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Original Article

Clinical sensitivity and specificity of serum total bilirubin – A study on thyroid status in clinically euthyroid non-obese, overweight, and obese type 2 diabetics

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ABSTRACT

Objective: The objective of this study was to evaluate the sensitivity and specificity of total bilirubin (serum) in determining thyroid status in clinically euthyroid non-obese, overweight, and obese type 2 diabetics

Subjects and Methods: Three anthropometry specific groups of clinically euthyroid type 2 diabetics were enabled, following enrolment: 153 non-obese (body mass index [BMI] = 18.5–24.99), 291 overweight (BMI = 25–29.99), and 126 obese type 2 diabetes mellitus (BMI ≥30). Total bilirubin (serum), glycemic status, insulin resistance (IR), and thyroid hormones, besides routine biochemistry, were estimated, as per International Federation of Clinical Chemistry approved procedures.

Results: Receiver operating characteristic curves for non-obese, overweight, and obese were plotted to assess the role of total bilirubin (serum) in determining thyroid status in clinically euthyroid type 2 diabetics. In overweight, the area under curve (AUC) for FT3 and postprandial sugar showed 0.621 and 0.531 with cutoff values of 2.02 pg/ml and 147.5 mg/dl, respectively, whereas for aspartate aminotransferase/alanine aminotransferase (De Ritis ratio), the AUC was 0.583. As regards, obese diabetics and the AUC for insulin and homeostatic model assessment IR were 0.657 and 0.709, respectively, with cutoff values of 16.06 mIU/L and 7.274, respectively, and for postprandial sugar 0.727, in the same group (obese) with cutoff value of 208.5 mg/dl.

Conclusion: Total bilirubin could predict thyroid status and IR in anthropometry specific clinically euthyroid type 2 diabetics.

Keywords: Bilirubin, Free thyroxine, free triiodothyronine, homeostatic model assessment of insulin resistance, insulin resistance, thyrotropin, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is an endocrine, metabolic disorder that has been assuming an alarming proportion worldwide. The complications of DM are significant and need to be addressed objectively. Three hundred and eighty-two million people representing nearly 8% of the adult population suffer from DM.[1] It is feared that by the year 2035, one adult in every 10 individuals would suffer from this metabolic disorder.[2] However, according to the report filed by International Diabetes Federation, this figure is supposed to be even higher. Such of those patients affected with type 2 DM (T2DM) possess an enhanced risk for developing cardiovascular abnormalities.[3]

Glycated hemoglobin (HbA1c) is a well-established marker for monitoring long-term glycemic control, besides predicting the risk of diabetic complications.[4,5] Diabetic dyslipidemia possesses a nexus with glycemic status and insulin resistance (IR).[6] Clear lines of evidence suggest that dyslipidemia is secondary to IR or factors closely related to IR, such as adiposity. It is believed that an exaggerated free fatty acid flux secondary to IR as well as pronounced increments in pro-inflammatory adipokines, cytokines released from adipose tissue might determine this interrelationship.[7,8] Chronic hyperglycemia leads to the generation of the highly dangerous reactive oxygen species (ROS) that have been implicated in vascular dysfunction. This is a recognized major underlying feature in diabetic complications.[9,10]

Earlier studies have reported that hyperglycemia could act as a predisposing factor to increased low-density lipoprotein (LDL) glycation and associated pathologic events eventually

culminating in atherogenesis and cardiovascular disease (CVD).[11] It is imperative that an early diagnosis of dyslipidemia in conjunction with good lipid control could be used as a firm prophylactic measure for promulgating long-term glycemic control.

It has been significantly observed that bilirubin, a biochemical substance endogenously produced possesses a pronounced antioxidative property that is largely attributed to its inherent ability to scavenge peroxyl radicals. This property of bilirubin enables the inhibition of the oxidation of LDL.[12] A rapidly emerging fact is that bilirubin could be involved in the pathologic process of cardiometabolic aberrations, in which oxidative stress is believed to play a cardinal role.[12-17]

In view of the possible role of bilirubin as mentioned above, we proceeded to assess total bilirubin levels specifically with reference to thyroid status since thyroid comorbidity is a documented feature in DM. Moreover, oxidative stress has been linked to DM and thyroid disorder, though in several isolated studies. We embarked on this study by addressing the possible role of bilirubin in determining the thyroid status in clinically euthyroid type 2 diabetics. This facet could bestow greater credence and provide an insight into the aspects associated with diabetes linked latent thyroid comorbidity that is frequently ignored in several studies.

Fasting (post-absorptive) state brings about changes in both thyroid hormone (TH) secretion and insulin signaling. It is imperative that DM and thyroid disease are the two most commonly encountered endocrine-related aberrations in the present day clinical practice.[18] THs enhance heme oxygenase-1 activity, which is the key enzyme involved in bilirubin production.[19] Furthermore, THs downregulate the catalytic activity of uridine diphosphate glucuronyltransferase which governs bilirubin conjugation, before secretion.[20] All of these have been taken into due account while planning the study.

Need for the present study and scope

Thus, it is certain that THs and insulin signaling are related, wherein an aberration or an obvious clinical abnormality in one can lead to alterations in the other.[21] Moreover, the contributions of THs to insulin signaling and glucose metabolism are evident even in euthyroid status as related to IR.[22-26] The pronounced antioxidative properties bestowed on bilirubin would explain *in toto* its cardioprotective effects and we undertook this study keeping this perspective in mind. Moreover, to assess the total bilirubin is certainly envisaged as an economical, reliable, and easily measurable endeavor in throwing light on the comorbidity status observed in T2DM, in anthropometry specified groups of diabetics (non-obese, overweight, and obese). Very few instances are available from South India citing as to whether circulating bilirubin could be related with thyroid function in clinically euthyroid type 2 diabetics. It is noteworthy to mention that earlier studies on thyroid status in T2DM have not particularly considered anthropometry in delineating the subtle differences by way of comparison of biomarkers in T2DM with apparently normal thyroid function (euthyroid state).

Subjects and Methods

The study was carried out on type 2 diabetics (both genders) at a tertiary health-care unit in Pondicherry, South India. Following the enrolment into the study (during June 2015–August 2017), three groups were segregated: 153 non-obese patients (body mass index [BMI] = 18.5–24.99), 291 overweight subjects (BMI = 25–29.99), and 126 obese T2DM (BMI ≥30).

Inclusion criteria

Sample size $n = 570$ – one proportion test. Consecutive patients (adult males and females in the age group 35–70) with T2DM and with no obvious clinical manifestations of thyroid disease were taken up.

Exclusion criteria

Patients with a documented history of thyroid diseases were promptly excluded. Patients with a known history of cardiac, liver, and muscle diseases were also excluded. Patients with other endocrine and chronic illnesses as well as such of those on drugs that might alter serum bilirubin levels were also excluded.

The study was duly approved by the Research Advisory Committee (vide minutes of the Research Advisory Committee dated December 26, 2014) and Institutional Human Ethics Committee (vide certificate of approval duly signed by the secretary, IHEC dated June 5, 2015).

Biochemical measurements

All the measurements were enabled through procedures fully compliant with the International Federation of Clinical Chemistry guidelines and methods. Stringent quality control (QC) was promulgated. The internal QC was enabled with the assistance of QC samples supplied by M/S Biorad, USA. External quality assessment was facilitated by the clinical biochemistry laboratory (accredited by NABL under ISO/IEC 15189), Christian Medical College, Vellore, under the aegis of ACBI. Fasting and postprandial blood glucose were estimated, based on glucose oxidase-peroxidase method. Fasting insulin (venous plasma) was estimated by automated electrochemiluminescence. HbA1C was estimated by high-performance liquid chromatography. The IR index was computed by the homeostatic model assessment and computed using the formula: Homeostatic model assessment IR (HOMA-IR) = (concentration of glucose in venous plasma (mmol/l) × concentration of insulin in venous plasma (mU/L)/22.5).[27] Triacylglycerols in serum were quantitated by

glycerol kinase method. Total cholesterol was quantitated by enzymatic method. High-density lipoprotein (HDL) cholesterol was quantitated by polyanion precipitation. LDL cholesterol was computed by Friedewald equation, i.e. LDL cholesterol = total cholesterol-(HDL cholesterol +very LDL [VLDL]) where VLDL = TAG/5. Small dense LDL particles were assessed using the surrogate marker (TAG/HDL). Free triiodothyronine (FT3), free thyroxine, and thyrotropin (thyroid-stimulating hormone [TSH]) in serum were measured by automated electrochemiluminescence method. Total and direct bilirubin was estimated by the method of Jendrassik–Grof.

Reference ranges for biochemical parameters

Fasting plasma glucose 70–110 mg/dL; fasting plasma insulin 0.7–9 µIU/ml. HOMA-IR 1.7–2. Others: 15–40 mg/dl for urea; 0.2–1.4 mg/dl for creatinine; 150–200 mg/dl for total cholesterol 75–150 mg/dl for triacylglycerols; 30–60 mg/dl for HDL. TSH: 0.27–4.2 µ/u//mL; 2–4.4 pg/ml for free T3 and 0.93–1.7 ng/dl for free T4; 5–40 IU/L for alanine aminotransferase (ALT) and aspartate aminotransferase (AST); 40–125 IU/L for ALP; 6–8 g/dl for total protein; 3.5–5 g/dl for albumin; 2.5–3.5 g/dl for globulin, 0.2–1 mg/dl for bilirubin (T); and <0.4 mg/dl for bilirubin (D).

Statistical analysis

Receiver operating characteristic (ROC) curves were established and area under curve (AUC) was plotted (Ref: pROC Version 1.13.0) and compared with regard to BMI in overweight, obese, and non-obese T2DM patients. The predictive value of bilirubin (total) was tested against THs, IR, and other parameters including those that reflect liver function.

Results

The predictor nature with reference to glycemic control, IR, liver function, and thyroid status utilizing total bilirubin as the dependent variable in the three anthropometry specified groups, namely non-obese, overweight, and obese was enabled and found to be statistically significant.

ROC curve for non-obese T2DM patients

We plotted a ROC curve based on sensitivity and specificity with reference to postprandial blood glucose (PPS), FT3, and HOMA-IR against total bilirubin. AUC for FT3 was 0.598 with cutoff value 1.71 and a less notable AUC for PPS 0.512 with cutoff value 187.5 mg/dl. These are depicted in Figure 1.

ROC curve for overweight T2DM patients

As regards overweight type 2 diabetics, we plotted a ROC curve based on sensitivity and specificity with reference to postprandial blood glucose, FT3, and AST/ALT against total bilirubin. AUC for FT3 was 0.621 and AST/ALT (ratio of AST to ALT – also known as De Ritis ratio) was 0.543 with cutoff value of 2.02 pg/ml and 0.583, respectively, and PPS 0.531 with cutoff value of 147.5 mg/dl. These are shown in Figure 2.

ROC curve for obese T2DM patients

We plotted a ROC curve based on sensitivity and specificity with reference to PPS, insulin, and HOMA-IR against total bilirubin. AUC for PPS was 0.727 with cutoff value of 208.5 mg/dl; for insulin, the AUC was 0.657 with cutoff value of 16.06 µIU/ml and HOMA-IR 0.709 with cutoff value of 7.27. These are depicted in Figure 3.

Discussion

Aberrations in carbohydrate metabolism and thyroidal functions are strongly associated.[28] THs affect insulin secretion either by attenuating glucose-induced insulin secretion or alternatively by reducing cell responsiveness that results from enhancements in cell mass in hypo- and hyper-thyroid states.[29,30] Adiponectin,

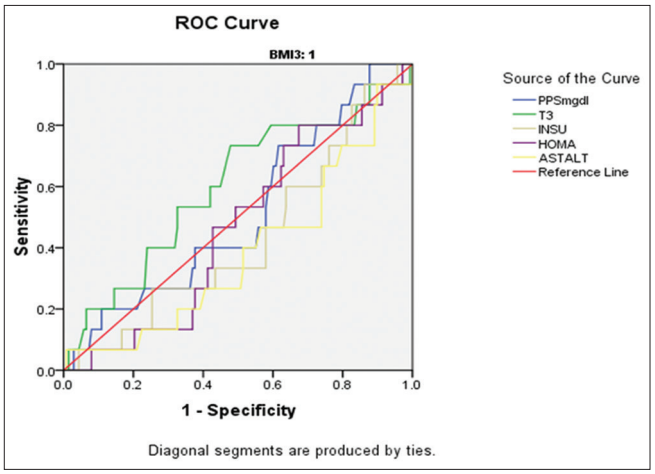

Figure 1: Receiver operating characteristic curve for non-obese type 2 diabetes mellitus patients

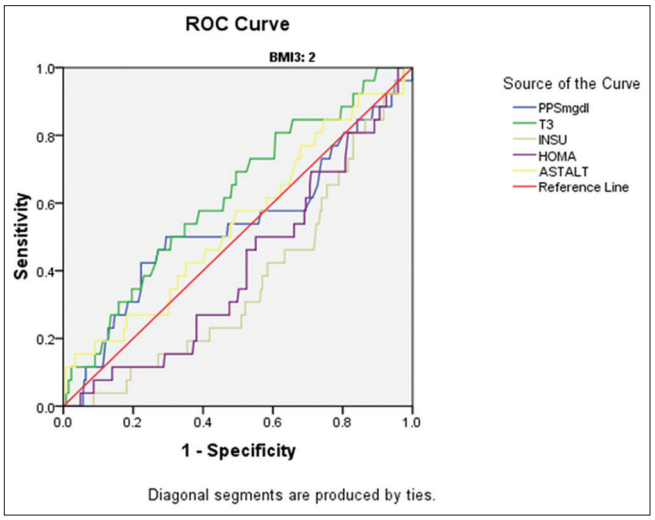

Figure 2: Receiver operating characteristic curve for overweight type 2 diabetes mellitus patients

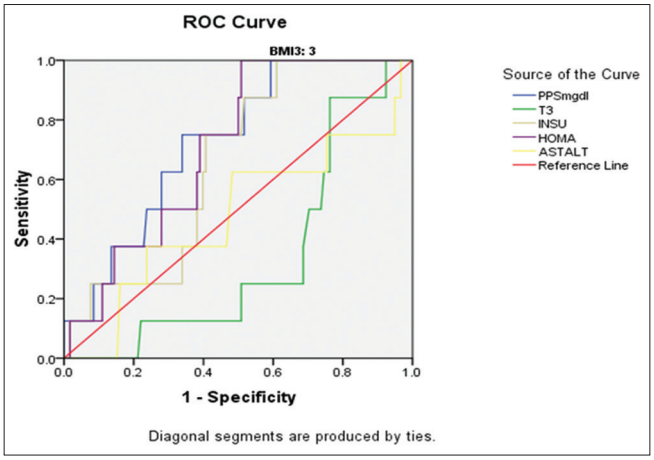

Figure 3: Receiver operating characteristic curve for obese type 2 diabetes mellitus patients

an important adipokine that enhances insulin sensitivity, is increased in hyperthyroidism.[31,32] HOMA-IR, an objective measure of IR, was higher in subjects with subclinical hypothyroidism in comparison with euthyroid subjects. This implies that decreased TH levels may be synonymous with IR. Moreover, TSH is positively associated with insulin and IR, whereas FT3 and thyroxine were inversely correlated with insulin and HOMA-IR.[33]

Earlier reports cite that an individual with apparently normal BMI can still exhibit central adiposity, a forerunner to IR.[34] We had deliberately included BMI as the anthropometric index to open up the additional group, namely overweight, which otherwise gets frequently ignored in the diabetic population. This is due to the fact that most of the studies are either focused on lean (non-obese) or obese diabetics.

The three facets, namely glycemic control (HbA1c), IR (HOMA-IR), and lipid status (dyslipidemia) in the three groups, namely non-obese, overweight, and obese, share a common feature, namely T2DM and since only a very few reports are available, wherein the thyroid status has been evaluated alongside, we undertook the study. As a novel attempt, we wanted to study the predictive role of total bilirubin levels (an endogenously derived antioxidant) with reference to (a) glycemic control – as revealed by HbA1c, (b) IR (as evaluated by HOMA-IR), (c) dyslipidemia (denoted by lipid profile). It is to be noted that bilirubin has been already linked to metabolic syndrome.[35]

In our present study, with particular reference to the thyroid status in the anthropometry specified groups of type 2 diabetics, we evaluated the predictive role of total bilirubin.

Originality of our study

We opine that the controversy that surrounds thyroid comorbidity is focused on the ambiguity of the relationship of IR with both hypo- and hyper-thyroidism. It is well documented that both micro- and macro-vascular complications are observed with either T2DM alone or in combination with altered thyroid status.

Furthermore, India being a large country with a huge diabetic population that is still in the underprivileged and economically poor bracket, we wanted in our own small way to contribute, through evident-based research, to identify an economical, reliable, and easily monitorable biochemical parameter that can be facilitated even in small- and medium-sized clinical laboratories that could serve as a predictor of thyroid status in T2DM, but as a function of anthropometry specified groups, namely non-obese, overweight, and obese, a hitherto less frequented area of research. The predictor, as per our study, is total bilirubin, an economical and reliable biochemical parameter that would promote objectivity in determining thyroid status in clinically euthyroid type 2 diabetics.

Before beginning this study, we had hypothesized that bilirubin could serve as a predictor differentially in the light of thyroid status and IR and with reference to anthropometric groups. Our results have unequivocally reflected the same. Our present study has enabled us to observe the association with total bilirubin in serum as against IR perceived in T2DM, but with special reference to thyroid status. Our study reveals that oxidative stress may be the common nexus between thyroid status and IR in otherwise clinically euthyroid type 2 diabetics. This is due to the fact that factors such as obesity, overweight, central obesity, and hypertriacylglycerolemia are common to both thyroid status and T2DM. Moreover, oxidative stress has been implicated as a common factor. Bilirubin, being an endogenously derived antioxidant, is significant in the light of the above-mentioned facts, and hence, we had studied bilirubin and its associations. Our results unequivocally demonstrate the utility and value of bilirubin (total) as a predictor of thyroid status and IR in overweight and obese type 2 diabetics.

As per an earlier report, serum bilirubin could be recognized as an early biochemical clinical marker for predicting the progression of chronic kidney disease in patients with T2DM and preserved renal function.[36] However, our study was focused on the purported ability of bilirubin (total) to act as a predictor of thyroid status as well as IR. This is due to the fact that the thyroid comorbidity is frequently observed in T2DM. Earlier reports had categorically stated that it is total bilirubin that is associated with the metabolic syndrome. However, as per a study depicting multivariable-adjusted model, the inverse associations with total and indirect bilirubin became less pronounced. As per an earlier study, the relation was observed particularly with direct bilirubin in the objectivized subgroups of metabolic syndrome. Therein, various inherent facets such as central obesity, hypertriacylglycerolemia, and hyperglycemia were taken into due consideration. The inverse association of metabolic syndrome was apparent and consistent with the conjugated bilirubin as per a previous report, unlike our study which has now implicated total bilirubin.[37]

As far as, the statistical test/model for predicting a morbidity or disease is considered, the characteristic features such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are taken into due consideration. However, such statistical measures are hampered by the inherent disadvantage, namely a single cutoff point. As per the single cutoff, we can envisage only an abstract assessment of the interplay between sensitivity and specificity. However, PPV and NPV are further influenced by prevalence in the population. Hence, our study with reference to bilirubin as a natural (endogenous) antioxidant had enabled us to study the predictive value with the focus on IR associated thyroid status. Furthermore, we wanted to evaluate the predictive value of bilirubin because oxidative stress has been implicated in altered thyroid status as well as in IR, as reported in literature. Taking all of the above facets into due consideration, we had embarked on the use of ROC curves to assess the predictive nature of total bilirubin in assessing thyroid status in type 2 diabetics who are afflicted with IR.

ROC curves confer the distinct advantage of providing a graphical depiction of the range of possible cutoff points with the corresponding false-positive rate. Every point in the ROC curves would, hence, represent a distinct sensitivity/specificity pair. We exploited these inherent merits in the delineation of the ROC curves concerning the predictive value of bilirubin. Furthermore, we used the pattern elaborated by ROC curves to determine the predictive value of bilirubin with reference to IR, thyroid status, and transaminase levels (aminotransferases) in clinically euthyroid type 2 diabetics [Figures 1-3]. Hence, in our study, we plotted a ROC curve for non-obese T2DM based on sensitivity and specificity with reference to PPS, T3, and HOMA-IR against total bilirubin. Furthermore, cardiovascular morbidity has been observed in IR as well as in altered thyroid status. A revelation that has emerged in recent years is the role of bilirubin in alleviating ischemic heart diseases, thanks to its role as a naturally occurring antioxidant. Reports are available to suggest that mildly elevated serum bilirubin levels are strongly associated with a lower prevalence of oxidative stress-mediated diseases that include diseases of the cardiovascular system. Serum bilirubin levels have been time and again depicted to be negatively correlated with CVDs and also other risk factors such as hypertension, DM, metabolic syndrome, and obesity. The key enzyme in the heme catabolic pathway, namely heme oxygenase, has also been projected in underlining the role of bilirubin as an antioxidant. This has opened up newer vistas in evidence-based diagnostic medicine that points to comprehensive information on both experimental and clinical evidence centered around the heme catabolic pathway and CVD. Even interlinked diseases promoting comorbidity such as diabetes, metabolic syndrome, and obesity are under effective consideration.[13] Our results could, thus, be acceded to the available literature.

We had wanted to study the extended role of bilirubin as an antioxidant that not only exerts effect on IR but also governs thyroid status and that for two reasons:

1. Thyroid diseases are observed as comorbidity in IR
2. Oxidative stress is linked to IR and bilirubin levels. Our study was primarily focused on the use of the routine parameters in clinical chemistry, namely bilirubin to act as a surrogate for IR and alterations in thyroid status – a novel concept that could assume clinical relevance.

However, conflicting reports are also available, wherein direct bilirubin and not total bilirubin are related to metabolic syndrome. In a longitudinal study, it has been observed that conjugated (direct) bilirubin is related to components of metabolic syndrome, with particular reference to obesity and dyslipidemia.[38]

Studies have shown that non-alcoholic fatty liver disease (NAFLD) is associated with patients suffering from T2DM. In this context, the liver enzymes assume great relevance, in the light of the IR. A study by Sheng *et al.* has revealed that the transaminases (aminotransferases) are associated with an increased HOMA-IR (measure of IR). A study has enabled the possibility of evaluating the degree of IR and hepatocellular steatosis in type 2 diabetes– a factor that could help in planning therapeutic modality for delaying the progression of T2DM and NAFLD.[39] Yet another study had delineated the fact that elevated levels of liver enzymes, but well within the normal (reference) range could independently predict T2DM in elderly men, associated with metabolic syndrome endearing with IR.[40] A recent study from South India has linked glucose intolerance and IR in NAFLD. The study concluded by stating a high prevalence of both prediabetes and diabetes in patients with NAFLD and is associated with increasing IR in higher grades of NAFLD.

Pyridoxine (B6) deficiency may interfere with adipogenesis, thereby affecting IR.[41] Pyridoxal phosphate can act as a free radical scavenger, and hence, deficiency of B6 will be linked to IR. Pyridoxal phosphate is required for cystathionine beta-synthase and, hence, linked to homocysteine high level in IR. Pyridoxal phosphate is required for transaminase, and hence, pyridoxine status related to IR can be surrogated through AST and ALT.[42]

Studies have also revealed that bilirubin thwarts the atherosclerotic process. Bilirubin is believed to attenuate a key factor, namely chemotactic activity of monocytes and pronouncedly, inhibits the adhesion of white blood cells to venule and production of pro-inflammatory cytokines. Bilirubin measurement is certainly inexpensive as compared to other marker and is performed routinely besides being accessible to most medical centers, small- and medium-sized clinical laboratories. Thus, serum bilirubin levels can be utilized easily by clinicians as one of the risk factors for the development of IR. However, it is underlined that additional studies must necessarily be affected to augment the optimal clinical potentials and utility of serum bilirubin as a marker

in monitoring IR. Detection at an earlier stage is deemed important in T2DM patients, especially with a low normal total bilirubin concentration and more so with altered thyroid status/comorbidity looming large in hastening insulin-resistant linked vascular complications.

The inherent limitations of the study include small sample size, lack of gender specificity, and total antioxidant status being not quantitated.

Conclusion

The serum total bilirubin levels might prove to be an early biochemical as well as clinical marker in the prediction of the progression of IR in type 2 diabetics with subclinical thyroid comorbidity. Hence, discretion is deemed appropriate when managing patients with T2DM and a low normal total bilirubin concentration. Further studies are absolutely deemed essential to determine as to whether the total bilirubin concentration could serve as a potential therapeutic target for the prevention of IR and associated thyroid status though the subjects may apparently be euthyroid as per the clinical criteria.

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Conflicts of Interest

The authors declare that there are no conflicts of interest in this study.

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References

1. Maghsoudi Z, Azadbakht L. How dietary patterns could have a role in prevention, progression, or management of diabetes mellitus? Review on the current evidence. J Res Med Sci 2012;17:694-709.
2. International Diabetes Federation. 6th ed. Brussels, Belgium: IDF Diabetes Atlas; 2013. p. 30. Available from: http://www.idf.org/diabetesatlas. [Last accessed on 2017 Feb 17].
3. Hayward RA, Reaven PD, Wiitala WL, Bahn GD, Reda DJ, Ge L, *et al.* Follow-up of glycemic control and cardiovascular outcomes in Type 2 diabetes. N Engl J Med 2015;372:2197-206.
4. American Diabetes Association. Erratum. Classification and diagnosis of diabetes. Sec 2. In standards of medical care in diabetes-2016. Diabetes care 2016;39(Suppl 1):S13-S22. Diabetes Care 2016;39:1653.
5. Ikeda F, Doi Y, Ninomiya T, Hirakawa Y, Mukai N, Hata J, *et al.* Haemoglobin A1c even within non-diabetic level is a predictor of cardiovascular disease in a general Japanese population: The Hisayama study. Cardiovasc Diabetol 2013;12:164-171.
6. Mullugeta Y, Chawla R, Kebede T, Worku Y. Dyslipidemia associated with poor glycemic control in Type 2 diabetes mellitus and the protective effect of metformin supplementation. Indian J Clin Biochem 2012;27:363-9.
7. Faghihimani Z, Mirmiran P, Sohrab G, Iraj B, Faghihimani E. Effects of pomegranate seed oil on metabolic state of patients with Type 2 diabetes mellitus. Int J Prev Med 2016;7:124.
8. Chehade JM, Gladysz M, Mooradian AD. Dyslipidemia in Type 2 diabetes: Prevalence, pathophysiology, and management. Drugs 2013;73:327-39.
9. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107:1058-70.
10. Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: Pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord 2008;9:315-27.
11. Singh AK, Singh SK, Singh N, Agrawal N, Gopal K. Obesity and dyslipidemia. Int J Biol Med Res 2011;2:824-8.
12. Vítek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. Adv Clin Chem 2007;43:1-57.
13. Vítek L. The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases. Front Pharmacol 2012;3:55-61.
14. Oda E, Aizawa Y. Total bilirubin is inversely associated with metabolic syndrome but not a risk factor for metabolic syndrome in Japanese men and women. Acta Diabetol 2013;50:417-22.
15. Novotný L, Vítek L. Inverse relationship between serum bilirubin and atherosclerosis in men: A meta-analysis of published studies. Exp Biol Med (Maywood) 2003;228:568-71.
16. Chan KH, O'Connell RL, Sullivan DR, Hoffmann LS, Rajamani K, Whiting M, *et al.* Plasma total bilirubin levels predict amputation events in Type 2 diabetes mellitus: The fenofibrate intervention and event lowering in diabetes (FIELD) study. Diabetologia 2013;56:724-36.
17. Ajja R, Lee DC, Sui X, Church TS, Steven NB. Usefulness of serum bilirubin and cardiorespiratory fitness as predictors of mortality in men. Am J Cardiol 2011;108:1438-42.
18. Waring AC, Rodondi N, Harrison S, Kanaya AM, Simonsick EM, Miljkovic I, *et al.* Thyroid function and prevalent and incident metabolic syndrome in older adults: The health, ageing and body composition study. Clin Endocrinol (Oxf) 2012;76:911-8.
19. Smith TJ, Drummond GS. Retinoic acid can enhance the stimulation by thyroid hormone of heme oxygenase activity in the liver of thyroidectomized rats. Biochim Biophys Acta 1991;1075:119-22.
20. Gartner LM, Arias IM. Hormonal control of hepatic bilirubin transport and conjugation. Am J Physiol 1972;222:1091-9.
21. Fernández-Real JM, López-Bermejo A, Castro A, Casamitjana R, Ricart W. Thyroid function is intrinsically linked to insulin sensitivity and endothelium-dependent vasodilation in healthy euthyroid subjects. J Clin Endocrinol Metab 2006;91:3337-43.
22. Kouidhi S, Berhouma R, Ammar M, Rouissi K, Jarboui S, Clerget-Froidevaux MS, *et al.* Relationship of thyroid function with obesity and Type 2 diabetes in euthyroid tunisian subjects. Endocr Res 2013;38:15-23.
23. Ortega E, Koska J, Pannacciulli N, Bunt JC, Krakoff J. Free triiodothyronine plasma concentrations are positively associated with insulin secretion in euthyroid individuals. Eur J Endocrinol 2008;158:217-21.
24. Prats-Puig A, Sitjar C, Ribot R, Calvo M, Clausell-Pomés N, Soler-Roca M, *et al.* Relative hypoadiponectinemia, insulin resistance, and increased visceral fat in euthyroid prepubertal girls with low-normal serum free thyroxine. Obesity (Silver Spring) 2012;20:1455-61.

25. Ren R, Jiang X, Zhang X, Guan Q, Yu C, Li Y, *et al.* Association between thyroid hormones and body fat in euthyroid subjects. Clin Endocrinol (Oxf) 2014;80:585-90.
26. Roos A, Bakker SJ, Links TP, Gans RO, Wolffenbuttel BH. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. J Clin Endocrinol Metab 2007;92:491-6.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC, *et al.* Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
28. Lin Y, Sun Z. Thyroid hormone potentiates insulin signaling and attenuates hyperglycemia and insulin resistance in a mouse model of Type 2 diabetes. Br J Pharmacol 2011;162:597-610.
29. Mitrou P, Raptis SA, Dimitriadis G. Insulin action in hyperthyroidism: A focus on muscle and adipose tissue. Endocr Rev 2010;31:663-79.
30. Stanická S, Vondra K, Pelikánová T, Vlcek P, Hill M, Zamrazil V, *et al.* Insulin sensitivity and counter-regulatory hormones in hypothyroidism and during thyroid hormone replacement therapy. Clin Chem Lab Med 2005;43:715-20.
31. Ağbaht K, Erdogan MF, Emral R, Baskal N, Güllü S. Circulating glucagon to ghrelin ratio as a determinant of insulin resistance in hyperthyroidism. Endocrine 2014;45:106-13.
32. Pontikides N, Krassas GE. Basic endocrine products of adipose tissue in states of thyroid dysfunction. Thyroid 2007;17:421-31.
33. Vyakaranam S, Vanaparthy S, Nori S, Palarapu S, Bhongir AV. Study of insulin resistance in subclinical hypothyroidism. Int J Health Sci Res 2014;4:147-53.
34. Yu Y, Venners SA, Wang B, Brickman WJ, Zimmerman D, Li Z, *et al.* Association of central adiposity with prediabetes and decreased insulin sensitivity in rural Chinese normal-weight and overweight women. Metabolism 2010;59:1047-53.
35. Jo J, Yun JE, Lee H, Kimm H, Jee SH. Total, direct, and indirect serum bilirubin concentrations and metabolic syndrome among the Korean population. Endocrine 2011;39:182-9.
36. Ahn KH, Kim SS, Kim WJ, Kim JH, Nam YJ, Park SB, *et al.* Low serum bilirubin level predicts the development of chronic kidney disease in patients with Type 2 diabetes mellitus. Korean J Intern Med 2017;32:875-82.
37. Chen Q, Xiao J, Zhang P, Chen L, Chen X, Wang S, *et al.* Association between serum direct bilirubin with metabolic syndrome and its components based on a longitudinal health check-up study. Zhonghua Liu Xing Bing Xue Za Zhi 2016;37:486-90.
38. Wannamethee SG, Shaper AG, Lennon L, Whincup PH. Hepatic enzymes, the metabolic syndrome, and the risk of Type 2 diabetes in older men. Diabetes Care 2005;28:2913-8.
39. Sheng X, Che H, Ji Q, Yang F, Lv J, Wang Y, *et al.* The relationship between liver enzymes and insulin resistance in Type 2 diabetes patients with nonalcoholic fatty liver disease. Horm Metab Res 2018;50:397-402.
40. Kitade H, Chen G, Ni Y, Ota T. Nonalcoholic fatty liver disease and insulin resistance: New insights and potential new treatments. Nutrients 2017;9:387-400.
41. Liu Z, Li P, Zhao ZH, Zhang Y, Ma ZM, Wang SX, *et al.* Vitamin B6 prevents endothelial dysfunction, insulin resistance, and hepatic lipid accumulation in apoe (-/-) mice fed with high-fat diet. J Diabetes Res 2016;2016:1748065.
42. Răcătăianu N, Leach N, Bondor CI, Mârza S, Moga D, Valea A, *et al.* Thyroid disorders in obese patients. Does insulin resistance make a difference? Arch Endocrinol Metab 2017;61:575-83.