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# CORRELATION OF SERUM BILIRUBIN, GLYCEMIC CONTROL AND ALBUMINURIA IN TYPE 2 DIABETES MELLITUS – A RETROSPECTIVE STUDY

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## ABSTRACT

Bilirubin, a product of the heme catabolic pathway was considered as a toxic compound for many years.But the recent studies show that mildly elevated bilirubin levels are beneficial and negatively associated with the oxidative stress Serum bilirubin has been consistently shown to be negatively correlated to cardiovascular diseases (CVD), diabetes mellitus (DM), metabolic syndrome, and obesity. Recent studies are showing that bilirubin is negatively correlated with renal dysfunction and glycemic control, Hemoglobin A1c (HbA1c) in Diabetic individuals. To explore this further, we did a retrospective observational study. To assess the renal function albumin-creatinine ratio (ACR) was used as a marker and to assess glycemic control HbA1c was used as a marker. After statistical analysis we demonstrated the following;

a. Negative association between bilirubin and HbA1c
b. Negative association between bilirubin and albuminuria
c. Positive association between glycemic control and albuminuria.

**KEYWORDS:** **Bilirubin, Albuminuria, Glycemic control, Diabetes mellitus.**

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# INTRODUCTION

Bilirubin, the end product of heme catabolism, is generally considered to be potentially toxic, lipophilic waste product that needs to be excreted. However, studies are showing that bilirubin is a potent endogenous antioxidant cytoprotectant[1]. Studies show that bilirubin, a major intravascular product of heme catabolism, is an effective antioxidant that effectively scavenges peroxyl radicals and suppresses the oxidation of lipids and lipoproteins and thus acts against plaque formation and subsequent atherosclerosis[2]. Free bilirubin, albumin-bound bilirubin, conjugated bilirubin, and unconjugated bilirubin were all noted to be effective scavengers of peroxyl radicals and to be able to protect low-density lipoprotein against peroxidation.[3] Besides being an antioxidant, bilirubin also has anticomplement properties that protects against inflammation[4]. All these support the beneficial role of bilirubin as a physiological, chain-breaking antioxidant. HbA1c is a reliable marker of chronic hyperglycemia and is the test of choice for the management of diabetes. Albuminuria is taken as a marker of renal funcion in diabetic individuals. Recent studies are showing that bilirubin is negatively associated with Hemoglobin A1c (HbA1c) and renal function in the general population. Information available on the association of serum bilirubin concentrations with kidney function and albuminuria is limited. It has been shown by Fukui et al[5]. that serum total bilirubin is positively associated with eGFR, and negatively with albuminuria in a hospital based sample of 633 Japanese type 2 diabetic patients[5]. This shows a potential renoprotective effect of bilirubin. This study was aimed at establishing the correlation between bilirubin, renal function and glycemic control.

# MATERIALS AND METHODS

This being a retrospective study, we obtained the serum total bilirubin, HbA1c and urine ACR of 100 Type 2 DM adults who were evaluated in our laboratory attached to hospital, during January 2013 to March 2013. All these patients had normal liver enzymes and their bilirubin levels were within normal limits. We evaluated the relationships among serum bilirubin concentration, HbA1c and urine ACR of Type 2 DM adults aged between 40 and 60 years.

# RESULTS

Data was collected from 100 Type 2 DM individuals who were on regular follow up. Of these, 52 were females and 48 were males (Table 1). The age group of the patients included in the study was between 40 and 60 years. Among the 100 study subjects, the mean of total serum Bilirubin was 0.727, the mean of ACR was 45.861 and the mean of HbA1c was 7.4916 (Table 2). Pearson correlation analysis showed statistically significant correlation between bilirubin and HbA1c ($r = -.617$, $p = .000$) (Figure 1). The relationship between bilirubin and HbA1c inverse and the level was significantly lower in patients with poor glycemic control. Similarly, we found that statistically significant inverse correlation was found between serum bilirubin level and ACR($r = -.395$, $p = .000$) (Figure2). In contrast to this, there was a positive association between $HbA1_C$ and ACR ($r = .604$, $p = .000$) (Figure 3). Results show that with increasing ACR, bilirubin level comes down and with increasing HbA1C level; ACR tends to increase showing the positive correlation between poor glycemic control and renal dysfunction. The association between serum bilirubin, albuminuria and HBA1c are independent of age, gender, duration of diabetes, BMI, smoking status, blood pressure and plasma lipids.

## *Frequencies*

**Table1**
***Number of male and female individuals chosen for study***

**sex**

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | f | 52 | 52.0 | 52.0 | 52.0 |
| | m | 48 | 48.0 | 48.0 | 100.0 |
| | Total | 100 | 100.0 | 100.0 | |

***Note: Data collected from 52 females and 48 males Type 2 DM individuals.***

## *Correlations*

**Table 2**
***Relationships between serum bilirubin concentration and other variables***

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Bilirubin(Total) | 0.727 | 0.1254 | 100 |
| ACR | 45.861 | 36.8250 | 100 |
| HbA1c | 7.4916 | 1.02375 | 100 |

**Table 3**
***An inverse correlation was found between bilirubin and HbA1c (r = -.617, p = .000)***

| | ACR | HbA1c |
|---|---|---|
| Bilirubin(total) | | |
| Pearson correlation | -.395 | -.617 |
| Sig.(2-tailed) | .000 | .000 |
| N | 100 | 100 |
| ACR | | |
| Pearson correlation | 1 | .604 |
| Sig.(2-tailed) | | .000 |
| N | 100 | 100 |
| HbA1c | | |
| Pearson correlation | .604 | 1 |
| Sig.(2-tailed) | .000 | |
| N | 100 | 100 |

***Note: ACR- albumin-creatinine ratio***
***Correlation is significant at 0.01 level (2-tailed)***

**Figure 1**
***An inverse correlation was found between bilirubin and HbA1c (r = -.617, p = .000)***

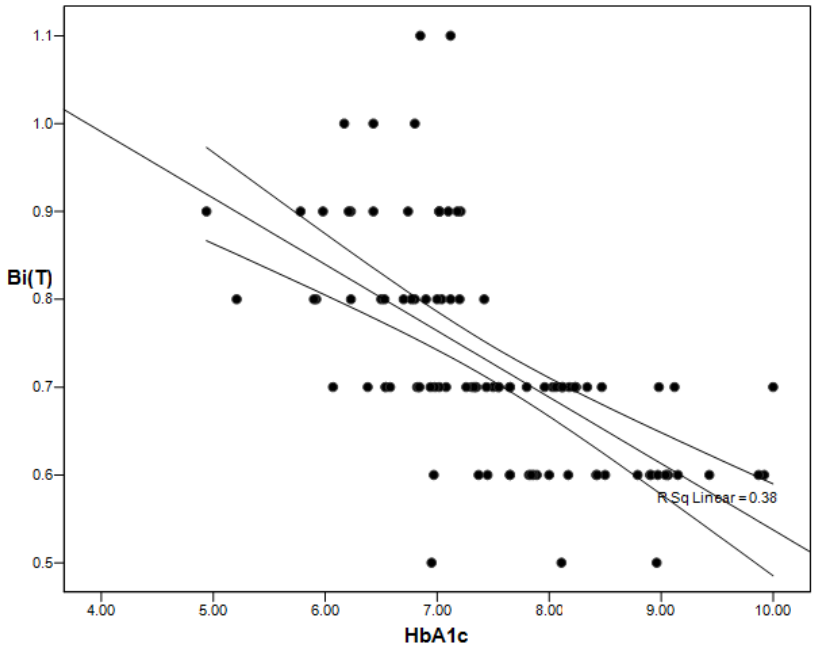



**Figure 2**
***An inverse correlation was found between serum bilirubin concentration and albumin-creatinine ratio (ACR) (r = -.395, p = .000)***

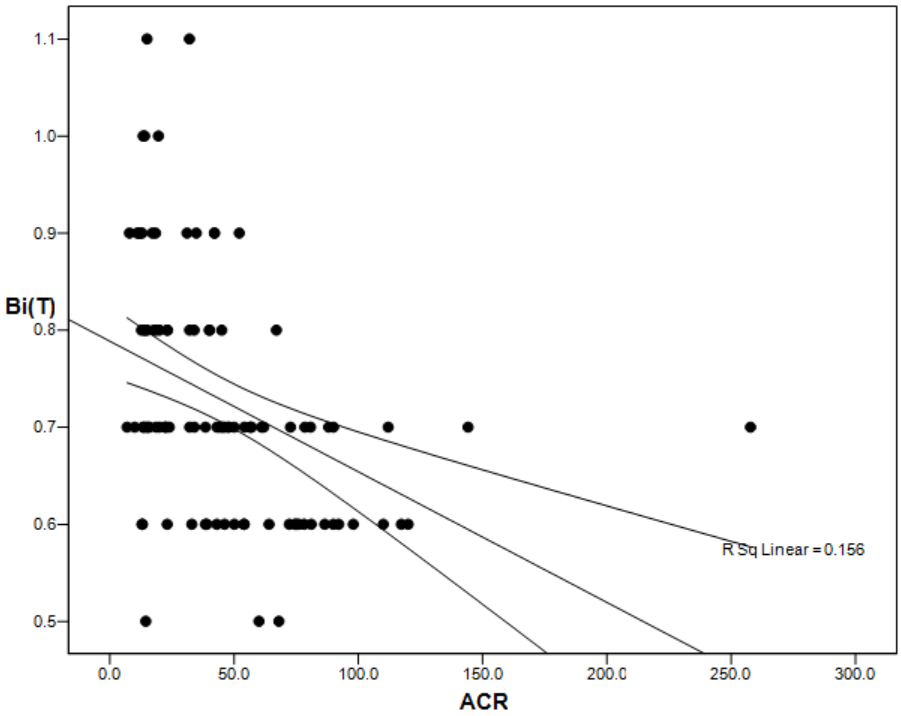
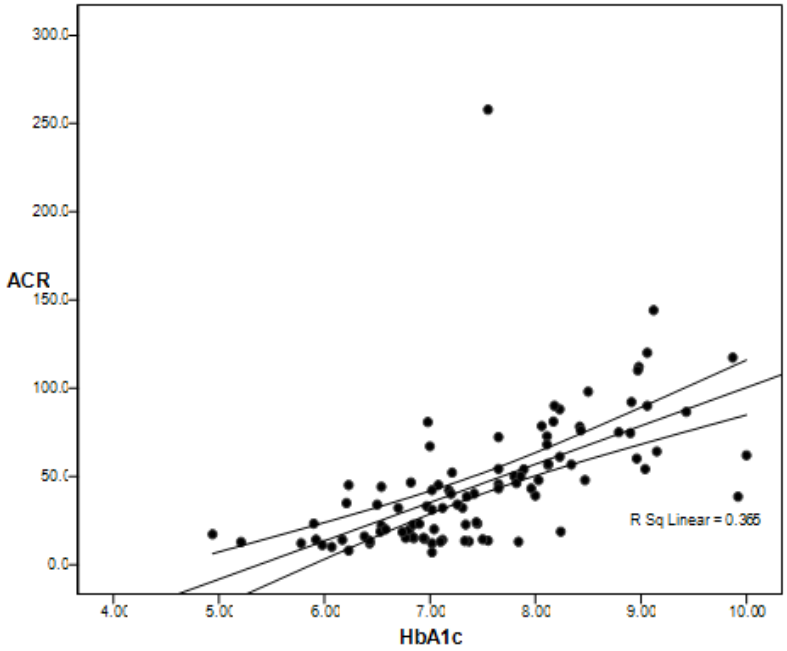


**Figure 3**
***Positive correlation was found between HbA1C and albumin-creatinine ratio (ACR) (r = .604, p = .000).***




## DISCUSSION

This is a hospital based study specifically aimed at analysing the associations between serum total bilirubin concentration, glycemic control and albuminura in Type 2 DM subjects attending our hospital. We retrospectively reviewed the data of 100 Type2 DM adult subjects aged between 40 and 60 years who were treated in our hospital, during January 2013 to March 2013. This study demonstrated that serum total bilirubin concentration was negatively correlated with HbA1c and albuminuria. Further, there was a positive correlation between HbA1c and albuminuria. Type 2 diabetes is the commonest form of diabetes and is associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species and oxidative stress. To counteract the harmful effects of free radicals, antioxidant defense mechanism operates to detoxify or scavenge these free radicals. There are many antioxidants which counteract the effects of oxidation induced damage. Bilirubin is one such physiological antioxidant which confers many benefits to the body at normal level. The antioxidant capacity of bilirubin and its ability to provide potent scavenging of peroxyl radicals have also led to suggestions that mildly increased circulatory bilirubin may have a physiologic function to protect against disease processes that involve oxygen and peroxyl radicals. Studies show that bilirubin is a potent endogenous antioxidant and an anti-inflammatory molecule that scavenges free radicals in vitro and in vivo. In addition to its free radical-scavenging activity, bilirubin has a potent inhibitory effect on the activity of NADPH oxidase, which is likely an important source of reactive oxygen species (ROS) production[6]. Wu T-W et al[7] have demonstrated that both unconjugated bilirubin and conjugated bilirubin can protect human low density lipoprotein (LDL) against oxidation by oxyradicals and bilirubin was more effective than ascorbate in preventing LDL oxidation. This shows the antiatherogenic effect of bilirubin and emphasises the beneficial role of bilirubin as an effective antioxidant. Recent studies reveal that bilirubin can protect cells from a 10,000-fold increase in oxidative stress caused by hydrogen peroxide[8,9] Oxidative stress and ROS activate multiple serine kinase

cascades, which potentially target the insulin signalling pathway, including the insulin receptor and its substrate proteins[10]. In addition, oxidative stress may contribute to progressive beta-cell damage. All these studies are showing that bilirubin has a protective effect against the development of DM and CVD by reducing oxidative stress. In our study, to establish the role of bilirubin as an antioxidant it has been correlated with HbA1c and albuminuria, which are considered to be worsening in the event of oxidative stress. In the present study, firstly, we examined the association between bilirubin and glycemic control. To assess glycemic control, HbA1c was taken as a marker. To demonstrate this, few studies and explanations have been taken in to consideration. Many studies have reported that high bilirubin concentration is inversely associated with the prevalence of hypertension [11] and type 2 diabetes [12].This shows that bilirubin plays a role in diabetes and its complications. Some researchers have studied the relationship between HbA1c and bilirubin. In a study of 4,180 members of the general population, total bilirubin was negatively associated with HbA1c independent of other cardiovascular risk factors[13]. However, this study was conducted with the general population, rather than with type 2 diabetes patients. Keizo Ohnaka et al[14] have also demonstrated that there is an inverse association between serum bilirubin and HbA1c in Type 2 DM. In our study also, serum bilirubin concentration correlated negatively and significantly with glycemic control. All these data suggest that bilirubin level may be low in poor glycemic control. There are also some explanations for the mechanism linking HbA1c and bilirubin. Bilirubin may inhibit the glycation of hemoglobin by reducing oxidative stress which is involved in the glycation reaction. Oxidative stress can facilitate the auto oxidation of glucose to dicarbonyl intermediates, which is an early step in the Maillard reaction. In addition to this, malondialdehyde, which is generated by lipid oxidation, is thought to enhance the process of protein glycation by acting as an anchor between sugar and hemoglobin moieties. Besides, bilirubin may play an important role in glycemic control. Increased expression of heme oxygenase-1, the enzyme responsible for the conversion of hemoglobin to bilirubin, is associated with enhanced insulin sensitivity and glucose metabolism. These explanations are well correlating with the results of our study. In another study done by Kumar et al[15], they have shown that serum bilirubin concentration is inversely correlated with oxidative stress and is positively correlated with antioxidative enzyme activities such as those of superoxide dismutase, catalase, and glutathione peroxidase. Also, clinical studies focused on the protective effects of serum bilirubin concentration on atherosclerosis are supporting this[16,17]. All these studies emphasise the role of bilirubin in reducing complications secondary to oxidative damage. Michiaki Fukui et al[18] have shown that Serum bilirubin concentration correlated negatively with degree of urinary albumin excretion and correlated positively with an estimated glomerular filtration rate (GFR), which suggests that bilirubin has a potential role for protection of diabetic nephropathy. To explore this further, we also correlated the bilirubin with albumin creatinine ratio and we found a negative correlation between these two parameters. There are many studies and explanations to establish this correlation between bilirubin level and kidney function. Bilirubin besides being an antioxidant, has anticomplement properties that protect against inflammation[19].Furthermore, bilirubin has been suggested to have cytoprotective properties through its regulatory role on protein kinase C. In vitro, protein kinase C increases the scavenger receptor expression in smooth muscle cells and therefore contributes to the formation of smooth muscle foam cells. The mechanisms described above are some of the important mechanisms of the development and progression of diabetic nephropathy[20,21]. Ollinger et al[22] have shown that bilirubin inhibited serum-driven smooth muscle cell-cycle progression at the $G_1$ phase in vitro[22]. This suggests that bilirubin is a natural inhibitor of vascular smooth muscle proliferation. All these point towards the negative association between serum bilirubin concentration and the degree of diabetic nephropathy. In our study there is a negative correlation between bilirubin and albuminuria.

This suggests that bilirubin has a potential role for protection of diabetic nephropathy. Hence, bilirubin could be considered as a marker of diabetic nephropathy in DM individuals and it can be utilized by clinicians to assess the kidney function. Lastly, our study results also reveal that there is a positive association between HbA1c and albuminuria stressing that poor glycemic control could contribute to renal dysfunction. This correlation is consistent with the above mentioned studies and explanations. Our study and all the other related studies suggest that hypobilirubinemia combined with poor glycemic control could result renal dysfuntion in DM. Limitations of our study are small sample size and it is a retrospective study. In addition, results of this study may not be applicable to the general population. Thus further prospective studies into this interesting correlations required.

## CONCLUSION

Our findings suggest that decreasing serum total bilirubin concentration is associated with poor glycemic control and increasing albuminuria in Type 2 DM.

## ABBREVIATIONS

ACR-albumin-creatinine ratio, DM- diabetes mellitus, CVD- cardiovascular diseases, HbA1c- Hemoglobin A1c

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