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Research Article

Association of serum bilirubin levels and glycemic measurements in Type 2 diabetic patients

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Abstract

Introduction: Bilirubin, a waste product of the heme catabolic pathway, has proven to be a natural antioxidant associated with a lower prevalence of oxidative stress-mediated diseases. This study evaluates the association of serum bilirubin levels with Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS) in diabetic patients. Methods: A retrospective analytical study was conducted at the Department of Haematology of the Sri Jayewardenepura General Hospital. Data was obtained through a laboratory information system of randomly selected 201 adult patients diagnosed with T2DM and grouped as poorly and well controlled using HbA1c values over and below 7%, respectively. Normal ranges for serum bilirubin, FBS, and PPBS were (0.3-1.2) mg/dL, (75-110) mg/dL and (80-140) mg/dL, respectively. Data were analyzed using (SPSS) version 20, descriptive statistical methods, Pearson's Correlation test and independent sample t-test. $P<0.05$ was considered significant. Results: A negative correlation between total bilirubin and HbA1c ($r = -0.234$, $p=0.001$) was observed. There was no statistically significant correlation between total bilirubin level and FBS ($p=0.131$), PPBS ($p=0.408$), and age ($p=0147$). However, the mean values of total bilirubin ($p=0.001$), FBS ($p<0.001$) and PPBS ($p<0.001$) were significant between diabetes well controlled and poorly controlled groups. A statistically positive correlation was observed between HbA1c and FBS ($p<0.001$) and PPBS ($p<0.001$), but not with age ($p=0.887$). Conclusion: There was a significant relationship between total bilirubin with FBS, and PPBS in both groups. A significant negative correlation was found between total bilirubin and HbA1c, suggesting a potential protective or modulatory role of bilirubin in glycemic control. No significant correlation was observed between total bilirubin levels and FBS, PPBS, or age in the total population.

Keywords: T2DM, HbA1c, bilirubin, FBS, PPBS

Introduction

Type 2 Diabetes Mellitus (T2DM) is an endocrine disorder. This is characterized by high levels of glucose in the blood resulting from variable degrees of insulin resistance and deficiency. Chronic hyperglycemia can lead to multi-organ damage resulting in many complications in renal, neurologic, and cardiovascular systems [1].

The worldwide prevalence of adult T2DM is 9% in men and 7.9% in women [2]. In 2005, a study conducted in four Provinces (Western, North Central, Southern, and Uva) in Sri Lanka reported a diabetes prevalence of 14.2% among men, and 13.5% among women in the adult population aged between 35 and 65 years [3].

Glycemic control is very important in reducing morbidity and mortality of the disease. Control of blood glucose levels in patients with diabetes can

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be determined by the estimation of Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS). HbA1c level remains the gold standard for the assessment of diabetes at follow-up [4].

T2DM is a chronic low-grade inflammation that causes a reduction of the antioxidant capacity of the human body. In T2DM, hyperglycemia is the main cause of the complications. Hyperglycemia causes the glycation of serum proteins. In glycation, free amino groups of proteins, lipids, and nucleotides are modified by monosaccharides. During the reaction, advanced glycation end products (AGEs) are formed. Chronic hyperglycemia accelerates the reaction between monosaccharides and proteins to form AGEs. HBA1c is also an AGE that the hemoglobin compound produced after glucose reacts with the amino group on a hemoglobin molecule forming a ketoamine [5].

HbA1c level indicates the average blood sugar over the past three months. Regardless of underlying treatment, HbA1c more than 7.0 % is associated with a significantly increased risk of both microvascular and macrovascular complications. Every 1% reduction in HbA1c is said to result in a nearly 20% decrease in the risk of death related to diabetes and more than 35% decrease in the risk of microvascular complications [6].

A few decades ago, bilirubin was believed to be only a waste product of the heme catabolic pathway and a potentially toxic compound. However, recent data has shown that mildly elevated levels of serum bilirubin are strongly associated with a lower prevalence of oxidative stress-mediated diseases. Bilirubin is a natural antioxidant of the body which has protective effects against free oxygen radicals. Bilirubin plays an important role in preventing glycation of proteins. This capability is an important benefit in T2DM and macrovascular diseases [7]. This study is designed to investigate the relationships between serum bilirubin levels and key glycemic indicators, namely HbA1c, FBS, and PPBS, in individuals diagnosed with diabetes.

Methods

Study Design

A retrospective analytical study.

Center of the Study

Department of Haematology of the Sri Jayewardenepura General Hospital.

Sample Population, Sample Size and Selection

The sample size for this study was determined through a simple random sampling technique, focusing exclusively on patients with available data for bilirubin, FBS, PPBS, and HbA1c within the Laboratory Information System (LIS). The decision to restrict the sample to individuals with bilirubin, FBS, PPBS, and HbA1c records in the LIS aimed to ensure a focused examination of the relationship between these specific parameters in the context of diabetes management.

The patients diagnosed with Type 1 diabetes mellitus, patients with hypothyroidism, Cushing's syndrome, chronic systemic illness, hepatic impairment, renal disorders, heart failure, pregnancy, and cancer were excluded from the study.

Our study population was grouped into two according to the HbA1c level. Subjects with HbA1c levels over 7% were grouped as poorly controlled, and 7% or lower were grouped as well controlled T2DM [7]. According to World Health Organization (WHO), the normal range for serum bilirubin for adults was (0.3-1.2) mg/dL. Normal ranges for FBS and PPBS were (70-100) mg/dL and (100-140) mg/dL, respectively.

Data Collection

Laboratory data, generated by the Mindray BS-480 Automated Chemistry Analyzer, Abbott Architect Plus Analyzer, and BIO RAD D10 Analyzer, was collected through the Laboratory Information System (LIS).

Statistical Analysis

Data were double-entered and were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistical methods were used to calculate the median, mean, and standard deviation of age, serum bilirubin, HBA1c, FBS, and PPBS. Correlations between study variables were assessed using Pearson's Correlation test. The p-value, lower than 0.05 was considered statistically significant. The coefficient of determination (R Sq) was used as a statistical measure of how close the data are to the fitted regression line. Two diabetes groups were compared by independent sample t-test.

Ethical approval was obtained from the Ethics Review Committee of Sri Jayewardenepura General Hospital and Post Graduate Training Centre, Thalapathpitiya, Nugegoda, Sri Lanka.

Results

Of the total of 201 patients' data analyzed, 136 were female and 65 were male. The age range was between 31 to 83 years and the mean ±SD age was 59.3±10.56 years. The data was categorized under six age categories as indicated in Table 1.

Table 1: Age categories of the patients

Age Category (Years)	Frequency
31 - 40	11
41 - 50	32
51 - 60	64
61 - 70	68
71 - 80	23
81 - 100	3

The mean±SD values of total bilirubin, HbA1c, FBS, and PPBS were 0.61±0.34 mg/dL, 7.85±1.84 %, 134.0 ± 54.6 mg/dL and 182.7± 83.4 mg/dL, respectively (Table 2).

The mean±SD value of total bilirubin was high in the (41-50) age category and it was 0.762±0.55 mg/dL. The age category (51-60) showed the highest mean±SD value for HbA1c (8.25±2%). However, the 61-70 age category and 81-100 age category showed the highest mean value of FBS (140.6±62.4 mg/dL) and PPBS (233.1±103.1 mg/dL), respectively (Table 3).

When Pearson's correlation test was applied to evaluate the significance of HbA1c with FBS, PPBS, and age of diabetes-controlled and poorly controlled patients (Table 4), there was a significant positive correlation between HbA1c and FBS (r=0.0.369, p<0.001) with the R Sq of 0.0.136 and showing a moderate linear relationship (Fig. 1).

A significant positive correlation between HbA1c and PPBS (r=0.436, p<0.001) showed a moderate linear relationship with R Sq of 0.19. There was no statistically significant relationship between HbA1c and age (p=0.887).

Table 2: Descriptive data of age, total bilirubin, Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS)

Variable	Unit	Minimum	Maximum	Mean	Std. Deviation
Age	Years	31.0	83.0	59.3	10.6
Total bilirubin	mg/dl	0.2	2.6	0.6	0.3
HbA1C	%	4.7	14.5	7.9	1.8
FBS	mg/dl	55.7	425.7	134.0	54.6
PPBS	mg/dl	59.0	486.0	182.7	83.4

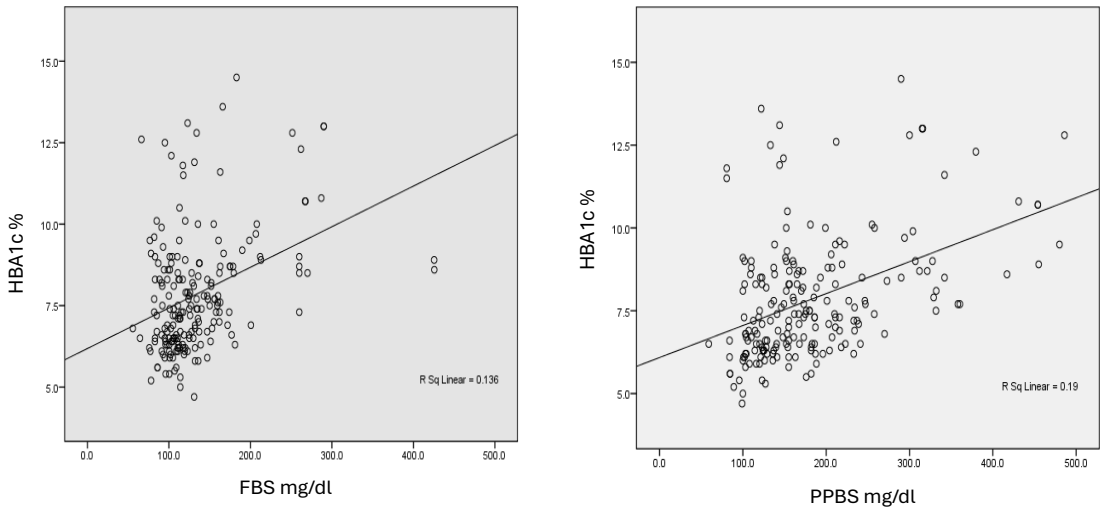

Figure 1: Scatter diagram of Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS) with Glycated Hemoglobin (HbA1c)

Our study population was grouped into two according to the HbA1c levels. There were 80 subjects in the well-controlled diabetes group and 121 were included in the poorly controlled group.

The independent sample t-test was done between well-controlled and poorly controlled diabetes groups to compare the mean values of total bilirubin levels, HbA1c, FBS, PPBS, and age. (Table 5).

The mean values of total bilirubin, FBS, and PPBS of two diabetes groups were compared and it showed a statistically significant relationship ($p<0.001$). However, there was no statistically significant relationship ($p=0.119$) between the mean values of age of the two diabetes groups.

Pearson's correlation was done to evaluate the effect of total bilirubin levels on the measurement of HbA1c, FBS, and PPBS in diabetes patients (Table 6). There was a significant negative correlation between total bilirubin and HbA1c ($r = -0.234$, $p=0.001$) which also revealed a low linear relationship (R Sq of 0.055, Fig. 2). There was no statistically significant relationship between the total bilirubin level and FBS ($p=0.131$), PPBS ($p=0.408$), and age ($p=0.147$).

When the mean values of HbA1c in the male (7.58%) and females (7.98%) were compared by independent sample t-test, there was no statistically significant difference between these variables ($p=0.965$).

Discussion

Glycemic control in diabetes mellitus is important in reducing morbidity and mortality of the disease. Monitoring HbA1c level, FBS, and PPBS play a major role in diabetes control. HbA1c level and daily monitoring of plasma blood glucose levels are very important to set up daily blood sugar testing goals to achieve HbA1c levels that have a low risk of diabetes outcomes. In resource-poor settings, it is difficult to perform HbA1c and there is no consensus on whether only FBS and PPBS are better predictors in diabetes assessment. On the other hand, HbA1c also neither captures blood sugar fluctuations in short periods nor provides any information on recent control of diabetes.

In our research, we were able to prove that, there was a statistically significant positive correlation between HbA1c with FBS and PPBS in diabetes-controlled and poorly controlled patients. The HbA1c test is more expensive than the conventional FBS and PPBS estimations and a very expensive analyzer should be in the

Table 3: Descriptive data for age categories

Variable	Age Category (Year)	Minimum	Maximum	Mean	Std. Deviation
Total Bilirubin (mg/dL)	30 - 40	0.3	0.7	0.49	0.15
	41 - 50	0.3	2.6	0.76	0.55
	51 - 60	0.2	1.2	0.57	0.25
	61 - 70	0.2	1.9	0.64	0.33
	71 - 80	0.2	0.8	0.52	0.16
	81 - 100	0.4	1.0	0.60	0.34
HBA1c (%)	30 - 40	5.9	10.0	7.59	1.21
	41 - 50	4.7	13.1	7.60	1.91
	51 - 60	5.3	13.6	8.25	2.00
	61 - 70	5.4	14.5	7.90	1.90
	71 - 80	5.6	9.6	7.11	1.13
	81 - 100	6.3	7.9	7.33	0.89
FBS (mg/dL)	30 - 40	55.7	153.0	108.79	27.76
	41 - 50	78.1	267.6	132.12	50.46
	51 - 60	66.3	290.0	136.02	52.93
	61 - 70	64.6	425.7	140.66	62.43
	71 - 80	77.3	270.1	126.69	51.75
	81 - 100	100.0	122.7	113.23	11.80
PPBS (mg/dL)	30 - 40	100.0	270.0	140.35	52.92
	41 - 50	84.0	454.0	164.16	83.78
	51 - 60	100.0	431.0	184.18	73.59
	61 - 70	59.0	486.0	199.74	96.69
	71 - 80	84.5	327.6	168.34	65.76
	81 - 100	124.0	329.0	233.13	103.14

Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS)

laboratory setup (eg; BIORAD D 10 analyzer). However, HbA1c has its advantage in that the sample collection for this test is more convenient to the patient as it only requires a random sample of EDTA blood at any time, unlike FBS and PPBS samples which have to be collected at specific periods [8].

A study by Haghighatpanah M et al, [11] showed that there was a moderate correlation between

Table 4: Pearson's Correlation between Glycated Hemoglobin (HbA1c) and mean results of Fasting Blood Sugar (FBS), Postprandial Blood Sugar (PPBS), and age

	Correlation (r-value)	**R Squared value**	**p-value**
Age	-0.010	1.019	0.887
FBS	0.369	0.136	0.000
PPBS	0.436	0.19	0.000

Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS)

Table 5: Comparison of glycemic parameters in two diabetes groups using independent sample t-test

Variable	Diabetes Group (HbA1c)	Frequency	Mean	Std. Deviation	p-value
Total Bilirubin (mg/dL)	=<7	80	0.686	0.44	0.001
	>7	121	0.572	0.23	
FBS (mg/dL)	=<7	80	113.885	26.02	0.000
	>7	121	147.421	63.82	
PPBS (mg/dL)	=<7	80	142.862	43.73	0.000
	>7	121	209.176	92.64	
Age (Years)	=<7	80	59.500	11.58	0.119
	>7	121	59.207	9.88	

Glycated Hemoglobin (HbA1c), Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar (PPBS)

HbA1c level and FBS (r=0.528), and PPBS (r=0.510), and this finding was consistent with the findings of Sikaris et al. [9] and Ketema et al. [10]. In this research, the correlation between PPBS and HbA1c had greater sensitivity, specificity, positive predictive value, and negative predictive value compared to the FBS in the South Indian T2DM population [11]. A study by Begum G S et al [12] also showed that both FBS and PPBS correlated significantly with HbA1c values. PPBS correlated more strongly with HbA1c in comparison with FBS. The findings of our study also showed more correlation between HbA1c and PPBS (r= 0.436) than FBS (r=0.369).

Oxidative stress occurs when there is an imbalance between free radicals and their counteracting agents, the antioxidant enzymes, in the body. Risk factors for developing oxidative stress include obesity, high-fat diets, excessive sugar consumption, processed foods, exposure to radiation, smoking, alcohol consumption, certain medications, and exposure to chemicals. This harmful process adversely affects various cellular structures, including membranes, lipids, proteins, lipoproteins, and DNA [13].

In diabetes, metabolic abnormalities lead to the overproduction of mitochondrial superoxide in endothelial cells of both large and small vessels, as well as in the myocardium, contributing to oxidative stress. This oxidative stress, in turn, plays a pivotal role in the development of complications associated with diabetes, affecting both microvascular and cardiovascular systems.

Beyond its traditional role as the final product of heme catabolism, bilirubin is now recognized as a fundamental substance with antioxidant and anti-inflammatory properties in the serum [14]. A study involving Korean Type 2 diabetes patients revealed a significant negative association

Table 6: Pearson's Correlation between total bilirubin and mean results of FBS, PPBS, and age.

	Correlation (r value)	**R Squared value**	***p* value**
Age	-0.103	0.011	0.147
FBS	-0.107	0.011	0.131
PPBS	-0.059	0.003	0.408
HbA1c	-0.234	0.055	0.001

between total bilirubin and HbA1c, independent of factors such as sex, age, abdominal circumference, smoking, and other risk factors [15].

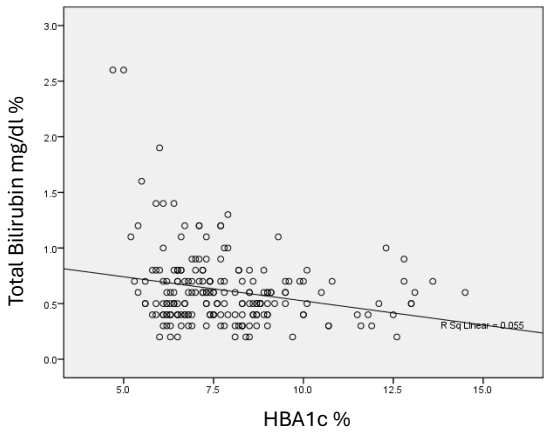

Figure 2: Scatter diagram of Glycated Hemoglobin (HBA1c) with total bilirubin

Furthermore, a review study highlighted that low serum bilirubin concentrations are linked to an increased risk of pathologic conditions such as cardiovascular diseases. In contrast, mildly elevated serum bilirubin levels were associated with protective effects. This underscores the emerging role of bilirubin beyond its conventional understanding, positioning it as a valuable agent in combating oxidative stress and inflammation [16].

Glycemic control in Type 2 diabetes is regulated by intricate mechanisms involving insulin resistance, beta-cell dysfunction, and glucose metabolism. Therefore, the influence of serum bilirubin on these complex processes may not manifest as a straightforward correlation with FBS and PPBS [16,17]. Blood sugar levels can fluctuate throughout the day and in response to various factors. A single measurement of serum bilirubin may not align with the specific time or conditions influencing FBS and PPBS, contributing to the absence of a significant relationship.

Conclusion

We conclude that, there was a significant positive correlation between HbA1c with FBS, and PPBS of controlled and poorly controlled diabetic patients. A significant relationship was observed between the total bilirubin, FBS, and PPBS in the well-controlled and poorly-controlled diabetes groups.

There was a significant negative correlation between total bilirubin and HbA1c in the diabetes patients. There was no statistically significant difference between the mean values of HbA1c in the male and female patients. In the conducted analysis, no statistically significant associations were observed between total bilirubin levels and FBS, PPBS, or age.

Limitations

This suggests that a potential protective or modulatory role of bilirubin in glycemic control. Further research may be warranted to explore the mechanisms underlying this correlation and its clinical implications.

One notable limitation of this study was the unavailability of information on key confounding factors, including Body Mass Index (BMI), smoking status, drinking status, and exercise habits of the patients. These factors are recognized contributors to diabetes and can exert a substantial influence on both glycemic control and bilirubin metabolism. The absence of data on BMI, smoking, drinking, and exercise habits restricts the comprehensive understanding of the potential interactions and influences these variables might have had on the observed relationships between total bilirubin and glycemic markers. Future research endeavors should consider incorporating a more extensive set of patient information to better elucidate the nuanced associations between bilirubin levels and diabetes outcomes, accounting for these essential confounding factors.

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