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Original Article

The impact of serum bilirubin on diabetic nephropathy and pulmonary function in type II diabetic patients

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Abstract

Introduction: Diabetes mellitus may be associated with many complications including diabetic nephropathy and pulmonary impairment, the pathogenesis and progression of these complications may be related to oxidative stress. Bilirubin, which is a non-polar molecule have antioxidant properties. The relationship between occurrence, development and prognosis of diabetic complications and bilirubin concentration had become a research focus. However, no study has evaluated the relationship between protective effect of bilirubin on both diabetic nephropathy and pulmonary impairment in type II diabetic patients.

Methods: The design of the study is a cross sectional study included 245 type II diabetic patients. Spirometry was done for all patients. Albumin/creatinine ratio (ACR), glycosylated hemoglobin, total serum bilirubin and serum glutathione reductase enzyme were measured.

Results: There was a significant statistical negative relationship between serum bilirubin and glutathione reductase enzyme with ACR and significant statistical positive relationship between serum bilirubin and glutathione reductase enzyme with lung function parameters (forced expiratory volume in the first second and forced vital capacity).

Conclusion: serum bilirubin levels had a protective effect against diabetic nephropathy and impairment of pulmonary function. If serum bilirubin levels were moderately high but within the normal range, this was related with decreased risk of diabetic complications and there was a parallel relationship between serum bilirubin levels and the glutathione reductase enzyme levels in type II diabetic patients.

Keywords:
Bilirubin;
Diabetes mellitus type II;
Diabetic nephropathy;
Pulmonary impairment;
Oxidative stress

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Introduction

Diabetes mellitus (DM) may be associated with extensive biochemical, functional and morphological defects that might be lead to many complications (Kaur and Agarwal, 2016). For example nephropathy, neuropathy, retinopathy and to some extent, cardiovascular diseases and diabetic foot (Lotfy et al., 2017). The major end-organ complication in diabetes is diabetic nephropathy, which can be defined as being there is more than or equal to 30mg albumin in

a urine sample collected per day (Beckman and Creager, 2016). The pathogenesis and progression of diabetic nephropathy (DN) may be related to oxidative stress, on the other word, antioxidants might inhibit the progression of kidney dysfunction (Jha et al., 2016).

Chronic hyperglycemia in diabetic patients can also lead to pulmonary complication. This results from glycation and leading to the formation of fibrotic tissue in the bronchial tree (Ali, 2014). Uncontrolled DM has a major effect on the respiratory system due to that it induce oxidative stress, systemic inflammation, structural changes in the lung tissue, hypoxemia and altered gas exchange (Gläser et al., 2015). Hyperglycemia also reduces the antioxidant defense mechanism in the lung and causes local biochemical changes in the lungs with consequent reduction in the lung volumes and elasticity (da Silva Almeida et al., 2016).

Bilirubin is an end-product of heme metabolism in the body that have elevated substantial attention in study over the latest decade, but recently, numerous studies have shown that bilirubin had advantageous outcomes on numerous diseases related to oxidative stress (Inoguchi et al., 2016; Zhang et al., 2017). The main source of bilirubin is hemoglobin (Hb) degradation, it is possible that the differences in the Hb concentration may be the cause of individual differences in bilirubin concentrations, also it is found that higher bilirubin levels results from an increase in red blood cell mass (Zhang et al., 2017). Bilirubin, which is a non-polar molecule have antioxidant properties (Vítek, 2012). Body displays two different antioxidant systems (hydrophilic and lipophilic) because that the water-soluble glutathione (GSH) principally defends water-soluble proteins, while the lipophilic bilirubin defends lipids from oxidation (Sahoo et al., 2016). Bilirubin in comparison with glutathione (water-soluble antioxidant) is more effective in preventing oxidation of lipids. While in comparison with vitamin E analog (lipid-soluble antioxidant) bilirubin is 30 times more potent in the protection of LDL from oxidation (Zhu et al., 2017).

In one recent study, it is reported that slight increase in serum bilirubin level has a protective impact on many diseases e.g. diabetes and cardiovascular diseases, so elevated serum bilirubin concentration may has a potential therapeutic role to improve many of these diseases (Gazzin et al., 2016).

Bilirubin produced via enzymatic reduction by biliverdin reductase as segment of the heme oxygenase cycle. Earlier studies had stated a negative correlation between diabetic microvascular complications and total bilirubin level (Wang et al., 2016). Moreover, persons with Gilbert syndrome displays slight increase in serum bilirubin concentrations also have a tendency to have a reduced prevalence of vascular complications (Rodrigues et al., 2012).

Bilirubin may be used as a biomarker of DN in addition to that it is considered one of the indexes of hepatic function. (Zhang et al., 2017). The linkage of the association between hemoglobin concentrations and diabetic nephropathy progression is serum concentration of bilirubin. It has been proved that anemia-induced renal hypoxia increase speed of the drop in renal function (Mashitani et al., 2014). Though numerous researches had been achieved, the precise mechanism behind the cause of the inverse association that connect the high bilirubin levels and the progression of DN stays unidentified (Li et al., 2017).

In a respiratory health, there are few studies about the advantageous effects of high serum bilirubin. If bilirubin concentrations were high, this might be associated with lower occurrence of chronic obstructive lung disease and lung cancer (American diabetes association, 2012). If serum bilirubin levels were moderately high but within the normal range, this was related with decreased risk of respiratory diseases (Horsfall et al., 2011). This study is an attempt to cover the relationship between the protective effect of bilirubin on both kidney and lung.

Materials and methods

Current study is a cross sectional study involved 245 types II diabetic. The study population includes 119 male and 126 female, aged 41-80 years with mean±SEM (57.43±0.54 years). All patients were collected from Al-Sadder Teaching Hospital, Annajaf Center for diabetic and endocrine during September 2017 to September 2018. All participants are type II diabetic patients and visit the center for routine follow-up; oral hypoglycemic agents and/or insulin treat them. Permission for carrying out the study was obtained from Ethical Committee of Medical Faculty, Kufa University. The patients that enrolled in this

Table 1: Demographic characteristics of the studied patients (n=245).

		No.(%)	Mean	SEM
Gender	Male	119 (48.6%)		
	Female	126 (51.4%)		
Duration of DM/years			8.74	0.34
HbA1c			8.28	0.10
ACR (mg/mmol)			9.19	0.80
FEV1 (%predicted)			67.38	1.48
FVC (%predicted)			66.38	1.43
S. Bilirubin (mg/dl)			0.75	0.02
Glutathione reductase (pg/ml)			5007.12	63.84

Table 2: The effect of serum bilirubin on ACR, FEV1 and FVC in type II diabetic patients.

Parameter	**Serum bilirubin mg/dl**		***P* value**
	<0.7 (N=109)	**0.7-1.2 (N=136)**	
	Mean±SEM	**Mean±SEM**	
ACR (mg/mmol)	17.66±1.43	2.39±0.04	<0.001
FEV1 (%Predicted)	45.67±1.52	84.79±0.80	<0.001
FVC (%Predicted)	45.09±1.41	83.44±0.76	<0.001

study signed informed consent form before the screening tests.

All patients underwent a complete assessment includes full history, chest radiography, physical examination and finally biochemical analysis. Respiratory disease patients, current or X- smokers, patients with renal disease other than diabetic nephropathy, patient with chronic liver disease, patients with hypertension (independent risk factor for nephropathy) and patients with heart failure were excluded from the study. All patients had undergone a comprehensive assessment including full history according to well-prepared questionnaire, including age, sex, height (to measure predicted value for lung function parameters), name, duration of DM, mode of treatment, history of smoking, history of hypertension and drug history. Specialist physician does complete chest examination for each member. Those with abnormal physical findings were excluded from the study. All patients were sent for Chest X-Ray to exclude chest problem.

Spiro metric parameters were measured by Spiro lab III (new 3rd generation), (del Maggiolono, Italy) a computerized diagnostic spirometer. The patient should be restful in sitting position. Spirometry was performed by trained and certified pulmonary technicians according to American Thoracic Society Guidelines (Hankinson and Bang, 1991), procedure should be repeated three times and choose the best one (highest one). The parameters that measured and recorded were forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1). The percentage of the predicted FEV1 and predicted FVC were also recorded for all patients. Urine sample was collected to test albumin/creatinine ratio (ACR) which was measured by auto-analyzer (BS-120 Chemistry Analyzer), (Mindray Biomedical Electronics Co., Ltd. China). ACR of ≤30 mg/g (≤3mg/mmol) considered normal, ACR between 30-300mg/g (3-30 mg/mmol) labeled as micro albuminuria and ACR of ≥300 mg/g (≥30mg/mmol) was defined as macro albuminuria (Gnudi et al., 2016).

Two milliliters (ml) of blood were collected from every patient which used to measure glycosylated hemoglobin (HbA1c) which is done using the D-10 Hemoglobin A1c testing system (Bio- Rad, USA) which utilizes principles of ion exchange high-performance liquid chromatography (HPLC). Total

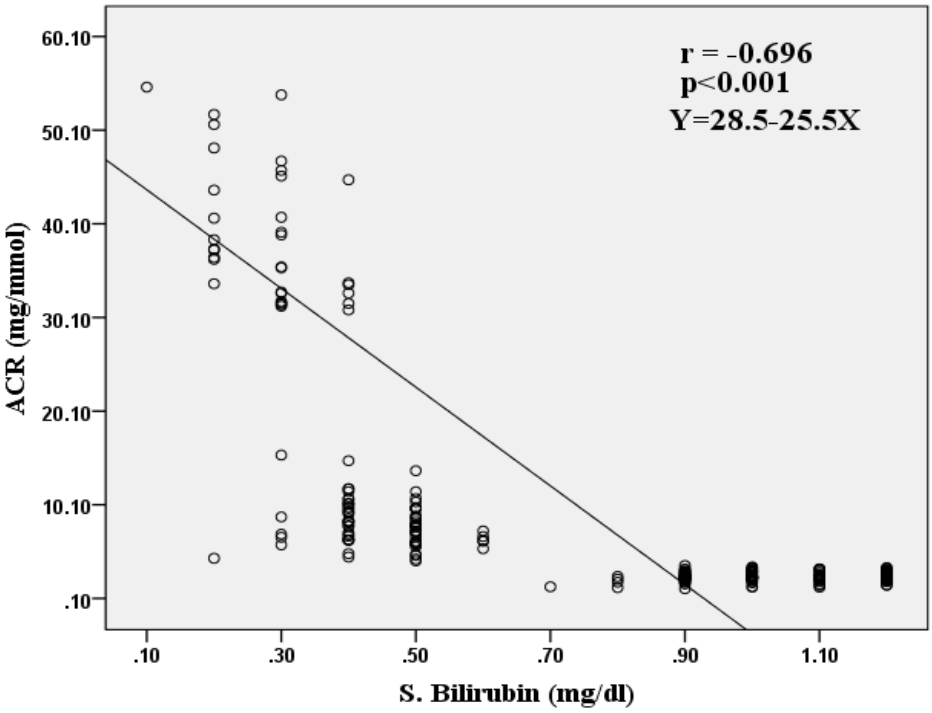

Fig.1. Correlation between serum bilirubin and ACR.

Table 3: Binary logistic regression for risk factors with ACR

	B	S.E.	Wald	df	P value	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
HbA1c	-0.793	0.535	2.197	1	0.138	0.452	0.158	1.291
Duration	-0.054	0.043	1.543	1	0.214	0.948	0.871	1.032
Bilirubin	-8.996	1.069	70.774	1	0.000	0.000	0.000	0.001

serum bilirubin and serum glutathione reductase enzyme also measured. Total Serum Bilirubin is measured by taking a 50-60µl of the serum using hematocrit capillary tube (heparinized) which was put in BR-501 dual wavelength total bilirubin meter (APEL, Japan), then record the reading which ranging from (0.1-1.2) mg/dl. Normal levels vary slightly from lab to lab, they range from about 0.1–1.2mg/dl (Arora et al., 2009; Charles and Bhupinder, 2018). Serum glutathione reductase enzyme measurement was done using glutathione reductase ELISA kit (Elabscince, China); this kit uses method of Sandwich- ELISA. The micro ELISA plate had been pre-coated with an antibody specific to human glutathione reductase, detection range was 78.13-5000pg/ml.

Statistical analysis

Statistical analysis done by using SPSS (statistical package for social sciences) version 20 using frequencies, percentages and mean with standard error as descriptive statistics. Independent sample t-test and Pearson correlation coefficient used for analysis of data. Binary logistic regression was done for risk factors. *P* value ≤0.05 regarded significant.

Results

There was a significant statistical negative relationship between serum bilirubin and ACR (*P*<0.001) which remain significant even after regression of other risk factors (duration and control of DM). In addition, there was a significant statistical positive relationship between serum bilirubin and FEV1 (% predicted) and serum bilirubin with FVC (% predicted) (*P*<0.001) as shown in Table 2 and also Figures 1, 2 and 3.

Study the effect of glutathione reductase on ACR,

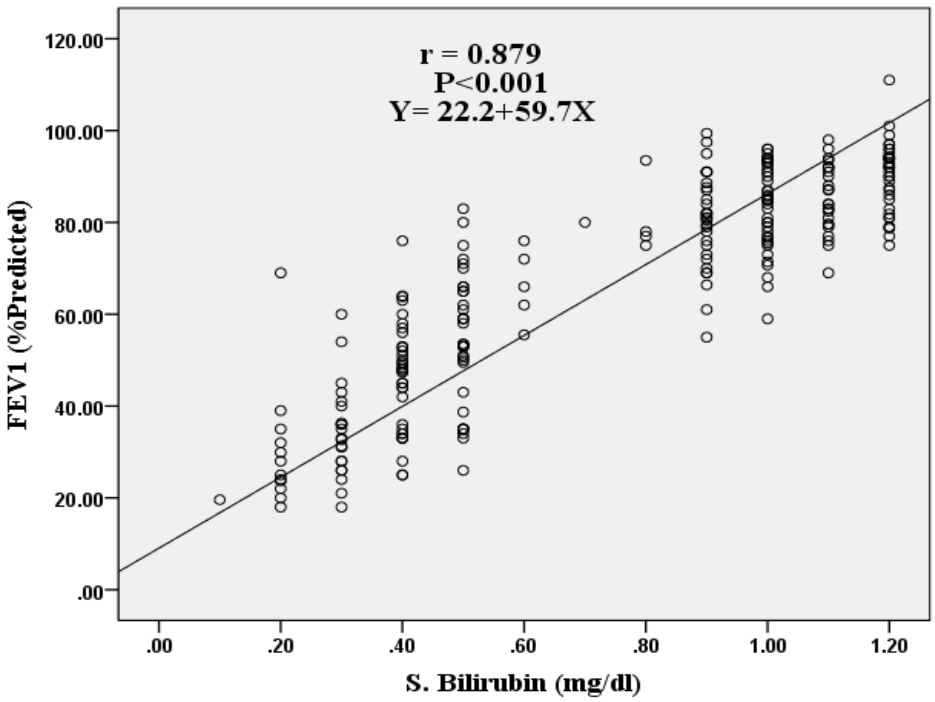

Fig.2. Correlation between serum bilirubin and FEV1 (%predicted).

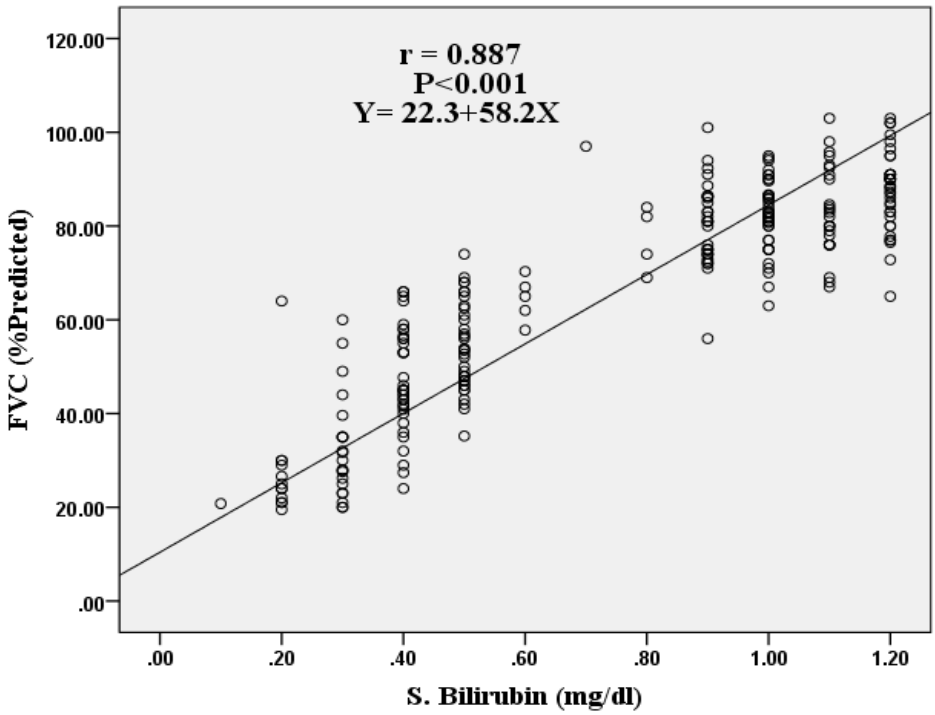

Fig.3. Correlation between serum bilirubin and FVC (%predicted).

FEV1 and FVC revealed negative association between serum glutathione reductase level and ACR (*P*<0.001) but positive correlation with FEV1 (%predicted) and FVC (% predicted) (*P*<0.001), as shown in Table 4. There was a significant statistical positive correlation between serum glutathione reductase and serum bilirubin, as shown in Figure 4.

Discussion

This study showed a significant inverse relationship between serum bilirubin levels and albuminuria (bilirubin have protective effect against DN), even after regression of other risk factors (duration and

Table 4: The effect of glutathione reductase on ACR, FEV1 and FVC in type II diabetic patients.

Parameter	Glutathione reductase Pg/ml		*P* value
	≤5000 (N=111)	>5000 (N=134)	
	Mean±SEM	Mean±SEM	
ACR (mg/mmol)	16.74±1.42	2.93±0.34	<0.001
FEV1 (%Predicted)	47.40±1.66	83.94±0.97	<0.001
FVC (%Predicted)	47.00±1.58	82.43±0.96	<0.001

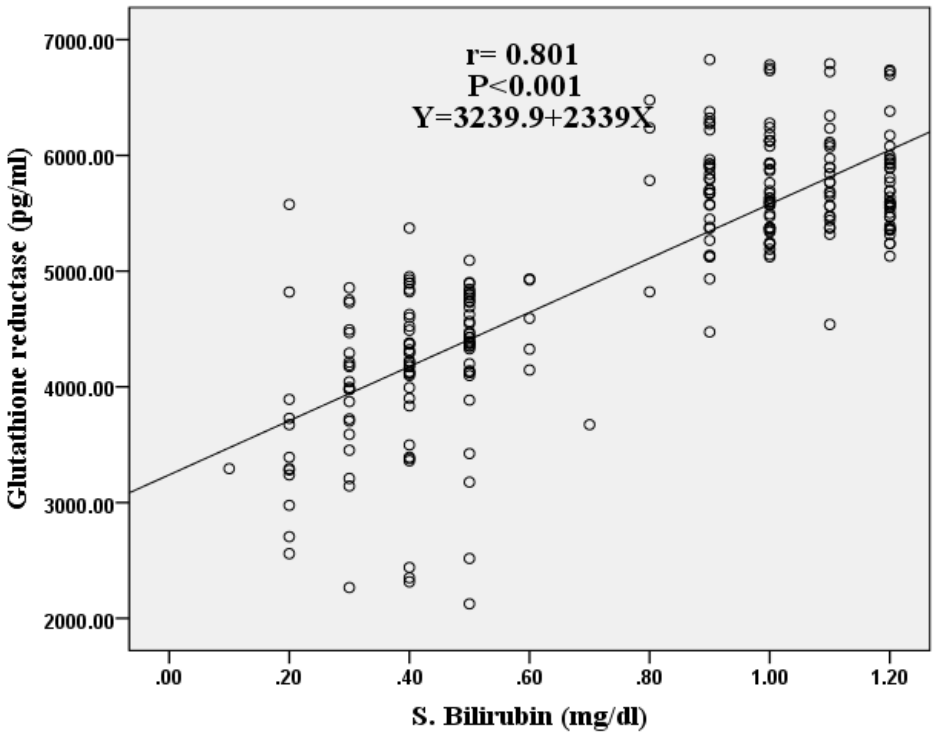

Fig.4. Correlation between serum glutathione reductase and serum bilirubin.

control of DM). The regression analysis showed no significant association between HbA1C and severity of albuminuria and no significant association between diabetes duration and severity of albuminuria, but bilirubin has a significant negative relationship with albuminuria. This result is agreed with Fukui et al. (2008) and Hamamoto et al. (2015). They showed that the serum bilirubin level is inversely associated with micro albuminuria in patients with type II diabetes. High serum bilirubin concentration had defensive effect on diabetic complications; this is proved by other researchers (Sekioka et al., 2015; Wang et al., 2016). While Han et al. (2010) did not notice any association between the level of serum bilirubin and complications of DM.

The results of this study concerning the impact of serum levels of bilirubin on lung function parameters show a significant positive relationship between serum bilirubin levels and FEV1 (% predicted) as well as FVC (% predicted). Which is agree with Curjuric et al., (2014) and Apperley et al. (2015) they observed that there was a significant positive relations between lung function parameters and serum bilirubin levels.

Possible explanations for the impact of bilirubin in decreasing diabetic complications may be that, hyperglycemia result in overproduction of mitochondrial superoxide in the large and small vessels, by these pathways: advanced glycation end products over production (Advanced Glycation End Products), increased formation of (AGEs) receptors, hexosamine pathway over activity, polyol pathway flux and protein kinase C isoforms activation. All these pathways are implicated in diabetic complications. Bilirubin inhibits peroxidation of lipid,

attenuate oxidation of LDL and decrease reactive oxygen species (ROS) levels. (Zhang et al., 2017).

Total antioxidant capacity in blood is belong to serum bilirubin. The major source for reactive ROS production, which is NAD (P) H oxidase, inhibited by bilirubin. It is reported that in rodents biliverdin and bilirubin inhibit the development of renal mesangial expansion and the progression of albuminuria in diabetes, in addition to the normalization of oxidative stress (Inoguchi et al., 2016). The first authors who systematically reviewed the observational studies and quantitatively assessed the association between total bilirubin level and the risk of DN were Zhang et al. (2017). This study revealed that Total Bilirubin Level in the Non Diabetic Nephropathy group was higher than that in the DN group and the results of this study are not affected by gender, age, study design, BMI and duration of diabetes mellitus.

In diabetes, it has been recommended that lung function may be changed via both free radical and oxidant exposure, whereas antioxidant vitamin consumption is positively correlated to lung function. Bilirubin is effectively antioxidant and cytoprotectant. Once hydrogen peroxide, an oxidant, is added to cells missing the biliverdin enzyme, the cells displayed considerably amplified sensitivity to hydrogen-peroxide-provoked cell death (Apperley et al., 2015).

This study also revealed an inverse association between serum glutathione reductase level and albuminuria but positive association between serum glutathione reductase and lung function parameters. This result is in agreement with Miranda-Díaz et al. (2016), who concluded that the markers of oxidative stress such as glutathione reductase, catalase and glutathione peroxidase (GPx) had significant differences when compared to the control group. Also in agreement with Singh and Singh (2017), that they found the activity of superoxide dismutase (SOD), GSH, glutathione reductase and GPx was significantly reduced in type II diabetes patients without and with micro albuminuria in comparison to healthy control group. A similar degree of significant decrease in SOD, GSH, glutathione reductase and GPx levels was also recorded in micro albuminuria with respect to patients without micro albuminuria. This is because that glutathione reductase is antioxidant enzyme and it decrease oxidative stress by changing oxidative products to hydrogen peroxide and finally to H_2O so, when it increase this lead to decrease incidence of DN. This is because that the pathogenesis and progression of DN may be related to oxidative stress, on the other word, antioxidants might inhibit the progression of kidney dysfunction (Jha et al., 2016).

In the lung, hyperglycemia also reduces the antioxidant defense mechanism and causes local biochemical changes in the lungs with consequent reduction in the lung volumes and elasticity (da Silva Almeida et al., 2016). This finding is agree with Liang et al. (2007) and Hu et al. (2014), they found that in diabetic lung, the activity of SOD was decreased. The current study revealed a significant positive correlation between serum glutathione reductase and serum bilirubin, as shown in Figure 4. This is because that both of them are antioxidants and may protect against complications of diabetes. Kumar et al. (2006) reported that serum bilirubin levels had a positive association with serum glutathione reductase, SOD and catalase levels.

Conclusion

Within normal range, serum bilirubin had a protective effect against diabetic nephropathy and impairment of pulmonary function in type II diabetic patients and there was a parallel relationship between serum bilirubin levels and serum glutathione reductase enzyme levels in type II diabetic patients.

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Conflict of interest

The authors declare that they have no competing interests.

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