                    | 36    | 23.68 | 32.5 $\pm$ 8.17  |         |
| 50-59                                    | 19    | 12.50 | 32.53 $\pm$ 8.28 |         |
| 60+                                      | 12    | 7.89  | 32.03 $\pm$ 6.6  |         |
| <b>Marital Status</b>                    |       |       |                  |         |
| Married                                  | 144   | 94.74 | 31.45 $\pm$ 7.69 | 0.616   |
| Unmarried                                | 8     | 5.26  | 30.24 $\pm$ 6.33 |         |
| <b>Education</b>                         |       |       |                  |         |
| No Education                             | 50    | 32.89 | 30.96 $\pm$ 8.22 | 0.700   |
| Primary                                  | 64    | 42.11 | 30.96 $\pm$ 7.4  |         |
| Secondary                                | 30    | 19.74 | 32.56 $\pm$ 7.82 |         |
| Higher secondary or more                 | 8     | 5.26  | 33.03 $\pm$ 4.27 |         |
|                                          |       |       |                  |         |

|                             |     |       |              |       |
|-----------------------------|-----|-------|--------------|-------|
| <b>Monthly income</b>       |     |       |              |       |
| Less than 5,000             | 6   | 3.95  | 29.5 ± 6.95  | 0.481 |
| 5,000-10,000                | 18  | 11.84 | 30.88 ± 7.44 |       |
| 10,001-15,000               | 64  | 42.11 | 32.49 ± 7.66 |       |
| More than 15,000            | 64  | 42.11 | 30.6 ± 7.69  |       |
| <b>Body mass index</b>      |     |       |              |       |
| Normal                      | 106 | 69.74 | 30.91 ± 7.64 | 0.433 |
| Overweight                  | 19  | 12.50 | 32.35 ± 6.92 |       |
| Obese                       | 1   | 0.66  | 32.25 ± 8    |       |
| <b>Work related factors</b> |     |       |              |       |
| <b>Working Year</b>         |     |       |              |       |
| Less than 1 year            | 13  | 8.55  | 31.01 ± 5.74 | 0.331 |
| 1-5 years                   | 53  | 34.87 | 31.03 ± 7.22 |       |
| 6-10 years                  | 21  | 13.82 | 29.09 ± 7.08 |       |
| More than 10 years          | 65  | 42.76 | 32.49 ± 8.33 |       |
| <b>Working Hours</b>        |     |       |              |       |
| 5-8 hours                   | 27  | 17.76 | 32.36 ± 6.5  | 0.411 |
| More than 8 hours           | 125 | 82.24 | 31.17 ± 7.84 |       |
| <b>Working Shift</b>        |     |       |              |       |
| Day only                    | 58  | 38.16 | 30.38 ± 7.76 | 0.356 |
| Night only                  | 1   | 0.66  |              |       |
| Both                        | 93  | 61.18 | 31.95 ± 7.51 |       |
|                             |     |       |              |       |

|                     |     |       |              |       |
|---------------------|-----|-------|--------------|-------|
| <b>Lifestyle</b>    |     |       |              |       |
| <b>Multivitamin</b> |     |       |              |       |
| Consumer            | 50  | 32.89 | 31.4 ± 7.62  | 0.948 |
| Non-consumer        | 101 | 66.45 | 31.38 ± 7.64 |       |
| <b>Cigarette</b>    |     |       |              |       |
| Smoker              | 100 | 65.79 | 31.26 ± 7.44 | 0.785 |
| Non-smoker          | 51  | 33.55 | 31.63 ± 8.01 |       |
| <b>Alcohol</b>      |     |       |              |       |
| Drinker             | 19  | 12.50 | 31.08 ± 4.98 | 0.797 |
| Non-drinker         | 133 | 87.50 | 31.43 ± 7.93 |       |

*Values are presented as mean ± standard deviation unless otherwise stated. p values were obtained using independent t-test or one-way ANOVA as appropriate. Vitamin D concentration expressed in ng/mL.*

Most participants were aged 30–39 years (33.55%), followed by 40–49 years (23.68%) and 18–29 years (22.37%). The overall mean score across age groups ranged from  $30.36 \pm 6.54$  to  $32.53 \pm 8.28$ , with no statistically significant difference observed among age categories ( $p = 0.683$ ). The majority of respondents were married (94.74%), while only 5.26% were unmarried. The mean scores between married and unmarried participants were comparable ( $31.45 \pm 7.69$  vs  $30.24 \pm 6.33$ ), showing no significant association ( $p = 0.616$ ). Most of the participants had primary education (42.11%), followed by no formal education (32.89%). Participants with higher secondary education or above constituted only 5.26% of the sample. Mean scores showed a slight increasing trend with higher education levels; however, the difference was not statistically significant ( $p = 0.700$ ). In terms of monthly income, 84.22% of respondents earned 10,001 BDT or more per month. Mean scores varied modestly across income categories, with no significant association observed ( $p = 0.481$ ). Based on body mass index (BMI), most participants were within the normal range (69.74%), followed by overweight (12.50%) and

obese (0.66%). No statistically significant difference in mean scores was found across BMI categories ( $p = 0.433$ ). Nearly half of the respondents (42.76%) had been working for more than 10 years, while 34.87% had 1–5 years of work experience. Although participants with longer working years showed slightly higher mean scores, the association was not statistically significant ( $p = 0.331$ ). The majority of participants (82.24%) worked more than 8 hours per day. Mean scores did not differ significantly between those working 5–8 hours and those working longer hours ( $p = 0.411$ ). Most respondents (61.18%) worked both day and night shifts, while 38.16% worked day shifts only. No significant difference in mean scores was observed by working shift ( $p = 0.356$ ). Approximately 32.89% of participants reported consuming multivitamins, while 66.45% were non-consumers. Mean scores were nearly identical between the two groups ( $p = 0.948$ ). Smoking was reported by 65.79% of participants, whereas 33.55% were non-smokers. No statistically significant difference in mean scores was observed between smokers and non-smokers ( $p = 0.785$ ). Alcohol consumption was reported by 12.50% of respondents. The mean scores between alcohol drinkers and non-drinkers were similar, with no significant association ( $p = 0.797$ ).

### **3.1 Vitamin D status of the participants:**

Vitamin D status of the 152 rickshaw pullers is summarized in Figure 1. None of the participants were classified as vitamin D deficient or toxic. A total of 66 participants (43.4%) had insufficient vitamin D levels, while 86 participants (56.6%) had sufficient levels.

The mean serum vitamin D concentration was  $31.39 \pm 7.61$  ng/mL, with a median value of 31.5 ng/mL. Serum vitamin D levels ranged from 15.7 to 49.3 ng/mL, with an interquartile range (IQR) of 11.98 ng/mL ( $Q1 = 25.28$  ng/mL,  $Q3 = 37.25$  ng/mL). No outliers were detected.

Assessment of data distribution showed that vitamin D levels were not normally distributed. The Shapiro–Wilk test indicated significant deviation from normality ( $W = 0.98$ ,  $p = 0.034$ ), which was further supported by the D’Agostino–Pearson test ( $p = 0.009$ ). The distribution demonstrated minimal skewness (0.06) and negative kurtosis ( $-0.77$ ).

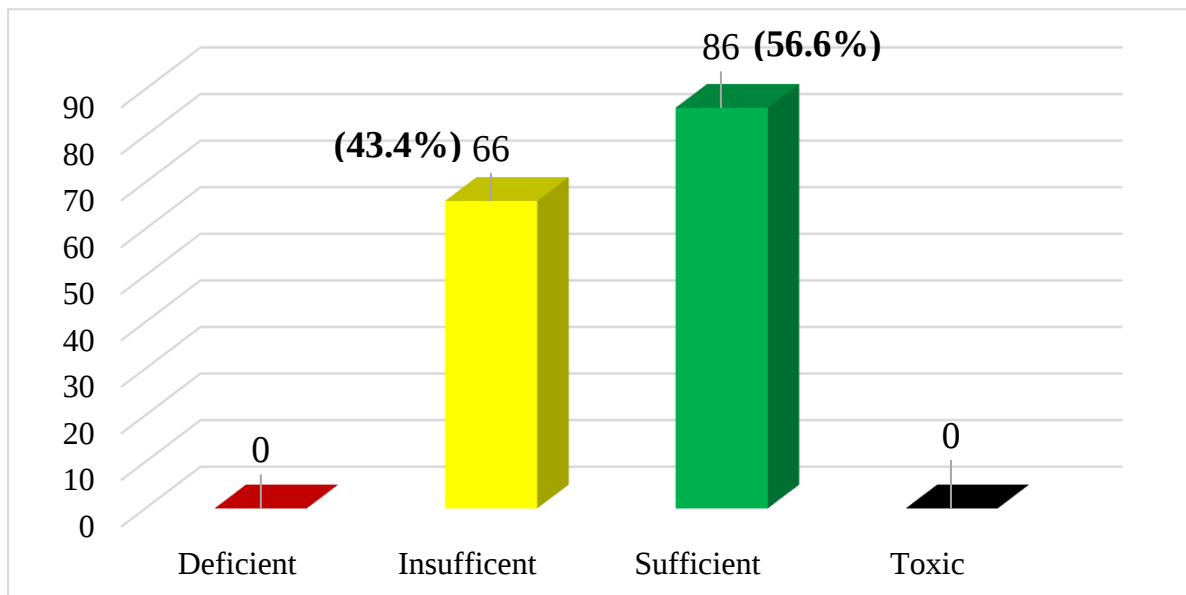


Fig 1. Distribution of vitamin D status among rickshaw pullers ( $n = 152$ ).  
*Vitamin D status was categorized according to predefined serum vitamin D cut-off values.*

### 3.2 Biochemical parameters of the participants:

Table 2 presents the distribution of biochemical parameters according to reference ranges. Most participants demonstrated normal liver function parameters, with over 94% showing normal levels of total bilirubin, ALT, AST, and ALP. Elevated liver enzymes were observed in a small proportion of participants, particularly for AST (5.9%) and ALT (5.3%).

Dyslipidemia was common among the study population. Elevated total cholesterol and triglyceride levels were observed in 34.2% and 36.8% of participants, respectively. Low HDL cholesterol levels were present in 41.4%, while elevated LDL cholesterol levels were observed in 13.2% of participants.

Regarding electrolytes, potassium levels were within the normal range for all participants. However, abnormalities were noted for calcium, with 40.8% below and 23.7% above the normal range. Mild deviations were also observed for chloride and CO<sub>2</sub> levels.

**Table 2: Distribution of biochemical parameters among participants (n = 152)**

**Liver function tests & lipid profile**

| Parameter                 | Below normal<br>n (%) | Normal / Optimal<br>n (%) | Above normal<br>n (%) |
|---------------------------|-----------------------|---------------------------|-----------------------|
| Total bilirubin (mg/dL)   | 0 (0.0)               | 148 (97.4)                | 4 (2.6)               |
| ALT (U/L)                 | 0 (0.0)               | 144 (94.7)                | 8 (5.3)               |
| AST (U/L)                 | 2 (1.3)               | 141 (92.8)                | 9 (5.9)               |
| ALP (U/L)                 | 0 (0.0)               | 147 (96.7)                | 5 (3.3)               |
| Total cholesterol (mg/dL) | —                     | 100 (65.8)                | 52 (34.2) *           |
| Triglycerides (mg/dL)     | —                     | 96 (63.2)                 | 56 (36.8) *           |
| HDL cholesterol (mg/dL)   | 63 (41.4)             | 82 (53.9)                 | 7 (4.6)               |
| LDL cholesterol (mg/dL)   | —                     | 105 (69.1)                | 47 (30.9) **          |

**Electrolytes**

| Parameter                | Below normal n (%) | Normal n (%) | Above normal n (%) |
|--------------------------|--------------------|--------------|--------------------|
| Calcium (nmol/L)         | 62 (40.8)          | 54 (35.5)    | 36 (23.7)          |
| Sodium (nmol/L)          | 1 (0.7)            | 143 (94.1)   | 8 (5.3)            |
| Potassium (nmol/L)       | 0 (0.0)            | 152 (100)    | 0 (0.0)            |
| Chloride (nmol/L)        | 16 (10.5)          | 124 (81.6)   | 12 (7.9)           |
| CO <sub>2</sub> (nmol/L) | 25 (16.4)          | 115 (75.7)   | 12 (7.9)           |

*\*Includes borderline high and high categories/ \*\*Borderline LDL = 0*

Values are presented as frequency and percentage. Classification was based on standard laboratory reference ranges. Borderline and high categories were combined as “above normal” where applicable for analytical clarity.

### 3.3 Association between vitamin D levels and biochemical parameters

Correlation and comparative analyses were performed to examine the association between serum vitamin D levels and biochemical parameters. No statistically significant associations were observed between vitamin D levels and most biochemical or lifestyle variables. However, a significant association was found between cigarette smoking status and serum triglyceride (TG) levels.

Smokers exhibited a significantly higher mean triglyceride level compared to non-smokers ( $203.12 \pm 101.67$  mg/dL vs  $151.08 \pm 106.76$  mg/dL). An independent two-sample *t*-test assuming unequal variances demonstrated that this difference was statistically significant ( $t = -2.88$ ,  $df = 96$ ,  $p = 0.004$ , two-tailed). The mean difference in triglyceride levels between smokers and non-smokers was 52.04 mg/dL (95% CI: 16.21–87.87 mg/dL).

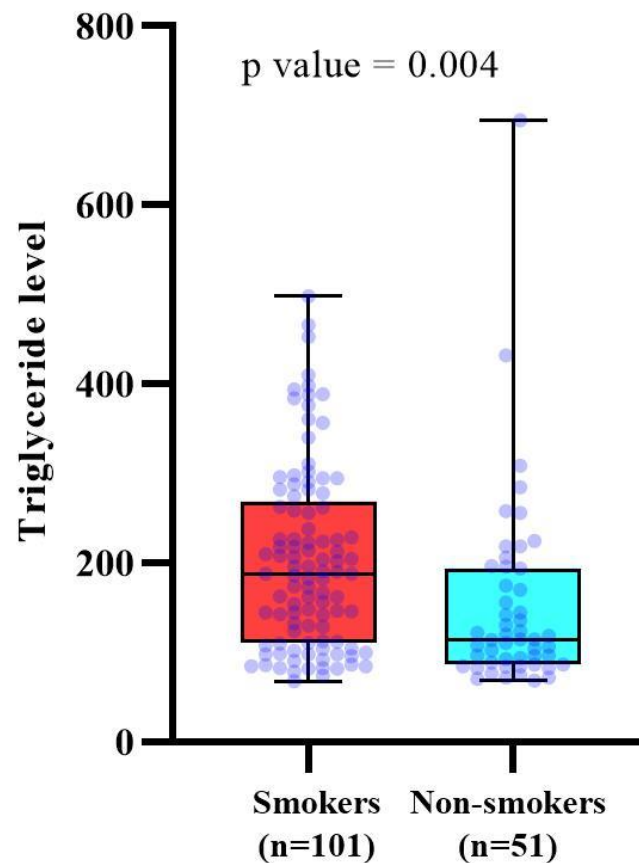
**Table 3. Comparison of serum triglyceride levels by smoking status**

| Smoking status      | Mean TG (mg/dL) $\pm$ SD | p value |
|---------------------|--------------------------|---------|
| Smoker (n = 101)    | $203.12 \pm 101.67$      | 0.004   |
| Non-smoker (n = 51) | $151.08 \pm 106.76$      |         |

*Independent two-sample t-test assuming unequal variances.*

To visually illustrate the distribution of triglyceride levels across smoking groups, a box-and-whisker plot with individual data points was constructed (Figure 2). The graphical representation further supports the statistical findings by demonstrating a higher median triglyceride level among smokers compared to non-smokers. Smokers also exhibited greater

variability in triglyceride levels, as indicated by a wider interquartile range. Although both groups showed the presence of outliers, the overall distribution of triglyceride levels was shifted toward higher values in the smoker group. The observed difference between groups was statistically significant, consistent with the results of the independent two-sample t-test ( $p = 0.004$ ).



**Figure 2. Comparison of serum triglyceride levels between smokers and non-smokers.** Box-and-whisker plots represent the distribution of triglyceride levels in smokers ( $n = 101$ ) and non-smokers ( $n = 51$ ). The central line within each box indicates the median, while the box boundaries represent the interquartile range (IQR). Whiskers denote the minimum and maximum values. Individual data points are overlaid to display participant-level variation. Smokers exhibited significantly higher triglyceride levels compared to non-smokers (independent two-sample t-test assuming unequal variances,  $p = 0.004$ ).

## **Chapter 4**

### **Discussion**

This study provides a comprehensive assessment of vitamin D status and biochemical markers among rickshaw pullers in Dhaka, Bangladesh. A key finding is the absence of vitamin D deficiency and the predominance of vitamin D insufficiency despite prolonged daily outdoor work. This contrasts with the high prevalence of deficiency reported in South Asian adults, where pooled data indicate that approximately two-thirds of adults in Bangladesh and neighboring countries are vitamin D deficient (serum 25(OH)D <20 ng/mL). A systematic review of studies encompassing over 44,000 adults across South Asia reported a pooled vitamin D deficiency prevalence of ~68%, with Bangladesh specifically estimated at ~67% considerably higher than observed in our occupational cohort (Siddiquee et al., 2021b).

#### **4.1 Vitamin D status in outdoor occupational groups**

The low prevalence of frank deficiency in this group likely reflects prolonged sun exposure inherent to rickshaw pulling, which increases cutaneous synthesis of vitamin D. Prior work suggests that outdoor laborers generally have higher serum 25(OH)D concentrations than indoor workers in the same geographic region, even when overall deficiency in the general population is high (Siddiquee et al., 2021b). However, the high rate of insufficiency (43.4%) in this cohort indicates that sun exposure alone may not guarantee optimal vitamin D status. Multiple factors documented in South Asia including skin pigmentation, clothing that limits skin exposure, atmospheric pollution, and limited dietary sources of vitamin D can blunt endogenous production (Khan et al., 2025). Dhaka's high levels of urban air pollution may notably attenuate ultraviolet B (UVB) radiation penetration, thereby reducing effective vitamin D synthesis despite outdoor activity (Yang et al., 2021).

## **4.2 Non-association with socio-demographic and occupational variables**

No significant association was observed between serum vitamin D levels and socio-demographic factors (age, education, income) or occupational conditions (years worked, hours per day, shift pattern). This suggests that uniform occupational sunlight exposure may homogenize vitamin D status across these categories, overshadowing effects of socio-economic determinants that are typically seen in general populations (Koh et al., 2022). Similarly, BMI was not significantly associated with vitamin D levels, possibly due to the low prevalence of obesity and the narrow BMI spectrum within this labor cohort.

## **4.3 Biochemical profiles and cardiometabolic risk**

Although liver function markers were largely normal, dyslipidemia was commonly observed, with elevated triglycerides in 36.8% and elevated total cholesterol in 34.2% of participants. These findings are consistent with population-level data from Bangladesh that document a high burden of dyslipidemia with elevated triglycerides, low HDL-cholesterol, and high LDL-cholesterol prevalent in adults across divisions of the country (Islam et al., 2023a). The high prevalence of multiple lipid abnormalities in this occupational group underscores a broader cardiometabolic risk that extends beyond micronutrient status.

Despite established biological links between vitamin D and lipid metabolism, no significant associations were found between vitamin D levels and lipid parameters in this study. Epidemiological findings on the vitamin D–lipid relationship have been mixed, and cross-sectional analyses with relatively narrow vitamin D distributions may lack power to detect modest metabolic effects (Islam et al., 2023b). More longitudinal studies are needed to clarify causal pathways.

#### 4.4 Smoking and triglycerides

A significant and robust association between *current smoking status and elevated triglyceride levels* was observed. This is consistent with a broad literature showing that smoking is linked to pro-atherogenic lipid profiles, including higher triglycerides and lower HDL-cholesterol (Syafii et al., 2024; van der Plas et al., 2023; Momayyezi et al., 2024). Meta-analytic evidence indicates that smokers tend to have significantly higher circulating triglyceride concentrations compared to non-smokers, likely due to nicotine-induced lipolysis and impaired lipid clearance mechanisms (Jain & Ducatman, 2018). Locally, smoking remains a common modifiable risk factor in Bangladeshi adult populations and has been independently associated with dyslipidemia in large national surveys (*Tobacco Smoking in Bangladesh*, n.d.). This underscores the importance of addressing smoking as a key target for metabolic risk reduction in this population.

The findings challenge the prevailing assumption that outdoor workers in South Asia will inevitably be vitamin D sufficient. While rickshaw pullers may be protected from overt deficiency due to sun exposure, a sizeable proportion remain insufficient, which may have implications for bone health, immune function, and chronic disease risk if unaddressed. A large population-based cohort study from Germany reported that vitamin D insufficiency and deficiency were associated with significantly increased mortality. Respiratory disease mortality was 2.1-fold higher in individuals with vitamin D insufficiency and 3.0-fold higher in those with deficiency compared with sufficient vitamin D status, with stronger associations observed among women. Importantly, 41% of respiratory disease-related deaths were statistically attributable to inadequate vitamin D levels, highlighting the potential public health impact of unaddressed insufficiency (Brenner et al., 2020). At the same time, the high burden of dyslipidemia and smoking-associated metabolic risk highlights the need for integrated occupational health interventions that extend beyond micronutrient supplementation. Programs

for this population may benefit from combining nutritional guidance, lipid screening, smoking cessation support, and education on sun exposure practices.

This study is one of the first to comprehensively evaluate vitamin D status and a broad panel of biochemical parameters in rickshaw pullers a socioeconomically marginalized group that is rarely sampled in health surveys. Using standardized laboratory assays and rigorous statistical analysis strengthens the validity of our findings.

However, the cross-sectional design precludes causal inference, and dietary vitamin D intake and quantitative sun exposure measures were not captured, which may introduce residual confounding. Additionally, convenience sampling may limit generalizability beyond similar urban outdoor worker populations.

## **Chapter 5**

### **Conclusion**

In conclusion, rickshaw pullers in Dhaka exhibited a low prevalence of vitamin D deficiency but a high prevalence of vitamin D insufficiency despite prolonged outdoor sun exposure. Serum vitamin D levels were not significantly associated with socio-demographic characteristics or most biochemical parameters. However, cigarette smoking was significantly associated with higher serum triglyceride levels, with smokers exhibiting markedly elevated triglyceride concentrations compared to non-smokers, indicating increased cardio metabolic risk among smoking individuals. These finding underscores smoking as a key modifiable lifestyle factor in this occupational group. Collectively, these results highlight the need for occupation-specific health strategies that address not only micronutrient status but also smoking-related metabolic risks to reduce long-term cardiovascular burden.

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