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Unique Glycemic and Cardio-Renal Protective Effects of Metformin Therapy among Type-2 Diabetic Patients with Association of High Non-Hdl-Cholesterol and Low Hba1c, Serum Creatinine and Spot Urine Protein: A Lesson from a Five Year Cross Sectional Observational Study of 1590 Patients

Special Issue on
Antidiabetic Drug-Metformin

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Abstract

Metformin is currently considered a first line therapy for managing type-2 diabetes for targeting insulin resistance and obesity. However, in usual busy clinical practice, the importance of metformin is sometimes ignored and patients are prescribed directly OHA or shifted to insulin therapy without metformin. Metformin has been shown to counteract dyslipidemia and as a drug for cardiac and renal protection as well. Current research was planned to enroll type-2 patients who were additionally prescribed metformin (with other OHA/insulins) compared to those who were not on metformin; and to compare HbA1c lipids, creatinine, spot urine protein and spot urine protein/creatinine between these two groups of patients. Study included 1590 patients and data were collected for the period of 5 years (January 2009 till January 2014) in the diabetology clinic ofAseer Diabetes Center at initial visits by standardized methodology. Type-1 DM, children (<13 years), pregnant, chronic renal failure, ESRD, and patients intolerant to metformin were excluded from the study. It was observed that 581 (36.6%) type-2 DM subjects were not prescribed metformin. Levels of HbA1c and BMI were lower in the metformin group compared to those without metformin (9.47 ± 2and 27.35versus 9.74± 2.4 and 30.25 ± 5.72; p-values 0.004 and < 0.0001 respectively). Non-HDL-C values were higher for the metformin group (152.6 ± 48 versus 140.4 ± 44; p<0.0001). Levels of creatinine, spot urine protein and spot urine protein/creatinine was higher for the group without metformin therapy (1.1 ± 0.31, 95.23 ± 138, 1.143 ± 3.39 versus 0.89 ± 0.2, 38.92 ± 82.17, 0.425 ± 1.25 with p-values <0.0001, 0.017 and 0.015 respectively). In the current study, metformin provided unique Glycemic and lipid control and cardio-renal protection among type-2 DM patients and should be prescribed to type-2 DM patients while managing diabetes to prevent complications of diabetes.

ABBREVIATIONS

BMI: Body Mass Index; CV: Cardiovascular; CVD: Cardiovascular Disease; DCCT: Diabetes Control and Complication Trial; DM: Diabetes Mellitus; DN: Diabetic Nephropathy; ESRD: End Stage Renal Disease; GLUT4: Glucose Transporter-4; HDL-C: High Density Lipoprotein Cholesterol; HTN: Hypertension; IHD: Ischemic Heart Disease; IGF: Insulin-like Growth Factors; IR: Insulin Resistance; LDL: Low-Density Lipoprotein; NCEP: National Cholesterol Education Program; OHA: Oral Hypoglycemic Agents; PRESTO: Prevention of Restenosis with Tranilast and its Outcomes; RECORD: Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia in Diabetes; ROS: Reactive Oxygen Species; SU: Sulphonylurea; T1DM: Type-1

Diabetes; T2DM: Type-2 Diabetes; TZD: Thiazolidinedione; UCFP: Urinary/Cerebrospinal Fluid Protein; UKPDS: United Kingdom Prospective Diabetes Study; UTI: Urinary Tract Infection Metformin with or without additional therapy

INTRODUCTION

Metformin, the only drug available for clinical use in the insulin sensitizing biguanide class, is now considered as a first line anti-diabetic and anti-hyperglycemic therapy for the management of type-2 diabetes. Metformin ameliorates the insulin resistance (IR) primarily in liver, muscle, and adipose tissue [1-4] by reducing the hepatic glucose output, largely due to a reduction in the rate of gluconeogenesis and glycogenolysis. The beneficial cardiovascular effects of metformin have been observed in the UK Prospective Diabetes Study (UKPDS) [5,6]. According to the results of this study, patients who received metformin benefited from clinically and statistically significant improvements in the risk of all-cause death, diabetes related death, myocardial infarction, and reduction of diabetes related complications. Also in UKPDS it has been shown that metformin provides protection from macrovascular diabetic complications independently of glycaemic lowering effects.

Regarding weight loss in obese diabetic subjects and proteinuria, concomitant and simultaneous treatment of diabetic dyslipidemia and treatment with metformin have all been shown to reduce urinary albumin excretion and ultimately reduction in microvascular and macrovascular complications independent of tight glycaemic control [7-9]. Additionally, marked decreases in proteinuria have also been observed in obese diabetics who lose weight. This reduction in body weight is associated with redistribution of fat from visceral depots to subcutaneous depots, which carry lesser cardiovascular (CV) risk [10].

Ischemic heart disease (IHD) remains the leading cause of death in the patients with type-2 diabetes mellitus (T2DM) and, considering this cardiac complication, it has been shown that metformin can exert several pleiotropic effects including beneficial changes in blood rheology, serum lipid profile, and putative anti-ischemic effects [11,12]. Although exact mechanism of metformin induced reduction of cardiovascular risk is still unclear, it has been demonstrated in rats that metformin has vaso-protective effects via increased activity of NO-synthase with its antihypertensive effects on the hypertensive rats [13-15]. Furthermore, regarding metformin induced cardio-protection, it has been shown that addition of the drug to the perfusate resulted in the attenuation of left ventricular post ischemic dysfunction (stunning) and infarct-limiting in the non-diabetic isolated rat heart model [16,17]. Additionally, in one study it was proven that regular oral administration of metformin increased myocardial tolerance to ischemia in streptozotocin induced type-1 diabetes mellitus (T1DM) rats [18]. Recently it has been also recommended to target non-HDL cholesterol for reducing cardiovascular morbidity and mortality. Before the recognition of low HDL-C as a risk factor, in 1963 Albrink already demonstrated that triglyceride was equally important with cholesterol in determining atherosclerotic risk in diabetes [19]. HDL-C is a strong inverse covariate of triglyceride [20]. Currently National Cholesterol Education Program (NCEP) has suggested a target for LDL-C to be < 100 mg/dl in patients with diabetes, followed by non-HDL cholesterol of < 130 mg/dl as a secondary target if triglyceride level remains elevated (> 200 mg/dl) [21]. American Diabetes Association (ADA) has issued similar guidelines [22]. These evidences indicate the importance of non-HDL-C as a potential marker of dyslipidemia.

Considering renal protection, diabetic nephropathy (DN) and metformin, it has been recently suggested that metformin can provide a protection against the deleterious consequences of hyperglycemia in kidney. These evidences come from the rodent models of diabetes as the Zucker diabetic fatty rats. In this model, Takiyama demonstrated that metformin treatment (250 mg/kg/d) during 9 to 39 weeks ameliorates tubular injury associated with hyperglycemia while no protective effect was observed with insulin [23]. Additionally, it has also been shown that metformin prevents gentamicin-induced acute renal failure, presumably by decreasing reactive oxygen species (ROS)-mediated lipid peroxidation [24]. Furthermore, it is now well established that hyperglycemia increases ROS production in diabetic podocytes, contributing to the development of diabetic nephropathy. Currently all attempts to reduce the ROS production in diabetic kidney has failed. However, Piwkowska demonstrated that metformin activates AMPK and decreases the NAD (P) H oxidase activity, ultimately leading to reduction of ROS production in cultured podocytes [25]. Under these experiments and observations, control of ROS production by metformin could be a new treatment strategy for controlling progression and management of diabetic nephropathy.

Development of proteinuria is an indicator of nephropathy. However, microalbuminuria alone may not provide optimal identification of risk of renal impairment, and thus identification of other risk factors is required [26-30]. Under these circumstances, spot urine protein and spot urine protein/creatine ratio is also considered an important marker of nephropathy and for monitoring its progression, especially when proteinuria is beyond microalbumin range (albumin excretion in urine in the range 30-299 mg/24-hour) [31-40]. As compared to 24-hour urine collection, which is cumbersome, time consuming and potentially misleading if done inaccurately, spot urine is valuable when proteinuria is gross, macroalbumin range (>300 mg albumin/ 24-hour) or in nephrotic range (≥3.5 g/day protein excretion). Hence spot urine protein can provide inexpensive and quick estimate of proteinuria in diabetic patients.

Under this research literature background and review, the purpose of the current study was to observe the clinical characteristics of the type-2 diabetic patients who were already on metformin as an additional therapy (with other diabetic medications; OHA/Insulins) and those without metformin therapy. This was achieved by observing different parameters and variables such as HbA1c (glycemic control status), total cholesterol and non-HDL-cholesterol (dyslipidemia measurement and cardiac protection status), serum creatinine and spot urine protein and spot urine protein/creatinine measurements (renal protection status). These have not been studied previously in research literature among diabetic subjects who were on metformin or without metformin therapy.

MATERIALS AND METHODS

Current research is a prospective observational cross

sectional study which was conducted in dialectology clinic of Aseer Diabetes Center of Aseer Central Hospital. Study included all type-2 diabetic patients who were referred from primary health care clinics to dialectologist for initial or annual evaluations, management and follow up. Total duration of study was 5 years, from January 2009 till January 2014. All type-1 DM patients, Children (less than 13 years), pregnant women, known cases of chronic renal disease (serum creatinine ≥1.5) and patients on end stage renal disease (ESRD) or dialysis were excluded from the study. Furthermore, patients demonstrating urinary tract infection (UTI), Proteinuria, or known cases of chronic kidney disease prior to the onset of diabetes were excluded from the study. Patients who were intolerant to metformin were also excluded from the study. Detailed history was taken for duration of diabetes, complications, co-morbidities (obesity, hypertension, IHD, CVA, diabetic nephropathy) and medications. Detailed clinical examination was done as well. Body mass index (BMI) was calculated as weight (kg)/height (m^2). BMI ≥30 kg/m^2 was considered obesity. All data were collected on the initial visits to the diabetologist. Patients on metformin as an additional therapy were analyzed separately as a group.

LABORATORY METHODS, COLLECTION AND DATA RETRIEVAL

All samples were collected in fasting state of not less than 12 hours and send to Aseer Central Hospital main laboratory. HbA1c was measured by A1c Flex® Reagent by the Dimension® clinical chemistry system, an *in vitro* diagnostic assay for the quantitative determination of both percent hemoglobin A1c and total hemoglobin, based on a turbidimetric inhibition immunoassay (TINIA) principle, and the measurement of total hemoglobin is based on a modification of the alkaline hematin reaction, an NGSP certified methodology (Siemens healthcare diagnostics Inc. Newark, DE 19714, USA). The percentage of total hemoglobin that is glycated was calculated and reported as %HbA1c (in g/dL), and final result has been standardized to the results obtained in DCCT.

Both serum creatinine and urine creatinine (mg/dl) was measured quantitatively by CREA method by Dimension® clinical chemistry system (Siemens healthcare diagnostics Inc. Newark, DE 19714, U.S.A). The technique for the measurement of creatinine in human plasma or urine involves such that in the presence of a strong base NaOH, picrate reacts with creatinine to form a red chromophore. The rate of increasing absorbance at 510nm due to the formation of this chromophore is directly proportional to the creatinine concentration in the sample and is measured using by a bichromatic (510,600 nm) rate technique. By this way, creatinine in the sample (plasma/urine) is determined quantitatively [41-43].

HDL-C (mg/dl) was measured directly in plasma by Automated High Density Lipoprotein (AHDL) method by the Dimension® clinical chemistry system and analyzer (Siemens healthcare diagnostics Inc. Newark, DE 19714, U.S.A), an in vitro diagnostic test intended for quantitative determination of high density lipoprotein cholesterol (HDL-C). Similarly, total cholesterol was measured directly by CHOL method (based on enzymatic procedures), a quantitative determination by the Dimension® clinical chemistry system and analyzer. Then non-HDL-C was calculated as total cholesterol – HDL-C.

Spot urine protein (mg/dl) was measured by UCFP (Urinary/Cerebrospinal Fluid Protein) method on Dimension® clinical chemistry system (Siemens healthcare diagnostics Inc. Newark, DE 19714, U.S.A). This is an in vitro diagnostic test intended for the direct quantitative determination of total protein in human urine and cerebrospinal fluid, which is an adaptation of pyrogallol red molybdenum method by Y.Fujita, I.Mori and S.Kitano [44]. In the reaction sequence, pyrogallol red combines with sodium molybdate to form a red complex with maximum absorbance at 470 nm. The protein in the sample reacts with this complex in acid solution to form a bluish-purple colored complex, which absorbs at 600 nm. The absorbance at 600 nm is directly proportional to the concentration of protein in the sample. The analyte concentration is determined by calculation using a logit curve fit on a previously stored calibration curve.

All sample requests and results were entered and retrieved by Natcom Hospital Information System (NATCOM HIS; National Computer System Co. Ltd) [45], a server based HIS software.

STATISTICAL METHODS

All the data was analyzed using SPSS® version 14 for Windows (SPSS® Inc. USA). All statistical tests were performed by standardized methodology in accordance with the reference to classical statistical methods and literature review while fulfilling all assumptions with their criteria [46-57]. For the variables, it was verified that they are normally distributed (requiring no data transformation) while observing the skewness and kurtosis values between -1 and +1 with no influential outliers. For testing normality normal Q-Q plots were used as well to assure that variables are approximately normally distributed. All data are presented as "mean± standard deviation". To compare different groups with or without metformin, t-test was utilized to rule out significant difference between the groups.

This study was designed to have a statistical power of 90% to detect significant changes. All p-values were two sided, and p-values less than 0.05 were considered statistically significant.

RESULTS

The results for demographic data with comorbidities and complications are presented in Table 1. It was observed that out of 1590 patients, 946 (59.5%) were males and 644 (40.5%) females; Regarding comorbidities and complications, 713 (44.8%) subjects were obese; 284 (17.9%) IHD; 709 (44.6%) HTN; 75 (4.7 %) CVA; and 527 (39.8 %) patients demonstrated diabetic nephropathy. Overall, 581 (36.6%) type-2 DM subject were not using metformin with other diabetic medications (OHA, Insulins). Table 2 demonstrates variables with Mean±SD.

Grouped variables (with or without metformin therapy) with Mean ± SD (95%CI) are presented in Table 3. According to the results produced by SPSS, it was observed that HbA1c for the patients without metformin therapy was on higher side (9.74± 2.4; 95% CI 9.6 to 9.9) as compared to those who were already on metformin therapy with other diabetic medications (9.47 ± 2; 95% CI 9.1 to 9.7). Similarly, BMI was on higher side for those

without metformin therapy (30.25 ± 5.72; 95% CI 29.9 to 30.58) as compared to those with additional metformin therapy (27.35 ± 5.9; 95% CI 26.6 to 28). Regarding lipids, non-HDL-C was found to be on lower side without metformin therapy (140.4 ± 44; 95% CI 133.7 to 147.2) as compared to those with metformin therapy (152.6 ± 48; 95% CI 149.7 to 155.6).

Regarding renal functions and proteinuria, it was observed that serum creatinine was on higher levels for the group without metformin (1.1 ± 0.31; 95% CI 0.93 to 1.29) as compared without metformin therapy (0.89 ± 0.2; 95% CI 0.87 to 0.895). Regarding proteinuria, it was observed that spot urine protein and spot urine protein/creatinine ration were on the higher levels for the group without metformin (95.23 ± 138; 95% CI 49.59 to 140.9 and 1.143 ± 3.39; 95% CI 0.575 to 1.71 respectively) as compared to those on metformin therapy (38.92 ± 82.17; 95% CI 32.54 to 45.3 and 0.425 ± 1.25; 95% CI 0.327 to 0.523 respectively).

Data for significant difference between two groups is presented in Table 4 with t-tests and p-values. It was found that HbA1c and BMI were significantly different between the group on metformin therapy and without it (p-value 0.004 and < 0.0001 respectively). There was significant difference between the two groups for Non-HDL-C (p-value <0.0001). Similar was the case with serum creatinine, spot urine protein and spot urine protein/creatinine ratio (p-values <0.0001, 0.017 and 0.015 respectively).

DISCUSSION

In this distinct study, we have focused the importance of metformin therapy in type-2 diabetic patients whether obese or non-obese. In the usual general busy practice, it was observed that role of metformin is often ignored and newly diabetic patients are prescribed OHA only, without metformin. Similarly, when uncontrolled diabetic patients are shifted to insulin therapy, often metformin in not prescribed.To some extent, only obese diabetic patients were considered to initiate metformin therapy at primary care level, leaving behind patients without obesity with other complications (HTN, IHD, CVA and DN). These facts were ruled out by detailed history given by patients. Commonly, patients' compliance also play role in the ignorance. When glycemic control becomes poor and diabetes remains uncontrolled, then at this stage metformin is prescribed or when the patients are referred to tertiary cared diabetes center. This fact has been mentioned in medical literature and reviews previously [58]. Hence, current study was planned to measure different parameters and variable among the group using

Table 1: Demographic Data of Diabetic Patients with comorbidities.

Parameters	Description with N (%) ; Totals = 1590 type-2	
Gender	Male	Female
	946 (59.5%)	644 (40.5%)
Obesity	Yes	No
	713 (44.8%)	877 (55.2%)
IHD	Yes	No
	284 (17.9%)	1306 (82.1%)
HTN	Yes	No
	709(44.6%)	881 (54.4%)
CVA	Yes	No
	75 (4.7 %)	1515 (95.3%)
Diabetic Nephropahty (DN)	Yes	No
	527 (39.8 %)	1063 (60.2)
Overall patients on metformin therapy	Yes	No
	1009 (63.4%)	**581 (36.6%)**

Abbreviations: CVA: Cerebrovascular Accidents; DN: Diabetic Nephropahty; IHD: Ischemic Heart Disease; HTN: Hypertension; OHA: Oral Hypoglycemic Agents.

Table 2: Variables with Mean ± SD (95% CI).

Variables	Mean±SD
Age	57.5 ± 14.3
Duration of diabetes	15.6 ± 9.8
BMI	29.6 ± 5.9
HbA1c	9.7 ± 2.16
Serum creatinine	0.986 ± 0.711
Total cholesterol	191.8 ± 49.5
HDL-C	41.7 ± 17.2
Non-HDL-C	150.3 ± 49.2
Spot urine protein mg/dl	98.8 ± 54.7
Spot urine creatinine	119.5 ± 72.3
Spot urine protin/creatinine ratio	0.56 ± 1.85

Abbreviations: BMI: Body Mass Index; HDL-C: High Density Lipoprotein Cholesterol

Table 3: Grouped variables; comparison between HbA1c, BMI, Non-HDL-C, serum creatinine, spot urine protein and spot urine protein/cratinine ratio in patients with and without metformin therapy.

Variables with Mean ± SD (95%CI)	
HbA1c of type-2 diabetics without metformin therapy	HbA1c of type-2 diabetics with metformin therapy
9.74± 2.4 (9.6 to 9.9)	9.47 ± 2 (9.1 to 9.7)
BMI of type-2 diabetics without metformin therapy	BMI of type-2 diabetics with metformin therapy
30.25 ± 5.72 (29.9 to 30.58)	27.35 ± 5.9 (26.6 to 28)
Non-HDL-C of type-2 diabetics without metformin therapy	Non-HDL-C of type-2 diabetics with metformin therapy
140.4 ± 44 (133.7 to 147.2)	152.6 ± 48 (149.7 to 155.6)
Serum creatinine of type-2 diabetics without metformin therapy	Serum creatinine of type-2 diabetics on metformin therapy
1.1 ± 0.31 (0.93 to 1.29)	0.89 ± 0.2 (0.87 to 0.895)
Spot urine protein of type-2 diabetics without metformin therapy	Spot urine protein of type-2 diabetics on metformin therapy
95.23 ± 138 (49.59 to 140.9)	38.92 ± 82.17 (32.54 to 45.3)
Spot urine protein/creatinine ratio of type-2 diabetics without metformin therapy	Spot urine protein/ creatininerationof type-2 diabetics on metformin therapy
1.143 ± 3.39 (0.575 to 1.71)	0.425 ± 1.25 (0.327 to 0.523)

Abbreviations: BMI: Body Mass Index; HDL-C: High Density Lipoprotein Cholesterol

Table 4: T-test for group of variables (HbA1c, BMI, Non-HDL-C, Creatinine, Spot urine protein and spot urine protein/creatinine ratio).

T-tests for group of variables	F-statistic	T-statistic	P-value
HbA1c with and without metformin therapy	8.25	1.77	0.004
BMI with and without metformin therapy	.804	7.36	< 0.0001
Non-HDL-C with and without metformin therapy	3.68	3.5	<0.0001
Serum creatinine with and without metformin therapy	243	- 12.6	<0.0001
Spot urine protein with and without metformin therapy	49.9	- 4.4	0.017
Spot urine protein/creatinine with and without metformin therapy	44.8	- 4.2	0.015

Abbreviations: BMI: Body Mass Index; HDL-C: High Density Lipoprotein Cholesterol.

metformin as an additional therapy and the group without it. We have extensively reviewed the research literature and confirmed the significant results by the current study. However, in the past no study is conducted to compare metformin therapy groups and to measure HbA1c, BMI, serum lipids and creatinine and spot urine urine proteins in such a manner. Therefore, current study is unique to rule out such facts for the first time in medical and diabetes literature.

Metformin is the only oral anti-diabetic drug which has been demonstrated to reduce diabetes-related and total mortality in obese type2 diabetes [57-61]. Metformin can also delay or reduce in the incidence of type 2 diabetes, a fact which is well known in medical research literature [62]. Metformin has been shown to improve β-cell function in type 2 diabetic patients as improved insulin sensitivity can enhance β-cell function by reducing glucotoxicity [63]. This effect of metformin is additional apart from other beneficial effects such as reduced fatty acid oxidation in adipose tissue and increasing the glucose transporter (GLUT4) translocation in muscle and fat, and reduce gluconeogenesis in liver [64,65]. Hence metformin is better termed "anti-diabetic" or "anti-hyperglycemic" rather than 'hypoglycaemic' because theoretically it does not induce hypoglycemia when prescribed as monotherapy. The glucose-lowering effect of metformin is additive when used in combination with a sulphonylurea (SU), a meglitinide, a thiazolidinedione (TZD) or an α-glucosidase inhibitor [66-89].The Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia in Diabetes (RECORD) study demonstrated a greater reduction of HbA1C with metformin compared with rosiglitazone when added to maximal sulphonylurea therapy for 18 months [90]. Furthermore, Addition of metformin to insulin based therapies and regimens have shown improved glycemic control, improved insulin sensitivity, reduced insulin requirements and dosages, weight control with reduced incidence of hypoglycemia [91-98]. This phenomenon of improved glycemic control has also been observed in the current study. BMI and HbA1c levels were lower for those prescribed metformin with other anti-diabetic agents, OHA or insulins. Those without metformin have shown poor control and elevated HbA1c. Hence metformin provided unique glycemic control in combination with other anti-diabetic medications, OHA and insulins.

The Prevention of Restenosis with Tranilastand its Outcomes (PRESTO) trial have shown that metformin was associated with significant improvements in cardiovascular outcomes, compared with those observed group of patients not receiving this drug [99]. This beneficial effect of metformin is due to the fact that it improves the lipid profile, the fact which is already known in medical literature [100-102]. In one study, metformin has been shown equally effective in reducing elevated lipid values when compared with statins [103]. Results from a double-blind, randomised, placebo-controlled crossover study in 27 type 2 diabetic patients treated with metformin for 12 weeks have demonstrated reductions in levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides while elevations of HDL-C levels to some degree [104]. This observation is consistent with our study findings, further confirming the benefits of metformin as cardio-protective agent.In this study, although majority of the referred patients with complications (IHD, HTN. CVA and DN) were already on statins, however they were not on metformin therapy and overall demonstrated lower levels of non-HDL-C .Contrary to this, patients without these complications were not on regular statins but instead were using metformin as additional diabetic therapy and have demonstrated higher levels of Non-HDL-C. Furthermore, while considering cardio-protection, metformin counteracts insulin resistance and ultimately reduces and abolishes the atherogenic effects of insulin resistance [105-108]. Much research has been done on metformin and its effect on cardiac muscle regarding limitation of infarct size via various distinct signaling pathways and mechanisms at molecular levels [109-126]. These studies are sufficient to prove its cardio-protection in diabetic state. Considering dyslipidemia, HDL-C is considered as a good cholesterol and elevated level are protective for coronary artery disease [21]. Non HDL cholesterol represents and provides a single index of apolipoprotein B-containing lipoproteins. Hence LDL-C alone is not sufficient to estimate atherogenic risk in patients with elevated triglycerides. Furthermore LDL-C can be misleading if triglycerides > 400 mg/dl. In fact it has been shown that non-HDL cholesterol was a somewhat better predictor of CVD than LDL cholesterol [127]. Hence in our study, Non-HDL-C was selected as a primary target for dyslipidemia estimation and was found to be higher among patients treated additionally with metformin, indicating the fact that metformin might increase this good cholesterol and provided cardio-protection in diabetics.

While considering metformin and renal protection, recently it has been demonstrated that hyperglycemia with lipotoxicity (increased levels of LDL-C, increased levels of triglycerides and low levels of Non-HDL-C) has deleterious effects on the progression of diabetic nephropathy [128-133]. Hence, reducing lipotoxicity by metformin to prevent diabetic nephropathy could be a new strategy for focusing diabetic kidney disease and diabetic nephropathy. It has been demonstrated that state of insulin resistance and metabolic syndrome are risk factors for

the development of microalbuminuria, cardiovascular mortality and nephropathy [134-142]. As mentioned in the introduction, microalbuminuria alone may not provide exact estimation of renal damage or proteinuria excretion estimation, especially when gross proteinuria or higher levels of proteinuria is present. In such cases, spot urine protein or spot urine protein/creatinine ration would be an ideal estimate of proteinuria. In the current study, spot urine protein with spot urine protein/creatinine ratio was considered as an estimate of proteinuria or renal damage.

Recent advanced growing research has also demonstrated that insulin resistance is associated with hemodynamic alteration in the kidney. Hyperinsulinemia has been reported to be able to raise glomerular hydrostatic pressure, increase renal vascular permeability, aggravate glomerular hyper filtration, and enhance renal sodium reabsorption [143-145]. Hence, metformin can be targeted as a novel drug to ameliorate insulin resistance and hyperinsulinemia and ultimately reducing renal damage.

Furthermore, it has been shown that metformin can have effect on reduction of cystic growth in the dominant polycystic kidney disease mice model, explained by AMPK activation by metformin and subsequent inhibition of both CFTR and mTOR pathways, demonstrating that the drug can target and modulate multiple molecular pathways in the kidney [146]. Under this literature review and agreement, the role of metformin in chronic kidney disease, its revision of clinical practice recommendations, especially the contra-indications of metformin use in chronic renal failure, is required to obtain its maximal beneficial effects on the renal system [147,148]. Furthermore, lactic acidosis reported with use of biguanides mostly involved phenformine, which was early withdrawn from the market. Lactic acidosis was 20 times more frequent with phenformine as compared with metformin. In cases of lactic acidosis, plasma metformin concentration has also not proved to be of any prognostic significance [149,150]. Additionally, as the risk of lactic acidosis is extremely low, Frid and others have recommended revising the use of metformin in renal insufficiency and determination of metformin dose by its plasma levels and glomerular filtration rate [151]. Similarly, a cohort study from the Swedish National Diabetes Register has demonstrated effectiveness and safety of metformin in 51,675 patients with type 2 diabetes with different levels of renal function that were followed for 4-years. They have concluded: "patients with renal impairment showed no increased risk of CVD, all-cause mortality or acidosis/serious infection. In clinical practice, the benefits of metformin use clearly outbalance the risk of severe side effects" [152].

Our data was consistent with above mentioned research findings. Patients using other medications in combination with metformin demonstrated lower levels of creatinine, spot urine protein, and spot urine protein/creatinine ratio, indicating the fact that metformin might reduce protein excretion in urine, prevent nephropathy and provide unique renal protection.

Although patients with carcinomas were neither included nor objectives in the current study, however it would be also advisable and valuable to document the role of metformin in the prevention of cancer. Recent prospective and case-control studies conducted on large cohorts have confirmed that type-2 diabetes is associated with significantly increased risk of cancer mainly affecting breast, colon, prostate, kidney and pancreas, which has been attributed mainly due to the growth promoting effect of chronic and persistent elevated plasma insulin levels in the presence of insulin resistance. Chronic exposure to the hyperglycemia state and hyperinsulinemia due to insulin resistance promote carcinogenesis directly through the insulin receptor or indirectly by increasing the levels of insulin-like growth factors (IGF), steroid sex hormones, inflammatory processes and derangements in adipokines homeostasis [153-155]. The impact of metformin on cancer risk reduction and its related mortality has been recently studied with a case control cohort of 12,000 type-2 diabetic patients [156]. In this study, it was demonstrated that metformin therapy was associated with a reduced risk of cancer (odds-ratio of any exposure to metformin was 0.79); a dose-response relationship between duration of exposure to metformin and cancer incidence was observed. The other studies have documented same phenomenon of cancer risk reduction with metformin [157-162]. These observations are consistent with *in vitro* and *in vivo* studies showing anti-proliferative action of metformin on various/specific cancer cell lines in animal models and via improvement of blood glucose and insulin levels [163-192].

In summary and regarding metformin therapy recommendations, metformin has been shown to be as effective as insulin or sulfonylureas when used as monotherapy in type-2 subjects, and its efficacy is independent of age, body weight, duration of diabetes, ethnicity, and insulin and C-peptide levels [5-7,193,194]. Metformin also has been proven to be effective in combination with insulin, sulfonylureas, and thiazolidinediones. This diabetes management strategy is important because single-drug therapy often fails to maintain normoglycemia, particularly as diabetes progresses [195-202]. Hence, considering metformin therapy in type-2 DM, it is always advisable to follow best available evidence based guidelines as currently metformin has been recommended as an agent of choice in the treatment of type 2 diabetes regardless of obesity [58,198,203-205].

Limitations of the current study included non-randomization, and there was no control group. Further studies at multicenter, randomized and controlled level are required to further explore metformin therapy benefits.

CONCLUSION

In the current observational and cross sectional study, it was demonstrated that the novel drug metformin has provided a unique cardiac and renal protection. The patients using metformin has demonstrated lower levels of HbA1c, BMI, creatinine, spot urine protein and higher levels of Non-HDL cholesterol. Hence, under these observations, metformin is recommended in all type-2 diabetic patients (in the absence of contraindications) for better glycemic control, and to provide cardio-renal protection. Primary care physicians should consider metformin as fist line agent while managing diabetes, and should continue metformin while shifting patients from OHA to various types of insulins to prevent or delay diabetes complications. Further randomized controlled studies are also required at multicenter level to confirm findings of the current study.

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