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Research Article

Fasting Plasma Total IGF-1 and Bile Acids Levels Correlate With the Disease Prognostic Indices in Type 2 Diabetic Patients

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ABSTRACT

Although they are pathophysiologically involved, evidences inconsistently highlight a relationship between type 2 diabetes mellitus (T2DM) and its prognosis with variation in circulating levels of each of bile acids (BAs) and Insulin-like growth factor-1 (IGF-1). We aimed to investigate the possible role of BAs and IGF-1 in the prediction of T2DM and its complications in Saudi patients. Their fasting plasma levels were correlated to each other and to the disease clinical and biochemical prognostic indices. This is an analytical cross-sectional Tertiary care center-based study. We consecutively included 184 hospital-diagnosed T2DM patients and 113 healthy controls from their accompanying relatives. Anthropometrics, demographics and disease history were recorded. Fasting plasma levels of total BAs, lipids and glucose were assayed colorimetrically, and, total IGF-1, hemoglobin A1c (HbA1c), insulin, and C-reactive protein (CRP) were quantitatively immunoassayed. Atherogenic index of plasma (AIP) and homeostatic model assessment of insulin resistance (HOMA-IR) were calculated. Diabetic patients were stratified by disease severity score and treatment score for association analysis. Correlations among parameters were assessed. Area under the receiver operating characteristic (ROC) curve (AUC) assessed the discriminative power of BAs and IGF-1 vs. prognostic indices. Our main outcomes were correlation among BAs and IGF-1 with diabetes prognostic indices. Majority of patients were overweight, females, <60 years old and had complications. Patients showed significant increase in all studied biochemical parameters than controls, except for total BAs; particularly with advancement of age and BMI. AUC showed that CRP, AIP, IR and HbA1c were better predictors of disease complications and that BAs level was the single best predictor of absence of complications. Binary logistic regression showed that only age and treatment score significantly predicted the presence of complications. In conclusion, IGF-1 can be a monitoring marker and BAs can predict the absence of complications in T2DM. Further prospective researches are needed to elucidate these conclusions in larger multicenter and longitudinal studies that take in consideration other limitations of this study.

Keywords: *Type 2 diabetes mellitus, bile acids, insulin-like growth factor-1, complications, disease duration, glycemic control, prognostic indices*

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an increasingly global socioeconomic burden that drains healthcare systems owing to its wide range of long-term complications affecting nearly all organs (Afroz *et al.*, 2018). In Saudi Arabia, the increasing trend of T2DM is alarming (Siddiqui *et al.*, 2018; IDF 2020). Despite appropriate adherence to lifestyle recommendations and treatment, T2DM complications seem inevitable, in majority of patients, indicating deficiency in our understanding of the pathogenesis of the disease and its complications.

Somatomedins or insulin-like growth factors 1 and 2 (IGF-1 and -2) alongside their binding proteins (IGFBPs 1-6) are secreted by many cell types (Frysa *et al.*, 2015). Although IGFBPs increase IGF-1 half-life in the blood, they lower its bioavailability to bind its receptor (IGFIR) that activates PI3 kinase/AKT kinase and RAF kinase/MAP kinase pathways.

IGF-1 as the prototype somatomedin is a 70-amino acid single polypeptide growth factor and shares structural homology with IGF-2 and proinsulin (Aguirre *et al*., 2016; Chen *et al.*, 2018). IGF-1, in an endocrine, paracrine, and autocrine manner, induces metabolic control, similar to that of insulin, and enhances insulin action. Albeit with varying affinities, IGF-1 and insulin bind each other receptors and their hybrid receptor. The latter binds IGF-1 stronger and its expression inversely correlates with insulin receptor in T2DM patients (Mughal *et al.*, 2019). During postprandial periods, insulin suppresses IGFBP-1 secretion to increase the free circulating level and anti-diabetogenic and β-cells supportive actions of IGF-1 (del Rincon *et al.*, 2007). Therefore, IGF-1 deficit is significantly associated with pathophysiology of insulin resistance (IR), metabolic syndrome (MetS), altered metabolic profile of diabetic patients and cardiovascular disease (Aguirre *et al*., 2016). Genome-wide association studies linked IGF-1 driver gene polymorphism with IR and T2DM (Dupuis *et al.*, 2010; Scott *et al.*, 2012; Shu *et al.*, 2017). Other studies did not find similar association (Chirita-Emandi *et al.*, 2019; Similä *et al.*, 2019). In mice and humans null for igf1 gene there are glucose intolerance, IR, and high blood insulin levels (Clemmons, 2004). IGF-1 perfects mitochondria integrity and function preserving it from the oxidative damage generated from augmented metabolism (García Fernández *et al.*, 2008). However, IGF-1 signaling pathway major functions are the specific long-term effects on cell fate; proliferation, migration, differentiation and apoptosis (Frysa *et al.*, 2015; Aguirre *et al*., 2016; Chen *et al.*, 2018).

Besides being lipid digestive/absorptive detergents, bile acids (BAs) play a major role in obesity, IR and T2DM by regulating lipid and glucose homeostasis through inducing incretins, and insulin secretion and sensitisation (Tomkin and Owens, 2016). Depending on their type, the hormonal metabolic regulation by BAs is specifically conducted by differential activation/antagonization of two receptors; the cell transmembrane Takeda G-protein-coupled receptor-5 (TGR5) and the nuclear transcription factor farnesoid X receptor (FXR) (Fouladi *et al.*, 2016). Alteration in BAs-FXR/TGR5 pathway may contribute to glucose intolerance (Yan *et al.*, 2017; Kuhre *et al.*, 2018). Dietary restriction and weight loss improve obesity and diabetes through normalizing the altered metabolism of BAs (Tomkin and Owens, 2016). TGR5 controls energy homeostasis, BAs homeostasis, glucose metabolism and induce secretion of insulin incretins; the anorexigenic glucagon-like peptide-1 and -2 (GLP-1/2) and peptide YY (PYY) from lower gut enteroendocrine L-cells (EELCs) (Velazquez-Villegas *et al.*, 2018; Donkers *et al.*, 2019; Schmid *et al.*, 2019; Finn *et al.*, 2019; Christiansen *et al.*, 2019). BAs induce growth, differentiation and incretin secretion from EELCs (Lund *et al.*, 2020). FXR signaling is very essential for glucose homeostasis against induction of obesity and utilizes FGF-19 and 21 and GLP-1 for that (Albaugh *et al.*, 2019; Pierre *et al.*, 2019). FGF-19, known to be reduced in patients with T2DM, enhances mitochondrial activity, improves hyperlipidemia, hepatic steatosis, and adiposity towards improving IR (Kir *et al.*, 2011; Barutcuoglu *et al.*, 2011). The increased serum 12α-hydroxy: non-12α-hydroxy BAs ratio correlates with key features of T2DM, namely, IR, high insulin, proinsulin, glucose, glucagon and triglyceride levels, and lower HDL-C, and may suppress fibroblast growth factor (FGF)-19-FXR pathway (Jiao *et al.*, 2018). However, lower 12α-hydroxy BAs level correlates with increased GLP-1 and improves glucose tolerance (Kaur *et al.*, 2015). Through modulating gut microbiome, metformin increases the hydrophilic glycol urso-deoxy-cholic acid (UDCA), which inhibits intestinal FXR and increase liver BAs synthesis to improve metabolic dysfunction including hyperglycemia (Sun *et al.*, 2018). Treatment with the core human gut Parabacteroides distasonis markedly increases litho-cholic acid (LCA) and UDCA that activate FXR pathway, repairs gut barrier integrity, and, reduce hyperlipidemia (Wang *et al.*, 2019).

In this study, we investigated the possible role of BAs and IGF-1 in the prediction of T2DM and its complications in Saudi patients. Their fasting plasma levels were cross-sectionally correlated to each other and to the disease clinical and biochemical prognostic indices.

MATERIALS AND METHODS

Study type and Setting: This analytical cross-sectional study was conducted at the Diabetes Care Unit, Prince Muteb General Hospital, and College of Medicine, Jouf University, Sakaka, Aljouf, Saudi Arabia in the period from December 1, 2017 to June 30, 2019.

Ethical consideration: It was ethically approved by The Bioethical Committee of Jouf University (13-3-21/38-39) and each participant signed an informed consent before participation.

Participants: We voluntarily enrolled 297 participants. Type II diabetic patients comprised consecutively enrolled 184 participants (46 males/138 females with a mean age of 48.7 ± 13.5 years) and age-, gender and BMI-matching patients' accompanying healthy controls comprised 113 participants (52 males/61 females with a mean age of 44.0 ± 14.3 years). Demographic data, anthropometric indices and disease history were anonymously recorded for each participant.

Exclusion criteria: Patients' exclusion criteria included immobilization for any reason, renal or hepatic failure, autoimmune diseases, acute infections, congenital and hemolytic anemias, and systemic inflammatory diseases, and patients on immunosuppressive or steroidal and non-steroidal anti-inflammatory for any reason.

Sampling and investigations: Morning fasting peripheral blood samples were collected on EDTA. After separating an aliquot of whole blood for HbA1c assay, plasma was recovered by centrifugation and was aliquot stored at -80 ^{o}C till used. Fasting plasma lipids and glycemic control indices were colorimetrically assayed (Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany) to calculate atherogenic index of plasma (AIP) and homeostatic model assessment of insulin resistance (HOMA-IR), respectively. HOMA-IR was calculated as glucose (mg/dL) x insulin (mIU/L)/405. AIP was calculated as log (triglycerides/high-density lipoprotein cholesterol; TAGs/HDL-C) in mM/L. Specific ELISA assay kits from Cloud-Clone Corp were used

to quantify hemoglobin A1c (HbA1c), insulin, and C-reactive protein (CRP) (Wuhan, China; cat# CEA190Hu, CEA448Hu, and SEA821Hu), and, from Sunlong Biotech Co. Ltd. was used to measure total IGF-1 (in acid displaced and ethanol precipitated samples) (cat# SL0940Hu; Zhejiang, China). Total BAs content was measured using enzymatic colorimetric assay kit from Egyptian Company for Biotechnology (Obour City, Cairo, Egypt; cat#305001).

In diabetic patients; the disease duration, disease severity score (no complications = 0, one complication = 1, two complications = 2, three complications = 3, and ≥4 complications = 4) and treatment score (no treatment = 0, metformin = 1, hypoglycemic ± metformin = 2, and insulin ± metformin = 3) were related to the investigated biomarkers. Age- and gender-stratified BMI (www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/metric_bmi_calculator/bmi_calculator.html) was calculated.

Statistical analysis

Statistical package for social sciences (SPSS) for Windows version 20.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA) was used for data analysis. Frequencies, percentages, mean and standard deviation was calculated for categorical and continuous variables respectively. Chi-square test was applied to evaluate differences between categorical variables. Independent t-tests was performed for comparing quantitative variables between diabetic and control groups, ANOVA with Tukey's HSD post-hoc test was performed when comparing three or more categories. Area under the receiver operating characteristic (ROC) curve (AUC) was calculated to assess the discriminative power of BAs and IGF-1. Correlation among different parameters with each group/subgroup was analyzed. Results were considered significant at a p value <0.05.

RESULTS

Of the 297-studied population, 63% was of the age group of 30-59 years. Mean age and the number of female population were significantly higher in the diabetic group compared to controls. Majority of the patients had complications - 40 patients only were complication-free (10 males/30 females). Almost half of them were having multiple complications (50.694%); 134 patients had neuropathy (28 males/106 females), 111 patients had ophthalmopathy (33 males/78 females), 13 patients had nephropathy (5 males/8 females), 3 male patients had ketoacidosis and 5 patients had myocardial infarction (2 males/3 females). All the biochemical parameters were significantly higher in the diabetic group except for BAs (Table 1).

Overall, biochemical parameters were significantly higher in the age group of >60 years except for BAs. Similar findings were observed for higher BMI categories with a further exception of IGF-1. HbA1c was significantly higher in patients with a history of 5-10 years of disease duration (Table 2).

Results of the correlation between independent variables (age and BMI) and biochemical parameters investigated in T2DM patients are shown in Table 3. Age showed a significant positive correlation with AIP, and HbA1c among diabetic patients. Similar correlations were observed for BMI with a further addition of a significant correlation with IR. CRP showed a positive correlation with age, BMI and all of the biochemical parameters and a negative correlation with BAs.

The relationships between disease duration and the studied biochemical parameters in T2DM patients are presented in Table 4. These investigations did not show any significant variations with disease duration.

Table 1:
Comparison between type 2 diabetic patients (T2DM; n = 184) and healthy non-diabetic controls (n = 113) for demographics, anthropometrics and investigated fasting plasma parameters.

Variable		Diabetics	Controls	P
[$]Gender	Mean age[*]	48.7 (13.5)	44.0 (14.3)	0.005
	Males	46	52	0.001
	Females	138	61	
[$]Age, years	≤29	16	21	0.020
	30-59	117	70	
	≥60	51	22	
[$]BMI categories	Underweight (<18.5)	2	0	.170
	Normal weight (18.5 - 24.9)	31	19	
	Pre-obesity (25.0 - 29.9)	76	51	
	Obesity class I (30.0 - 34.9)	50	18	
	Obesity class II (35.0 - 39.9)	24	17	
	Obesity class III (>40)	1	0	
[*]Biochemical Parameters	CRP, mg/dL	3.39 (1.0)	1.86 (1.1)	0.001
	AIP	0.39 (.18)	0.17 (.21)	0.001
	HbA1c %	8.17 (1.04)	6.93 (.83)	0.001
	Total IGF-1, ng/mL	98.39 (150.77)	70.34 (86.82)	0.043
	Total BAs, μM/L	4.14 (5.13)	4.59 (2.86)	0.390
	HOMA-IR	3.90 (2.60)	1.41 (1.31)	0.001

Data shown are frequency (n), mean (± SD) and P value. [*]Independent t-test; [$]Chi-square test. CRP = C-reactive protein. AIP = Atherogenic index of plasma. IGF-1 = Insulin-like growth factor-I. BAs = Bile acids. HOMA-IR = Homeostatic model assessment of insulin resistance.

Table 2: The investigated fasting plasma parameters in type 2 diabetic patients (n = 184) stratified for age, body mass index (BMI), disease duration (DD) and treatment and complication scores.

Characteristic		CRP	AIP	IR	HbA1c %	IGF-1, ng/mL	BAs, µM/L
Age, years	≤29 (n = 16)	2.0 (1.1)	0.22 (0.31)	1.7 (1.3)	6.9 (0.87)	53.4 (45.7)	4.3 (3.5)
	30-59 (n = 117)	2.8 (1.3)	0.30 (0.19)	3.2 (2.7)	7.8 (1.21)	103.4 (155.6)	4.2 (4.2)
	≥60 (n = 51)	2.9 (1.2)	0.36 (0.20)	2.8 (2.2)	7.7 (0.92)	64.9 (69.6)	4.4 ± 5.2
	P	**0.001***	**0.010***	**0.004***	**0.001***	**0.020***	**0.960**
BMI, kg/m^2	Underweight (<18.5)	1.92 (.88)	0.20 (0.17)	3.40 (0.42)	7.45 (.21)	29.60 (5.87)	2.26 (0.001)
	Normal weight (18.5–24.9)	2.42 (1.34)	0.24 (0.17)	2.08 (1.55)	7.12 (1.01)	88.56 (135.12)	4.23 (3.77)
	Pre-obesity (25.0–29.9)	2.52 (1.22)	0.28 (0.23)	2.65 (2.26)	7.48 (.98)	85.78 (123.23)	3.89 (2.80)
	Obesity class I (30.0–34.9)	3.33 (1.23)	0.36 (0.21)	3.84 (2.92)	8.16 (1.11)	96.29 (145.92)	5.54 (7.26)
	Obesity class II (35.0–39.9)	3.32 (1.48)	0.36 (0.23)	3.33 (2.76)	8.28 (1.15)	82.80 (131.16)	3.92 (2.82)
	Obesity class III (>40)	4.01	0.32	11.80	12.21	42.91	2.65
	P	**0.001**	**0.020**	**0.001**	**0.001**	**0.910**	**0.189**
DD, years	<5	3.28 (1.08)	0.37 (0.17)	3.77 (1.98)	7.94 (.96)	104.71 (131.35)	3.12 (1.51)
	5-10	3.38 (1.08)	0.38 (0.18)	3.93 (2.92)	8.33 (1.15)	91.42 (136.15)	4.74 (6.33)
	>10	3.49 (1.07)	0.44 (0.17)	4.04 (2.80)	8.31 (0.88)	113.28 (185.36)	4.39 (5.90)
	P	**0.610**	**0.110**	**0.870**	**0.050***	**0.730**	**0.140**
Treatment	Metformin	3.33 (0.95)	0.34 (1.49)	3.73 (1.51)	7.82 (0.63)	93.17 (95.88)	3.03 (1.47)
	Oral hypoglycemics (OH)	3.40 (0.85)	0.44 (0.18)	5.10 (3.38)	8.43 (1.54)	84.41 (144.61)	3.70 (1.85)
	Insulin	3.42 (0.84)	0.41 (0.18)	3.78 (3.29)	8.19 (.99)	104.62 (180.01)	4.77 (7.16)
	Metformin + OH	3.26 (1.09)	0.34 (0.16)	3.78 (2.16)	8.04 (1.15)	89.70 (130.55)	4.38 (4.68)
	Metformin + Insulin	3.48 (1.36)	0.42 (0.19)	3.54 (2.38)	8.36 (0.79)	109.77 (177.24)	4.35 (6.23)
	P	**0.730**	**0.090**	**0.23**	**2.300**	**0.890**	**0.890**
Complications	No complications	3.34 (1.07)	0.57 (1.15)	3.33 (1.33)	7.62 (0.58)	95.72 (154.42)	3.19 (1.70)
	One complication	3.32 (1.07)	0.50 (0.97)	3.74 (2.23)	8.13 (0.89)	99.45 (143.69)	3.90 (4.83)
	More than one	3.54 (1.09)	0.41 (0.18)	4.30 (3.06)	8.47 (1.18)	106.32 (162.38)	4.45 (5.86)
	P	**0.373**	**0.518**	**0.098**	**0.001***	**0.925**	**0.395**

Data shown are frequency (n), mean (± SD) and P value (ANOVA comparison of means and, *Post-hoc Turkey's LSD significant at <0.05).

Table 3: Correlation between independent variables (age and BMI) and biochemical parameters investigated in type 2 diabetic patients.

	BMI, kg/m^2	**CRP**, mg/dL	**AIP**	**IR**	**HbA1c %**	**IGF-1**, ng/mL	**BAs**, µM/L
Age	0.326 (0.000)	0.166 (0.004)	0.174 (0.003)	0.109 (0.062)	0.199 (0.001)	0.002 (0.970)	0.022 (0.703)
BMI	-	0.282 (0.000)	0.221 (0.000)	0.245 (0.000)	0.395 (0.000)	0.026 (0.658)	0.049 (0.404)
CRP	-	-	0.467 (0.000)	0.465 (0.000)	0.547 (0.000)	0.173 (0.000)	-0.054 (0.350)

Data shown are r (P) values

Area under the receiver operating characteristic (ROC) curve (AUC) analysis showed that plasma levels of CRP, AIP, IR and HbA1c were better predictors of disease complications. CRP has a sensitivity of 85% and a specificity of 45% with 0.69 (AUC) at a value of 2.32 mg%. HbA1c has a sensitivity of 91% and a specificity of 50% with 0.78 AUC at a value of 7.14 mg% (Figure 1).

AUC showed that fasting plasma total BAs level (µM/L) was the single best predictor of absence of complications in the studied population. At a BAs value of 4.42 µM/L, the specificity was 79% and a low sensitivity of 35% with 0.63 of AUC (Figure 2A). AUC showed that total IGF-1 (ng/mL) did not predict the absence of complications in the studied population (Figure 2B).

Using binary logistic regression for effect of independent variables (age, BMI, disease in years, treatment score, CRP, AIP, IR, HbA1c, IGF-1, BAs, Gender), Nagelkerke R Square was 0.625 with non-significant Hosmer and Lemeshow Test (P = 0.722). Only age and treatment score significantly predicted the presence of complication among diabetics (Table 5).

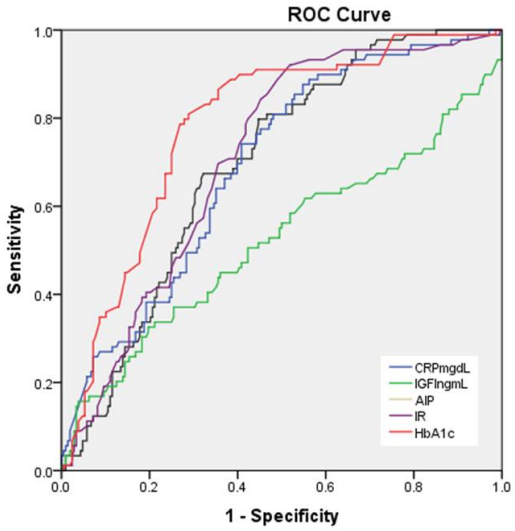

Figure 1:
Area under the receiver operating characteristic (ROC) curve (AUC) analysis for the biochemical indices as predictors of presence of complications among type II diabetic patients (n = 184). CRP AUC = 0.69 (0.63 – 0.75) and P <0.01. HbA1c AUC = 0.78 (0.72-0.83) and p <0.01. IR AUC = 0.71 (0.65 – 0.77) and P <0.01. AIP AUC = 0.69 (0.63 – 0.75) and P <0.01. Diagonal segments were produced by ties.

Table 4:
Effect of disease duration on the studied biochemical parameters in type 2 diabetic patients.

Parameters	Disease duration	n	Mean	SD	F	P
CRP, mg/dL	<5	66	3.287	1.080	0.492	0.612
	5-10	78	3.386	1.081		
	>10	44	3.494	1.070		
	Total	188	3.377	1.075		
AIP	<5	66	0.371	0.175	2.170	0.117
	5-10	78	0.387	0.181		
	>10	44	0.441	0.176		
	Total	188	0.394	0.179		
IR	<5	66	3.776	1.988	0.129	0.879
	5-10	78	3.935	2.921		
	>10	44	4.021	2.804		
	Total	188	3.899	2.589		
HbA1c %	<5	66	7.945	0.965	2.918	0.057
	5-10	78	8.332	1.151		
	>10	44	8.310	0.883		
	Total	188	8.191	1.040		
IGF-1, ng/mL	<5	66	104.712	151.555	0.309	0.735
	5-10	78	91.426	136.154		
	>10	44	113.283	185.363		
	Total	188	101.206	153.611		
BAs, µM/L	<5	66	3.130	1.516	1.922	0.149
	5-10	78	4.745	6.334		
	>10	44	4.395	5.903		
	Total	188	4.096	5.085		

Data shown are frequency (n), mean, SD, and F and P values. Analysis of variance (ANOVA) test was applied

DISCUSSION

The combined causation by genetic/epigenetic susceptibility and environmental exposures makes T2DM a highly heterogeneous disease. Saudi Arabia is 2nd Middle Eastern and 7th worldwide country for the rate of diabetes (Al Dawish *et al*., 2016). A majority of the population being obese with sedentary lifestyle, high consumers of obesogenic energy and fast unhealthy foods, and smokers could account for the later surge in national T2DM prevalence (Alsenany and Al Saif, 2015). Secretion of different incretins from lower gut EELCs is responsive to luminal nutrient and non-nutrient chemical cues (Kim and Jang, 2015). Our study analyzed the relationship of circulating levels of total BAs and IGF-1 vs. prognostic indices in T2DM Saudi patients. Majority of our patients were overweight, <60 years old, had diabetic complications and females. The latter could be reasoned to two factors; males being able to seek better healthcare services outside of the area and/or for females having a greater enthusiasm for follow up at the cost-free governmental centers. However, sample size-wise, equal female number to the used males was sufficient. Higher incidence of T2DM was reported among females attending the governmental primary healthcare centers although the prevalence is known to be higher among males (Albargawi *et al*., 2016; Alotaibi *et al*., 2017).

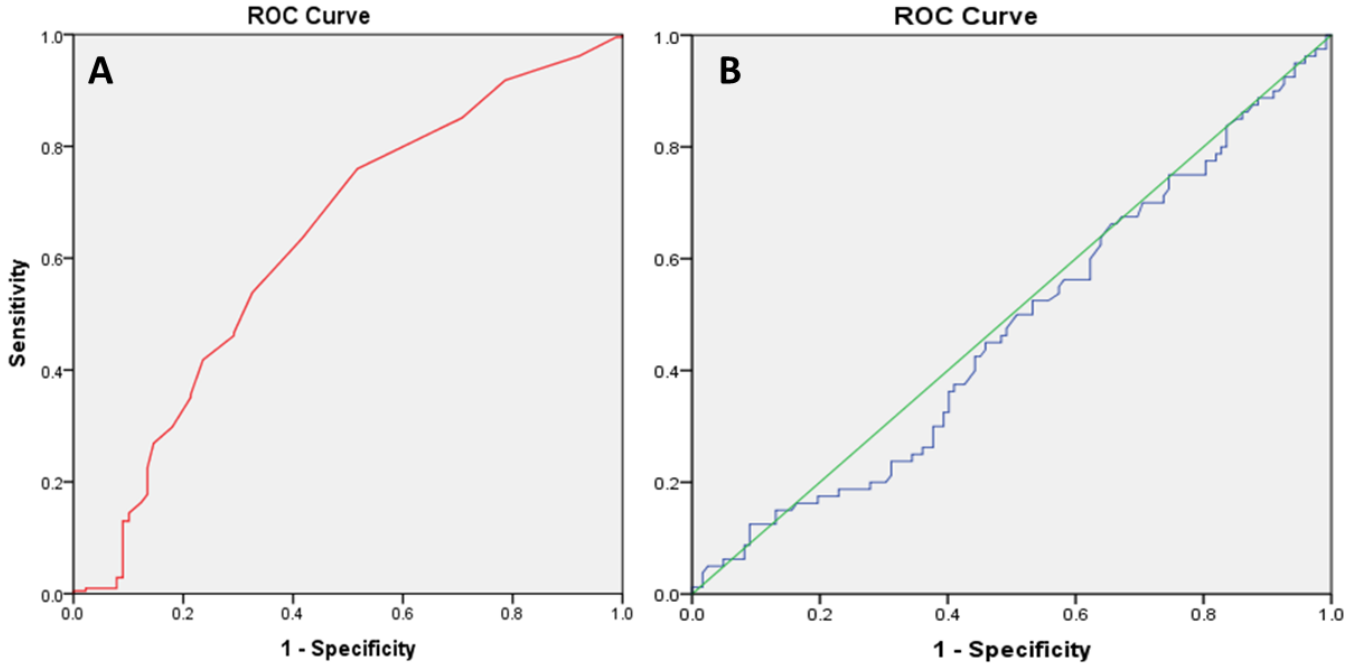

Figure 2:
A) Total fasting plasma bile acids level as a predictor of absence of complications among type II diabetic patients. AUC = 0.632 (0.56 – 0.70); P <0.01. Diagonal segments were produced by ties. B) Total IGF-1, ng/mL as a predictor of absence of complications among type II diabetic patients. AUC = 0.473 (0.392 – 0.554); P <0.01. Diagonal segments were produced by ties.

Table 5:
Binary logistic regression for effect of independent variables (age, BMI, disease duration in years, treatment score, CRP, AIP, Insulin Resistance, HbA1c, IGF-1, BAs, and Gender).

Model Summary			
Step	**-2 Log likelihood**	**Cox & Snell R Square**	**Nagelkerke R Square**
1	99.829[a]	0.394	0.625

a. Estimation terminated at iteration number 7 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test			
Step	**Chi-square**	**df**	**Sig.**
1	5.328	8	0.722

Variables in the Equation

Step 1[a]	**B**	**S.E.**	**Wald**	**df**	**Sig.**	**Exp (B)**	**95% C.I. for EXP(B)**	
							Lower	**Upper**
Age	**0.072**	**0.030**	**5.802**	**1**	**0.016**	**1.074**	**1.013**	**1.139**
BMI	0.124	0.075	2.744	1	0.098	1.132	0.978	1.310
Disease in years	0.159	0.111	2.076	1	0.150	1.173	0.944	1.457
Treatment score	**1.585**	**0.445**	**12.659**	**1**	**0.000**	**4.878**	**2.037**	**11.677**
CRP	-0.235	0.268	0.774	1	0.379	0.790	0.468	1.335
AIP	-0.067	0.219	0.094	1	0.759	0.935	0.609	1.435
IR	0.128	0.186	0.474	1	0.491	1.137	0.789	1.639
HbA1c	0.753	0.456	2.730	1	0.098	2.124	0.869	5.190
IGF-1	0.001	0.002	0.098	1	0.754	1.001	0.997	1.004
Bile salts	-0.019	0.080	0.057	1	0.812	0.981	0.839	1.148
Gender[(1)]	-0.659	0.672	0.962	1	0.327	0.517	0.138	1.932
Constant	-13.810	3.448	16.045	1	0.000	0.000	-	-

To explore the link between plasma total levels of BAs and IGF-1, and, T2DM prognostic indices, we compared changes in these parameters among the study groups. Previous studies that profiled BAs in T2DM patients have yielded inconsistent results (Brufau *et al*., 2010; Steiner *et al*., 2011). While our observation of insignificant higher BAs levels in healthy controls was consistent with some studies (Brufau *et al*., 2010; Steiner *et al*., 2011; Wewalka *et al*., 2014; Sun *et al*., 2016; Matysik *et al*., 2011; Haeusler *et al*., 2013), it contrasted others reporting higher levels (Sun *et al*., 2016) and rate of synthesis (Steiner *et al*., 2011) in insulin-resistant T2DM, and, in metabolic syndrome and T2DM patients though was confounded with BMI and triglycerides, respectively. We measured fasting BAs levels rather than the induced postprandial level (Matysik *et al*., 2011). 12α-hydroxylated BAs synthesis may not be expressed in steady-state plasma levels of BAs since their plasma level is predominantly specified by reabsorption and rate of liver re-uptake (Haeusler *et al*., 2013). Experimental Roux-en-Y gastric bypass in rat model of T2DM significantly improved glucose tolerance and insulin sensitivity correlating increased levels of total BAs, reduced hepatic expression of key gluconeogenic and lipogenesis proteins, and upregulated expression of FXR and PPAR-α and fatty acid β-oxidation (Yan *et al*., 2019). Metformin-induced inhibition of BAs reuptake is thought to be the cause of its ability to increase circulating GLP-1 levels. The metabolic beneficial effects of energy restriction weight loss and bariatric gastric by-pass surgery are in-part due to changes in BAs homeostasis towards their increase at the distal gut, increases in incretin section and changes in the profile of microbiotal metabolites (Rohde, 2016; Flynn *et al*., 2019). Epigenomic analysis for T2DM susceptibility loci in monozygotic twins showed a strong causative involvement of MALT1 gene hypermethylation that had consequent changes in insulin, glycaemic pathways, and taurocholate levels in blood (Yuan *et al*., 2014). Susceptibility to T2DM is associated with lower baseline proliferation-related BAs (UDCA and cheno-deoxycholic acids; CDCA) and higher apoptosis-related BAs (DCA and Glyco-DCA) after developing T2DM (Zeng *et al*., 2019). Higher circulating concentration of DCA increases T2DM risk, whereas, genetic variant that reduces its concentration, lowers T2DM risk (Fall *et al*., 2016). Rectal insulin suppositories combined with different types of BAs (namely, sodium cholate ± deoxycholate, sodium taurocholate or sodium taurodeoxycholate) was able to induce maximum hypoglycemic effect in dogs (Hosny *et al*., 2001). In support, dose-dependently, rectal taurocholate (the most potent GLP-1 secretagogue among BAs) increases secretion of GLP-1 and PYY from L-cells and insulin secretion from β-cells, and, decreases blood glucose and food intake in obese T2DM volunteers; abrogatable by bile acid-sequestering resins (Adrian *et al*., 2012; Wu *et al*., 2013; Brighton *et al*., 2015; Brønden *et al*., 2018). Moreover, the antidiabetic drugs affect BAs synthesis and turnover through hepatic insulin sensitization or other secondary effects (Camilleri and Gores, 2015).

We detected significantly higher IGF-1 levels in patients. We were not planning to measure IGFBPs and confirmed the previous reports of increased total IGF-1 levels in T2DM patients (Ezzat *et al*., 2008). However, the literatures examining IGF-1 levels in diabetic patients are ambiguous with various results showing decreased (Teppala and Shankar, 2010; Suda *et al*., 2016; Aleidi *et al*., 2019), normal

(Rajpathak *et al*., 2008), or elevated levels (Schneider *et al*., 2011; Friedrich *et al*., 2012). These conflicting conclusions can be partly due to the age of the studied groups (van den Beld *et al*., 2019), genetic characteristics of the patients (Frayling *et al*., 2002), metabolic control differences (Bang *et al*., 1994), dietary habits; given the significance of protein and energy intake in IGF-1 regulation (Isley *et al*., 1983), magnitude of the inflammatory status (Frost *et al*., 2000), measurements of total vs. free IGF-1 (Frystyk, 2004), presence of complications (Merimee *et al*., 1983) and different assay methods used. The control of circulating IGF-1 levels by IGFBPs, insulin and GH further complicate the interpretation (Frost *et al*., 2000). Diabetic patients with mean age similar to ours were reported to have relatively high IGF-1 levels (van den Beld *et al*., 2019). We could neither relate IGF-1 level to age since our patients were overwhelmingly <60 nor to complications since our patients were vastly complicated. The increased platelet activation in poorly controlled T2DM was reasoned to increased expression of IGF-1 receptor (Gligorijevic *et al*., 2019). T2DM patients exhibit lower serum total IGF-1 levels (Rui-Hua *et al*., 2019). As a ratio to IGFBP-3, T2DM had lower IGF-1 levels (Huang *et al*., 2015). Reduction in serum level of IGF-1 in T2DM patients correlates liver steatosis and fibrosis (Miyauchi *et al*., 2019). Similä *et al*. did not detect changes in IGF-1 and IGFBP-3 in T2DM Finnish male smokers (Similä *et al*., 2019). EPIC-Potsdam Study showed that higher IGFBP-3 levels might raise T2DM risk independent of the indiscriminative IGF-1 levels (Drogan *et al*., 2016). In T2DM women aged 50-75 years, IGF-1 and IGFBP-3 levels correlated positively with the cardiovascular risk-promoting LDL-C and Lp(a) (Leinonen *et al*., 2002). Serum free IGF-1 in T2DM was lower in the patients with complications than healthy controls (Garay-Sevilla *et al*., 2000). The control of circulating IGF-1 levels by IGFBPs, insulin and GH further complicate the interpretation (Frystyk, 2004). Diabetic patients with mean age similar to ours were reported to have relatively high IGF-1 levels (van den Beld *et al*., 2019). 82.1% of our diabetic patients had IR; previously reported to induce hepatic IGF-1 synthesis (Böni-Schnetzler *et al*., 1991). Transition from pre-diabetes to diabetes correlates with a decrease in hepatic and peripheral insulin sensitivity and an increase in IGF-1 levels (Ezzat *et al*., 2008). Friedrich *et al*. detected a U-shaped association between IGF-1 levels and elevated HOMA-IR values (Friedrich *et al*., 2012).

Secondly, we analyzed the link between BAs, IGF-1 and biochemical T2DM prognostic parameters. After stratification of diabetic patients by age, BMI, disease duration, and, treatment and disease severity scores, there were significant increase in CRP that reflects an inflammatory status (Hsueh *et al*., 2004; Ge *et al*., 2016), AIP, IR, HbA1c and IGF-1 in the age group of >60 that point to the progressive increase in glucose intolerance, and aggravation of biochemical parameters of DM with age (Szoke *et al*., 2008; Yakaryılmaz and Öztürk, 2017). The similar findings identified for higher BMI categories, with a further exception of IGF-1, supports the role of obesity - as a key threat to metabolic regulation, in IR and DM (Sonmez *et al*., 2019). Notably, HbA1c revealed a significant increase with disease duration >10 years and presence of more than one complication. Time-dependent impairment of glycemic control and long-term glycemic variability monitored as HbA1c have a strong association with the development of complications (Chehregosha *et al*., 2019). Correlation between the independent variables (age and BMI) and biochemical parameters investigated in this study supported our conclusions; as age had a significant positive correlation with AIP and HbA1c. Similar correlations were observed for BMI with a further addition of a significant correlation with IR. Obesity potentiates IR and promotes the atherogenic dyslipidemia by inducing inflammatory cytokines, CRP and leptin release (Funahashi and Matsuzawa, 2007). CRP showed a positive correlation with age, BMI and all of the biochemical parameters that agree with previous reports (Festa *et al*., 2000; Yudkin *et al*., 2004). CRP negatively correlated with BAs levels among diabetic patients which may be due to suppression of hepatic FGF-19-cholesterol 7α-hydroxylase pathway that decreases BAs synthesis - on top of other FGF-19-BAs signalling pathways (Kong *et al*., 2012; Zhang *et al*., 2017; Sun *et al*., 2020). CRP was negatively correlated with FGF-19 levels that causes decreased fatty acid oxidation and atherogenesis (Barutcuoglu *et al*., 2011). Diabetes positively correlates with decreasing IGF-1 levels in US patients aging <65 years (Teppala and Shankar, 2010). Studying 615 subjects aging 45 - 65 years, low serum IGF-1 levels had a positive association with diabetes or glucose intolerance (Sandhu *et al*., 2002). However, another study with 922 T2DM patients found no association between IGF-1 and diabetes (Rajpathak *et al*., 2008).

Our total BAs level demonstrated insignificant relationship vs. patients' demographics and anthropometrics. Variance among the reported levels and relationships with BAs was reasoned to BAs profile measured and whether basal vs. induced levels, study population, anti-diabetic medications, glycemic control, and, severity and duration of the disease (Steiner *et al*., 2011). Mechanistically, low insulin and hyperglycemia induce degradation of forkhead box transcription factor O1 (FoxO1). The latter inactivates CYP8β1 and results in lower synthesis of 12α-hydroxylated BAs (Haeusler *et al*., 2010; Haeusler *et al*., 2016). Swine baseline total hyocholic acid, reflecting a metabolic state of altered CYP3α4 activity, adversely correlates with BMI and IR and predisposes to T2DM (Chávez-Talavera *et al*., 2017; Chávez-Talavera *et al*., 2020). Reportedly, plasma C4, a marker of the classical pathway for synthesis of BAs, correlates positively with both BMI and HOMA-IR, while it correlates negatively with adiponectin (Steiner *et al*., 2011; Haeusler *et al*., 2016). Metformin increases the level of liver BAs that improves metabolic dysfunction including hyperglycemia. It does so through modifying gut microbiome to increase the hydrophilic glycol-urso-deoxycholic acid that inhibits intestinal FXR and increases incretin secretion (Sun *et al*., 2018).

We observed insignificant relationship between IGF-1 and BMI, DD, treatment protocol or presence of complications. It was higher in the age group of <60 years which agree with van den Beld *et al*. (2019). T2DM leads to significantly increased circulating levels of IGF-1 and IGFBP-3, with no correlation with BMI, glycemic control and kidney function (NeamŢu *et al*., 2017). Another study proved the absence of significant

associations between IGF-1 level and any of the gender, age, BMI, or DD (Aleidi *et al*., 2019). Oppositely, a premature and progressive age-related decrease in serum IGF-1 levels was reported in T2DM (Janssen and Lamberts, 2002). Similarly, IGF-1 levels negatively correlated with age, DD, IR and waist circumference (Song *et al*., 2019). In uncontrolled T2DM Japanese patients, serum IGF-1 levels significantly decreased that might be due to impaired insulin secretion may be the underlying mechanism (Suda *et al*., 2016). IGF-1 levels were found to be independently negatively associated with BMI, waist to hip ratio, blood pressure, and also independently correlated with glucose intolerance in both diabetic/non-diabetic persons (Sesti *et al*., 2005). Six months of metformin treatment did not alter the levels of IGF-1 in T2DM patients (Krysiak *et al*., 2016). The influence of multiple factors, including insulin secretion, obesity, IR and the nutritional intake, caused the controversy considering IGF-1 correlation in T2DM (Frystyk *et al*., 1999).

As a third approach, we tried to investigate the power of chosen biochemical parameters in predicting diabetic complications. ROC curve analysis revealed that plasma levels of CRP, AIP, IR and HbA1c were better predictors of disease complications. In support, treatment normalizing glucose levels prevents or delays the long-term complications of DM (Janssen and Lamberts, 2002). For us, BAs was the single best predictor of absence of complications in the studied population. BAs regulate lipid and glucose metabolism (Steiner *et al*., 2011) through TGR5-GLP-1 (Kuhre *et al*., 2018), FXR-FGF19 axes, insulin sensitivity (Bauer and Duca, 2016) and adipocyte browning by thyroxine activation through TGR5-cAMP-deiodemase-2-peroxisome proliferator-activated receptor-γ coactivator-1α-uncoupling protein 1 (UCP1) pathway (Jin *et al*., 2019). However, insulin was shown to increase level of BAs due to IR in diabetic patients (Sun *et al*., 2016). Moreover, circulating BAs had a positive correlation with IR in persons with or without T2DM (Cariou *et al*., 2011). Differentially, increased serum 12α-hydroxy/non-12α-hydroxy BAs ratio can lower HDL-C, and suppress FGF19-FXR pathway (Jiao *et al*., 2018; Haeusler *et al*., 2016). Our IGF-1 findings showed that it had no power for prediction of absence of complications. In support, no significant association was observed between IGF-1 levels and any of the age, gender, BMI, glycemic control levels or DD (Aleidi *et al*., 2019). On the contrary, the survival effect of IGF-1 on heart is affected by the decrease in IGF-1 bioactivity in T2DM patients (Janssen and Lamberts, 2002), and, the altered IGF-1 levels are associated with cardiovascular risk in well-controlled T2DM patients (Hjortebjerg *et al*., 2014). Discordance of our results vs. previous reports, and among these reports could be reasoned to patients/antidiabetic therapy characteristics, measurement of total vs. individual and fasting basal vs. kinetic levels BAs, IGF-1 total vs. free, and study population ethnic and genetic background.

Surprisingly, the predictive power of BAs, to exclude complications, was no longer significant upon binary logistic regression analysis for effect of independent variables (age, BMI, DD, treatment score, CRP, AIP, IR, HbA1c, IGF-1, BAs, and gender). Only age and treatment score significantly predicted the presence of complication among diabetics. Taking into consideration the study limitations that may confound the results, these data suggest that BAs levels are affected by many aspects in T2DM in addition to IR. Although they are related to glucose homeostasis, plasma levels of BAs do not predict the onset of diabetes in patients with impaired glucose intolerance (Chávez-Talavera *et al*., 2020). More specifically, changes in total BAs level are more closely related to changes in fatty acid utilization and ketogenesis, whereas, changes in BAs subtypes may be an important feature of IR, and their effects may be dissociable from the effects of alterations in total BAs levels (Haeusler *et al*., 2013). Rats with T2DM exhibited characteristic increased ratio of serum 12α-OH to non-12α-OH BAs due to up-regulation of hepatic Cyp8b1 expression with a negative effect on glucose homeostasis via inhibiting TRG5/FXR-mediated pathways in colon, liver and pancreas (Zhang *et al*., 2019). Consequently, individual BAs-regulated pathways are affected by specific endogenous BAs ligands; while some are inducible by a wide range of types of BAs, others are very BAs type-specific, and, still different types of BAs may work antagonistically (Ahmad and Haeusler, 2019).

Our findings of the power of age and treatment protocol in predicting the presence of complications among diabetics agree with the previous studies (Tesfaye *et al*., 2005; Wang *et al*., 2016; Jaiswal *et al*., 2017; Su *et al*., 2018). Literatures proved associations between many risk factors and diabetic complications including DD (Su *et al*., 2018), IR (Kim and Feldman, 2015), visceral adiposity and dyslipidaemia (Callaghan *et al*., 2012), HbA1c (Selvin *et al*., 2004), CRP (Ge *et al*., 2016; Funahashi and Matsuzawa, 2007), and IGF-1 (Janssen and Lamberts, 2002; Hjortebjerg *et al*., 2014). It is conceivable to explain our result by the progressively increased glucose intolerance and IR with age (Yakaryılmaz and Öztürk, 2017) with long-term glycemic variability that increase oxidative stress (Chang *et al*., 2012), mediate tissue damage through the polyol pathway, advanced glycation end-products overproduction, activated protein kinase C isoforms and hexosamine pathway (Brownlee, 2005), increased systemic inflammation (Chang *et al*., 2012), and DNA damage with p53 activation (Schisano *et al*., 2011).

The limitations of the present study are 1) the cross-sectional observational study design did not allow us to conclude causation associations, 2) DD, treatment protocol, and genetic/lifestyle background were unavoidable confounding factors, 3) fasting, and not the induced post-prandial, and total and not individual types of BAs were measured. This could have masked some important differences, 4) total and not the more sensitive free IGF-1 was measured, and, 5) the over whelming majority of our patients had complications with high HbA1c that precluded the relevant patient subgrouping for statistical analysis. Further prospective longitudinal studies are needed to clarify the exact/precise relationship between BAs levels and composition, IGF-1 and its binding proteins and T2DM complications.

In conclusion, the present study highlighted the increased total IGF-1 levels in T2DM patients. So it is possible to use IGF-1 as a biomarker for monitoring T2DM patients. In addition, BAs can predict the absence of complications in

T2DM but these conclusions should be interpreted in the shadow of the study limitations.

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