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Association Between Serum Bilirubin Levels and Depressive Symptoms Among University Students in Aden, Yemen: A Cross-Sectional Study

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Abstract

Background: Depressive symptoms are common among university students and have been linked to increased oxidative stress and impaired antioxidant defense. Bilirubin, an endogenous antioxidant, has recently emerged as a potential biomarker in psychiatric research; however, evidence regarding its association with depressive symptoms in university student populations remains limited and inconsistent. This study investigated the association between serum total and direct bilirubin levels and depressive symptoms among university students.

Methods: A cross-sectional study was conducted among 422 university students aged 18–25 years who were recruited from public and private universities in Aden, Yemen. Sociodemographic and lifestyle characteristics were collected using a structured questionnaire. Depressive symptoms were assessed using the Patient Health Questionnaire-9 (PHQ-9). Fasting venous blood samples were collected to measure serum total and direct bilirubin concentrations using the cobas c 311 clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Data were analyzed using descriptive statistics, correlation analyses, one-way analysis of variance (ANOVA), and multiple linear regression analyses.

Results: Depressive symptoms were identified in 88.2% of the participants, with mild symptoms being the most frequent category (32.0%), followed by severe (23.9%), moderate (22.0%), and moderately severe symptoms (10.2%). The mean serum total and direct bilirubin concentrations were 0.510 ± 0.247 mg/dL and 0.115 ± 0.056 mg/dL, respectively. PHQ-9 scores showed significant inverse correlations with total bilirubin ($r = -0.595$, $p < 0.001$) and direct bilirubin ($r = -0.452$, $p < 0.001$). After adjustment

for age, sex, marital status, field of study, monthly income, and academic stress, both total bilirubin ($\beta = -0.568$, $p < 0.001$) and direct bilirubin ($\beta = -0.431$, $p < 0.001$) remained significantly associated with PHQ-9 scores. The model including total bilirubin explained a greater proportion of the variability in depressive symptom scores than the model including direct bilirubin (adjusted $R^2 = 0.417$ vs. 0.287).

Conclusions: Lower serum bilirubin levels were significantly associated with greater depressive symptom severity among university students, with total bilirubin showing a stronger association than direct bilirubin. These findings support an association between serum bilirubin levels and depressive symptoms rather than a causal relationship. Prospective longitudinal studies are warranted to determine the temporal direction and clinical significance of this association.

Keywords: Depression; Depressive symptoms; Total bilirubin; Direct bilirubin; PHQ-9; University students; Oxidative stress.

Key Messages

What is already known?

Oxidative stress has been implicated in the pathophysiology of depressive symptoms. Serum bilirubin, an endogenous antioxidant, has been associated with depression in several clinical and population-based studies, although findings remain inconsistent.

What does this study add?

This study found that lower serum total and direct bilirubin levels were significantly associated with greater depressive symptom severity among university students. Total bilirubin showed a stronger association with depressive symptoms than direct bilirubin after adjustment for relevant sociodemographic and psychosocial factors. The findings provide evidence from a young, non-clinical university population in a resource-limited setting.

Implications for policy and practice

The findings support strengthening mental health screening and support services for university students, particularly in resource-limited settings. Further longitudinal studies are needed to determine whether serum bilirubin could have clinical utility as a biomarker for depressive symptoms and to better understand the temporal relationship between bilirubin levels and depression.

Introduction

Depression is one of the most common mental health disorders among university students worldwide and is associated with poor academic performance, impaired social functioning, and an increased risk of suicide (1,2). Its pathogenesis is multifactorial, involving genetic, psychological, environmental, and biological factors. Increasing evidence also suggests that oxidative stress and impaired antioxidant defense mechanisms may contribute to the development of depressive symptoms (3,4,5,6).

Bilirubin, a product of heme catabolism, has traditionally been used as a biochemical marker of liver function. However, growing evidence indicates that bilirubin also acts as an endogenous antioxidant with anti-inflammatory properties, potentially protecting neural tissues from oxidative damage (3,7). Altered serum bilirubin levels have been reported in several neuropsychiatric disorders, including major depressive disorder, bipolar disorder, and treatment-resistant depression (8,9).

Epidemiological and clinical studies have reported an association between serum bilirubin concentrations and depressive symptoms. Analyses of data from the National Health and Nutrition Examination Survey (NHANES), as well as findings from other observational studies, have shown that lower total bilirubin levels are associated with both the presence and severity of depressive symptoms after adjustment for potential confounding factors (10,11,12). In addition, variations in direct bilirubin concentrations have also been associated with depressive symptoms, suggesting that bilirubin may have potential value as a biomarker in depression research (2).

Several Mendelian randomization studies have further explored this relationship and have suggested a possible causal association between lower bilirubin levels and an increased risk of depression in specific populations, including postpartum women and individuals with diabetes (4,11,13). Proposed mechanisms include increased oxidative stress, neuroinflammation, and reduced antioxidant capacity. Although these findings provide biological plausibility, the underlying mechanisms linking bilirubin and depression remain incompletely understood (3,4,6).

Despite the growing body of evidence, most previous studies have focused on the general population or specific clinical groups. To the best of our knowledge, no published study has specifically examined the association between serum bilirubin levels and depressive symptoms among university students. University students in

Yemen are exposed to multiple psychosocial stressors, including prolonged political instability, economic hardship, and limited access to mental health services, all of which may increase vulnerability to depressive symptoms (14). Investigating this association in this population may provide additional insight into the relationship between endogenous antioxidant status and depressive symptoms during early adulthood. Therefore, this study aimed to investigate the association between serum total and direct bilirubin levels and depressive symptoms among university students in Aden, Yemen.

Subjects and Methods

Study design and setting

This was a cross-sectional, analytical study carried out at many public and private universities in Aden, Yemen.

Study Population

The study population included undergraduate university students aged 18 to 25 who were enrolled in several faculties at universities in Aden, Yemen.

Sample size calculation

The sample size was determined using Cochran's formula for cross-sectional studies:

$$n = \frac{z^2 \times p\%(1-p\%)}{d^2}$$

where n is the required sample size, Z is the standard normal deviate corresponding to a 95% confidence level (1.96), P is the estimated prevalence of depressive symptoms, and d is the margin of error (0.05).

Based on a previous study of Yemeni medical students, which found a prevalence of depressed symptoms of 48.4% (14), a conservative prevalence estimate of $P = 0.48$ was utilized for computation. Therefore, the sample size was computed as:

$$n = \frac{(1.96)^2 \times 0.48\%(1-0.48\%)}{(0.05)^2} \quad n = \frac{3.84 \times 0.249}{0.0025} = 382$$

To account for a potential non-response rate of 10%, the final required sample size was increased to 422 participants.

Sampling technique

A simple random sample technique was utilized to recruit participants from different faculties across selected universities, guaranteeing enough representation of various academic specialties.

Inclusion and Exclusion Criteria

The study population comprised undergraduate university students from public or private colleges in Aden, Yemen, aged 18 to 25 years, who provided written consent. The exclusion criteria for participation in the study included a medical history of liver conditions, such as viral hepatitis, cirrhosis, or cholestatic disorders, and hematological and genetic conditions affecting bilirubin metabolism, such as sickle cell disease, thalassemia, Gilbert's syndrome, and Crigler-Najjar syndrome. Other exclusion criteria were patients having been diagnosed with any psychiatric condition in the past, having been diagnosed and/or treated for depression or other mood-related disorders, consumption of drugs that have an impact on bilirubin metabolism (for instance, anticonvulsants such as valproate and phenytoin, rifampicin, some antibiotics, and chemotherapy), recent infections or sicknesses in the last two weeks, and pregnancy.

Data Collection Procedures:

The data collection procedure was done in an organized manner, involving several steps. First, the participant was required to fill out a sociodemographic and lifestyle questionnaire. This questionnaire included information on age, gender, marital status, year of study, area of specialization, smoking behavior, quality of sleep, and academic stress level. This questionnaire included information on age, gender, marital status, year of study, field of specialization, smoking habits, self-reported sleep quality, and self-reported academic stress level.

Second, Depressive symptoms were then assessed using the Patient Health Questionnaire-9 (PHQ-9), which is a validated questionnaire based on DSM criteria for major depressive disorder (15). The PHQ-9 consists of nine items, each assessed on a 4-point Likert scale ranging from 0 ("not at all") to 3 ("nearly every day"), for a total score of 0 to 27, with higher scores indicating more severe depressive symptoms. The severity of depression was classified as minimum, mild, moderate, moderately severe, or severe.

Finally, anthropometric measures were conducted to take into consideration potential physiological variables that could interfere with the analysis. Body weight was measured using a calibrated digital scale, while height was recorded using a stadiometer. The body mass index (BMI) was calculated from these parameters and was used as a covariate in the statistical analysis.

Sample Collection and Laboratory Procedures:

Venous blood samples were collected under standardized conditions. To minimize the influence of dietary intake on serum bilirubin concentrations, only participants who had fasted for 10–12 hours were included. All samples were obtained between 8:00 and 10:00 a.m. to reduce potential diurnal variation. A trained phlebotomist collected approximately 5 mL of venous blood from the antecubital vein using standard aseptic techniques. Blood was transferred into serum separator tubes (SSTs) and allowed to clot at room temperature for approximately 30 minutes before centrifugation at 3000 rpm for 10 minutes. The separated serum was transferred into appropriately labeled aliquot tubes identified by unique participant codes to ensure confidentiality. Hemolyzed samples were excluded because hemolysis may interfere with bilirubin measurement. To minimize photodegradation, serum samples were protected from direct light during collection, processing, and analysis.

Serum total and direct bilirubin concentrations were measured immediately after sample processing using the cobas c 311 clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions (16). The analyzer performs automated photometric clinical chemistry assays using dedicated reagents and standardized calibration procedures. Bilirubin concentrations were determined using a diazo-based colorimetric method, in which bilirubin reacts with diazotized sulfanilic acid to produce colored azobilirubin compounds that are quantified spectrophotometrically (17).

Before analysis, calibration of the analyzer was performed using manufacturer-provided calibrators according to the recommended procedures. Internal quality control was routinely performed using commercially available control materials at different concentration levels to monitor assay precision and accuracy. Analytical results were accepted only when control values were within the predefined acceptable ranges, in accordance with laboratory quality assurance principles (18). The reliability and standardization of bilirubin measurements were further supported by established recommendations for bilirubin assay standardization and analytical performance evaluation (19).

Data Analysis:

Data were analyzed using IBM SPSS Statistics software (version 25). Continuous variables were presented as mean $\pm$ standard deviation (SD), while categorical variables were summarized as frequencies and percentages. The normality of continuous variables was assessed using the Shapiro–Wilk test, supported by visual inspection of histograms and Q–Q plots. Homogeneity of variances was evaluated using Levene’s test.

Pearson’s correlation coefficient was used to assess associations between continuous variables, including serum total and direct bilirubin levels, age, BMI, and PHQ-9 scores. Point-biserial correlation was applied for dichotomous variables, while Spearman’s correlation was used for ordinal variables. One-way analysis of variance (ANOVA) followed by the Games–Howell post hoc test (when the assumption of homogeneity of variances was violated) was performed to compare serum bilirubin levels across categories of depressive symptom severity. Effect sizes were reported using eta squared (η^2).

Multiple linear regression analyses were conducted to examine factors independently associated with PHQ-9 scores after adjustment for potential confounding variables. Regression results were presented as unstandardized coefficients (B), standardized coefficients (β), 95% confidence intervals (CI), and p-values. Multicollinearity among independent variables was assessed using variance inflation factor (VIF) values, with VIF <5 considered acceptable. A two-sided p-value <0.05 was considered statistically significant.

Results

The mean age of the 422 participants was 22.84 ± 4.17 years, with the majority aged 20–24 (60.4%). Females accounted for 50.7% of the sample, and most participants were single (84.1%) and medical students (55.0%). The majority were enrolled in bachelor’s programs (83.4%). The second academic year had the highest proportion (26.3%). The mean BMI was 24.8 ± 4.2 kg/m². The majority of subjects (76.8%) did not smoke, had poor sleep quality (53.3%), and most participants reported moderate academic stress (38.2%), and were financially dependent on their parents (78.2%) (Table 1).

Table 1: Baseline Characteristics of Participants (N = 422)

Category	Frequency (n)	Percentage (%)
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Age (years): Mean $\pm$ SD = 22.84 $\pm$ 4.17		
≤ 19	58	13.7%
20-24	255	60.4%
≥ 25	109	25.8%
Sex		
Male	208	49.3%
Female	214	50.7%
Marital Status		
Single	355	84.1%
Married	67	15.9%
Field of Study		
Medical	232	55.0%
Non-medical	190	45.0%
Education level		
Bachelor	352	83.4%
Master	70	16.6%
Year of Study		
First	108	25.6%
Second	111	26.3%
Third	102	24.2%
Fourth or more	101	23.9%
BMI		
Mean $\pm$ SD (24.8 $\pm$ 4.2)		
Smoking status		
Yes	98	23.2%
No	324	76.8%
Sleep quality		
Good	197	46.7%
Poor	225	53.3%
Stress level		
Low	101	23.9%
Moderate	161	38.2%
High	160	37.9%
Income		
Independent income	92	21.8%
Depends on parents	330	78.2%

221 The mean $\pm$ SD values among the study participants were 0.510 $\pm$ 0.247 for total
222 bilirubin, 0.115 $\pm$ 0.056 for direct bilirubin, and 12.34 $\pm$ 7.54 for PHQ-9 scores (Table
223 2).

224 **Table 2. Mean and Standard Deviation of Total Bilirubin, Direct Bilirubin, and**
225 **PHQ-9 scores among Study Participants**

Variable	Mean $\pm$ SD	Rang
Total bilirubin (mg/dL)	0.510 $\pm$ 0.247	0.11-1.9
Direct bilirubin (mg/dL)	0.115 $\pm$ 0.056	0.0-0.70

PHQ-9 score	12.34 ± 7.54	0-27
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Depressive symptoms, as assessed by the PHQ-9, were identified in 88.2% of participants. Mild depression was the most common category (32.0%), followed by severe depression (23.9%) and moderate depression (22.0%), whereas only 11.8% reported no depressive symptoms (**Figure 1**).

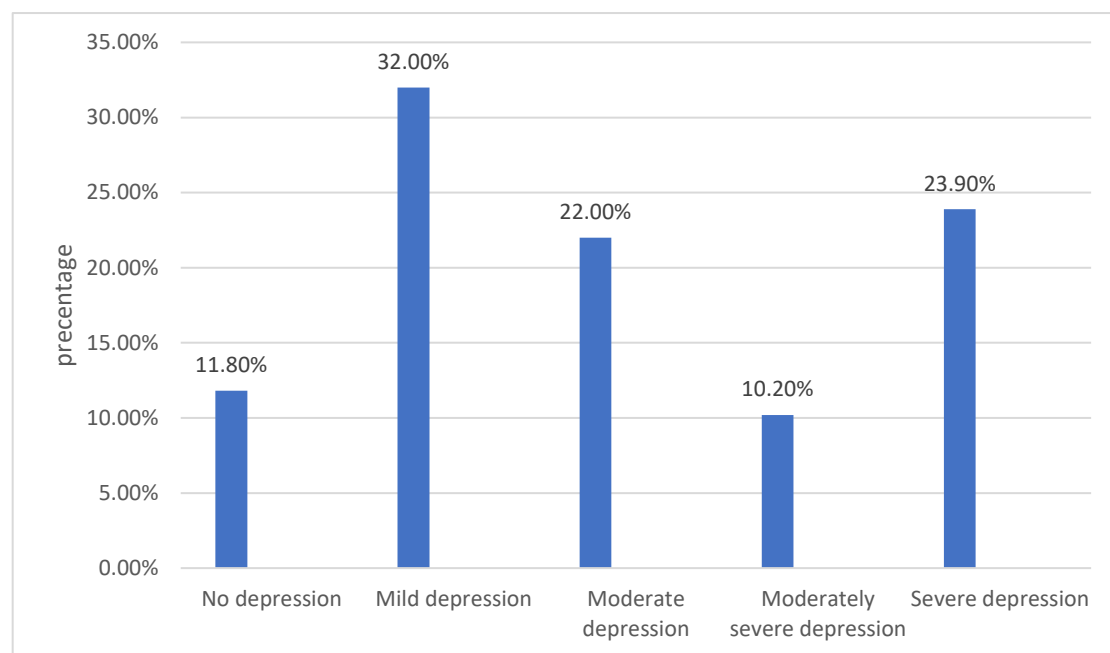


Figure 1. Distribution of depression severity among university students according to PHQ-9 scores (N = 422)

Correlation analyses showed significant inverse associations between PHQ-9 scores and serum total bilirubin ($r = -0.595$, $p < 0.001$) and direct bilirubin ($r = -0.452$, $p < 0.001$). Significant associations were also observed between PHQ-9 scores and several sociodemographic and academic variables, including field of specialization, sex, income status, age, academic stress, and marital status (Table 3).

Table 3: Correlation of study variables with PHQ-9 scores among university students (N = 422)

Variable	Correlation coefficient	p-value
Total bilirubin	-0.595**	<0.001
Direct bilirubin	-0.452**	<0.001
Field of specialization	0.208**	<0.001
Students gender	0.204**	<0.001
Students income	-0.184**	<0.001
Age	-0.118*	0.015
Self-reported academic stress	0.124*	0.011

Marital status	-0.114*	0.019
Year of study	-0.014	0.775
Educational level	-0.014	0.768
Body mass index	-0.026	0.599
Smoking status	0.009	0.859
Sleep quality	0.011	0.826

Pearson's correlation was used for continuous variables, point-biserial correlation was used for dichotomous variables, and Spearman's correlation was used for ordinal variables.

One-way ANOVA revealed statistically significant differences in both total and direct bilirubin levels across depression severity groups ($p < 0.001$). Levene's test indicated a violation of homogeneity of variances for both total bilirubin ($p < 0.001$) and direct bilirubin ($p < 0.001$); therefore, Games–Howell post hoc tests were applied. Total bilirubin levels generally decreased across increasing depression severity categories, from 0.769 ± 0.274 mg/dL in the non-depressed group to 0.326 ± 0.082 mg/dL in the severe depression group. A similar pattern was observed for direct bilirubin levels. Post hoc analysis showed that participants without depression had significantly higher bilirubin levels compared to all depressed groups ($p < 0.001$). Significant differences were also observed between most depression severity categories ($p < 0.05$), except for some comparisons involving adjacent severity levels. (Table 4)

Table 4: Comparison of Bilirubin Levels Across Depression Severity Categories

Depression severity category	N	Total bilirubin (Mean $\pm$ SD)	95% CI	Direct bilirubin (Mean $\pm$ SD)	95% CI
No depression	50	0.769 ± 0.274	0.692–0.847	0.158 ± 0.056	0.142–0.174
Mild depression	135	0.625 ± 0.261	0.581–0.670	0.136 ± 0.063	0.125–0.146
Moderate depression	93	0.497 ± 0.132	0.470–0.524	0.109 ± 0.030	0.102–0.115
Moderate-severe depression	43	0.307 ± 0.102	0.276–0.339	0.084 ± 0.100	0.053–0.114
Severe depression	101	0.326 ± 0.082	0.310–0.342	0.074 ± 0.024	0.069–0.079
Statistical tests					
Test		Total bilirubin		Direct bilirubin	
Levene's test		$p < 0.001$		$p < 0.001$	
One-way ANOVA (F)		69.567 ($p < 0.001$)		30.466 ($p < 0.001$)	

Effect size (η^2)	0.40	0.22
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Full post hoc results are provided in Supplementary Table S1.

Figure 2 demonstrates a progressive decline in both total and direct bilirubin levels with increasing depression severity. Participants without depression showed the highest bilirubin levels, whereas those with moderate-severe and severe depression exhibited the lowest levels

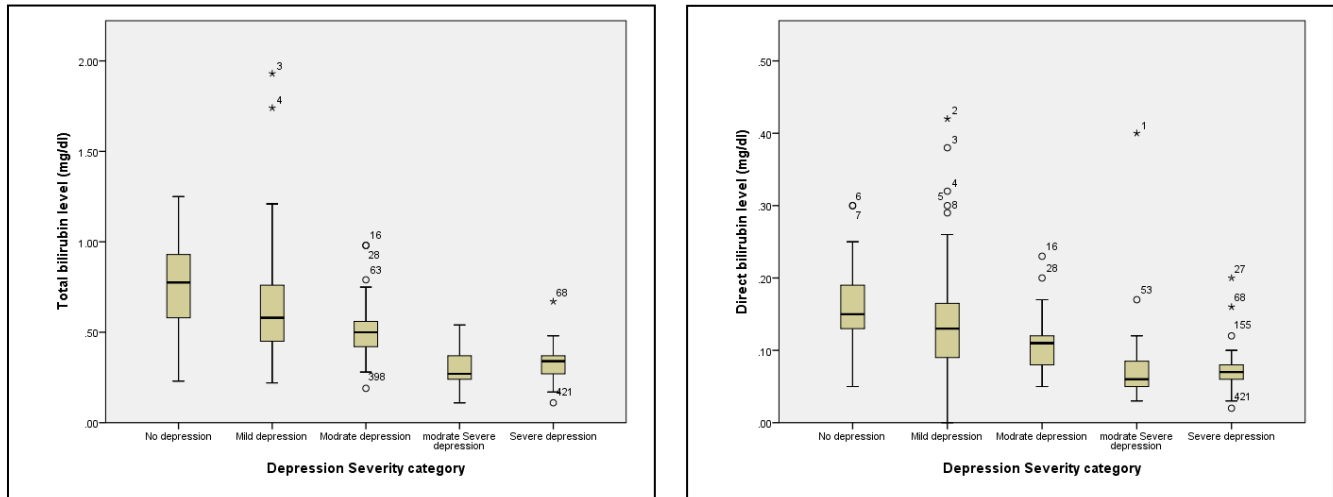


Figure 2: Distribution of total bilirubin and Direct bilirubin levels across depression severity categories.

Multiple linear regression analysis was performed to assess the association between serum total bilirubin levels and PHQ-9 scores after adjusting for potential confounding variables. Variance inflation factor (VIF) values were examined to assess multicollinearity among the independent variables, and no evidence of significant multicollinearity was observed. Lower total bilirubin levels were significantly associated with higher PHQ-9 scores ($B = -17.282$, $\beta = -0.568$, 95% CI: -19.556 to -15.007 , $p < 0.001$). Female sex, non-medical specialization, and independent income were also significantly associated with PHQ-9 scores, whereas age, marital status, and academic stress were not significant contributors. The model accounted for 42.6% of the variance in PHQ-9 scores ($R^2 = 0.426$; adjusted $R^2 = 0.417$) (Table 5).

Table 5: Multiple Linear Regression Analysis of Factors Associated with PHQ-9 score (Total Bilirubin Model)

Predictor	B	β	95% CI	p-value
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Total bilirubin (mg/dL)	-17.282	-0.568	-19.556 to -15.007	<0.001
Age	-0.323	-0.026	-1.320 to 0.673	0.524
Female sex	1.287	0.085	0.157 to 2.418	0.026
Married	-1.414	-0.069	-3.019 to 0.192	0.084
Non-medical specialization	2.905	0.192	1.774 to 4.036	<0.001
Independent income	-1.478	-0.084	-2.939 to -0.016	0.048
Academic stress	0.392	0.036	-0.424 to 1.209	0.345

Model fit: $R = 0.653$, $R^2 = 0.426$, $adjusted\ R^2 = 0.417$, $F(7, 414) = 43.941$, $p < 0.001$.

Multiple linear regression analysis was conducted to examine the association between serum direct bilirubin levels and PHQ-9 scores. Assessment of multicollinearity using VIF values showed no significant collinearity among the included predictors. Direct bilirubin was significantly associated with PHQ-9 scores after adjustment for potential confounders. ($B = -52.643$, $\beta = -0.431$, 95% CI: -62.629 to -42.656 , $p < 0.001$). Female sex ($B = 2.037$, $p = 0.001$), non-medical specialization ($B = 2.968$, $p < 0.001$), and independent income ($B = -1.771$, $p = 0.032$) were also significantly associated with PHQ-9 scores, whereas age, marital status, and academic stress were not. The model explained 29.9% of the variance in PHQ-9 scores ($R^2 = 0.299$; $adjusted\ R^2 = 0.287$; $F = 25.224$, $p < 0.001$) (Table 6).

Table 6: Multiple Linear Regression Analysis of Factors Associated with PHQ-9 score (Direct Bilirubin Model)

Predictor	B	β	95% CI	p-value
Direct bilirubin (mg/dL)	-52.643	-0.431	-62.629 to -42.656	<0.001
Age	-0.351	-0.029	-1.453 to 0.751	0.532
Female sex	2.037	0.135	0.797 to 3.277	0.001
Married	-1.352	-0.066	-3.127 to 0.423	0.135
Non-medical specialization	2.968	0.196	1.718 to 4.219	<0.001
Independent income	-1.771	-0.101	-3.386 to -0.157	0.032
Academic stress	0.545	0.050	-0.357 to 1.447	0.235

Model fit: $R = 0.547$, $R^2 = 0.299$, $adjusted\ R^2 = 0.287$, $F(7, 414) = 25.224$, $p < 0.001$.

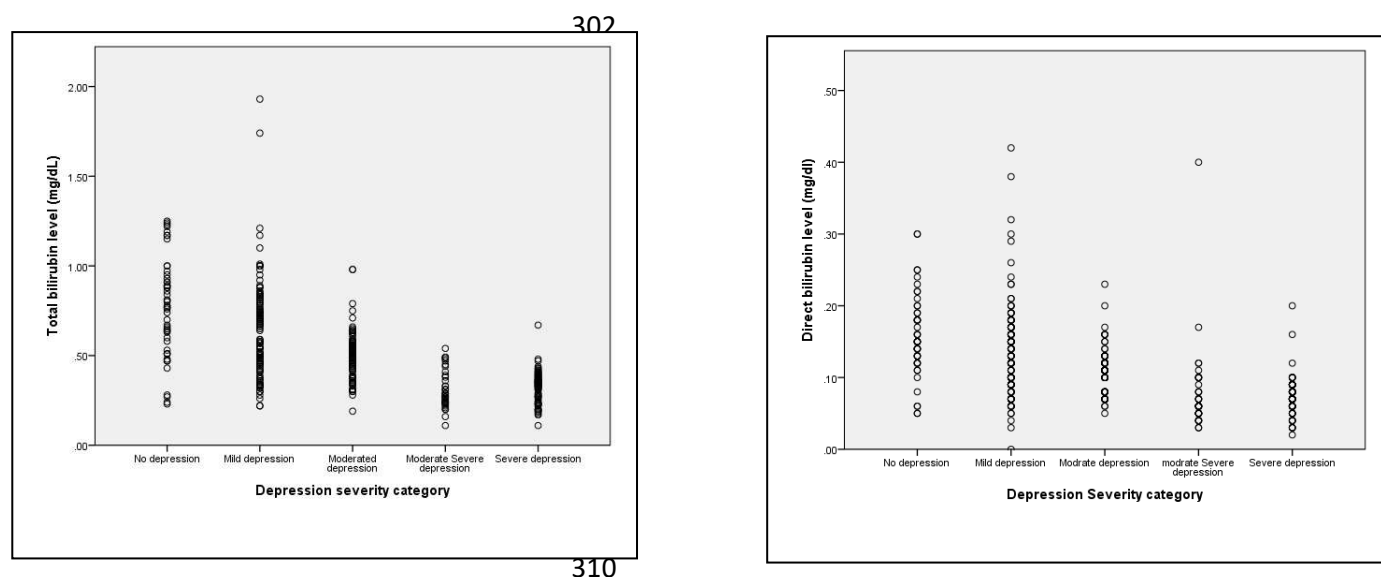


Figure 3: Dot plot showing individual bilirubin measurements according to depression severity

Figure 3 shows the distribution of individual bilirubin values among depression severity groups. As the severity of depression increased, both total and direct bilirubin levels gradually decreased. Despite a few statistical outliers, the overall trend remained consistent, supporting the inverse association observed in the correlation and regression analyses.

Discussion

The present study found that depressive symptoms were prevalent among university students, with 88.2% of participants screening positive for depressive symptoms based on PHQ-9 scores. Mild depressive symptoms were the most common category (32.0%), followed by severe (23.9%), moderate (22.0%), and moderately severe symptoms (10.2%). The high proportion of students with depressive symptoms indicates a considerable mental health concern that warrants further attention among university populations, particularly in settings exposed to multiple psychosocial stressors.

Compared with previous studies conducted in Yemen, the prevalence observed in the current study was higher than that reported among Yemeni medical students nationwide (48.4% using the PHQ-9) (14) and among medical students in Sana'a (73.67%) (20). Conversely, the prevalence was lower than that reported in Taiz, where depressive

symptoms were observed among all participants and 40% had extremely severe symptoms (21). Differences between studies may be related to variations in study populations, sample size, geographic setting, assessment tools, and the timing of data collection. In addition, factors such as ongoing social challenges, economic difficulties, academic pressure, and limited access to mental health services may contribute to differences in the prevalence of depressive symptoms across regions.

Different studies conducted among university students in Arab countries revealed different prevalence rates of depressive symptoms, such as in Saudi Arabia, ranging from 30.9% to 77.6% (22), while other studies have reported a prevalence of 54% (23). In the United Arab Emirates, the prevalence is 34.2% (24); in Palestine, it is estimated at 40%; and in Sudan, it is 77.9% (25). Such differences may reflect variations in socioeconomic conditions, educational environments, availability of psychological support services, and methodological approaches used for assessment.

Globally, the prevalence observed in the present study was higher than that reported in several international studies. A recent meta-analysis estimated the overall prevalence of depressive symptoms among medical students at approximately 31% (26), while another meta-analysis conducted in low- and middle-income countries reported a prevalence of 24.4% (27). The relatively higher prevalence observed in this study may be related to the combined influence of academic demands, economic uncertainty, social stressors, and limited access to mental health resources. However, differences in screening instruments, study populations, and contextual factors should be considered when comparing prevalence estimates.

The present study demonstrated a significant inverse association between serum bilirubin levels and depressive symptoms. Both total and direct bilirubin levels remained significantly associated with PHQ-9 scores after adjustment for relevant sociodemographic and psychosocial factors, suggesting that lower bilirubin concentrations are associated with greater severity of depressive symptoms. Notably, total bilirubin showed a stronger association with PHQ-9 scores than direct bilirubin, as indicated by its larger standardized coefficient and greater proportion of explained variance in the regression model.

These findings add to the existing evidence by examining this association in a young, non-clinical university population, which remains relatively underrepresented in biomarker-based mental health research. Previous studies have reported altered bilirubin levels among individuals with depressive disorders, including major depressive disorder and postpartum depression, suggesting that variations in endogenous antioxidant status may be related to depressive symptomatology. However, available evidence remains heterogeneous due to differences in study populations, methodologies, and clinical characteristics.

The inverse association observed in the current study is consistent with previous reports showing lower serum bilirubin levels among individuals with depressive disorders. For example, individuals with major depressive disorder have been reported to exhibit lower bilirubin concentrations compared with healthy controls, suggesting that alterations in antioxidant status may be involved in depressive symptom expression (1). Similarly, lower bilirubin levels have been associated with an increased likelihood of postpartum depression in previous studies (13). Large-scale investigations using different analytical approaches have also identified bilirubin among biochemical markers associated with depressive symptoms (5). Furthermore, studies using biochemical profiling and machine learning approaches have suggested that bilirubin-related metabolic markers may be involved in biological pathways associated with depression (6,7). Clinical and population-based studies have also reported associations between bilirubin concentrations and depressive symptom severity, supporting a potential relationship between bilirubin metabolism and mental health outcomes (3,8,11,12).

In addition to depression-related findings, bilirubin metabolism has also been investigated in other psychiatric disorders, including schizophrenia and bipolar disorder, although the available evidence remains inconsistent. Some studies have reported associations between lower bilirubin levels and metabolic disturbances in schizophrenia (28), whereas others have not demonstrated significant relationships (29). Differences in unconjugated bilirubin concentrations have also been reported between schizophrenia and bipolar disorder, suggesting potential differences in metabolic pathways across psychiatric conditions (30). Collectively, these findings

indicate that bilirubin alterations may reflect broader neurobiological and metabolic processes rather than being specific to depressive disorders.

Bilirubin has been recognized as an endogenous antioxidant involved in the regulation of oxidative balance and inflammatory responses. It can neutralize reactive oxygen species and reduce lipid peroxidation, potentially contributing to cellular protection against oxidative damage (31). Alterations in bilirubin concentrations may therefore reflect changes in antioxidant status that could be relevant to biological mechanisms associated with depressive symptoms. Additionally, the heme oxygenase–biliverdin–bilirubin pathway has been implicated in cellular stress responses and neuroprotective mechanisms (32). Dysregulation of this pathway may influence oxidative–antioxidant balance and inflammatory processes, which are increasingly recognized as contributing factors in depression-related biological pathways (33,34,35).

The present study also identified significant associations between depressive symptoms and several sociodemographic factors. Female sex, non-medical specialization, and income status were associated with PHQ-9 scores in the adjusted regression models, whereas sleep quality and academic stress did not remain statistically significant after adjustment. These findings are partially consistent with previous studies emphasizing the influence of academic environment and socioeconomic conditions on the mental well-being of university students (36,37,38). Differences in the impact of individual factors may reflect variations in population characteristics and contextual circumstances.

Strengths and Limitations

There are several advantages associated with this study. These include a large sample size and assessed bilirubin levels across several levels of depression severity. and control of several demographic and psychological factors, which increased the validity of the results obtained.

However, certain limits must be noted; First, since this is a cross-sectional study, causative associations between bilirubin and depressed features could not be determined. Secondly, the population for this study was limited to university students only, which may limit its applicability to other age groups or clinical populations. Third, indirect bilirubin and inflammation markers were not evaluated, thus limiting the analysis of mechanistic processes. Finally, residual confounding by eating choices,

physical activity, and unmeasured health issues cannot be ruled out. Future longitudinal studies are required to determine whether decreased bilirubin concentrations precede the onset of depression or are the consequence of oxidative stress induced by depression. Future research that involves inflammatory cytokines, oxidative stress markers, and neuroimaging data may contribute to the understanding of the molecular mechanisms involved in the association between bilirubin and depression. In addition, future prospective studies should examine the possibility of using bilirubin as an available biomarker of depression in college-aged individuals.

Conclusions

The findings of this study demonstrated a statistically significant inverse association between serum total and direct bilirubin levels and depressive symptoms among university students. Lower bilirubin concentrations were associated with greater depressive symptom severity. These significant association were confirmed after adjustment for potential confounding factors by using multivariable regression analysis. Our findings support the hypothesis regarding associations between oxidative stress, antioxidant status, and depressive symptoms. Further longitudinal studies are needed to clarify the temporal relationship and to evaluate whether serum bilirubin has potential utility as a biomarker of depressive symptoms.

Acknowledgments

The authors would like to express their sincere appreciation to all university students who participated voluntarily in this study for their time and honest responses.

Declarations

Human Ethics Approval and Consent to Participate

Human Ethical approval for the study was obtained from the Research Ethics Committee of the University of Science and Technology, Aden, Yemen (MEC /AD0137). Our research was conducted in accordance with principles of the ethical Declaration of Helsinki. Written informed consent was obtained from all participants after they were provided with detailed information about the study's objectives, procedures, and potential risks. The confidentiality and anonymity of all participants were strictly maintained throughout the study, ensuring that personal information and responses were handled securely and reported only in aggregate.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated during this study are included in this manuscript. Additional information can be made available upon reasonable request.

Conflict of interest

The authors declared no conflicts of interest.

Funding

Not applicable

Authors' Contributions

HAAM conducted the research and prepared the manuscript. ANMG supervised the research and provided critical guidance and revision of the manuscript.

AI use statement

It is acknowledged by the authors that Grammarly and ChatGPT were used to correct language and improve grammar, while QuillBot was used to paraphrase and make documents clearer. All AI-assisted technologies were utilized with the authors' oversight. The authors examined and validated all outputs and are solely responsible for the manuscript's accuracy, integrity, and final content.

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