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Effect of Metformin on C-reactive Protein in Type 2 Diabetes Mellitus Patients

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Diabetes mellitus (DM) is a complex endocrinology disease which requires a meticulous understanding of its pathogenesis and its complications to subdue it. It has been riddled with extensive micro and macro vascular complications which by itself has its own set of pathogenesis. There is a link between DM and cardiovascular disease (CVD), which is the most important cause of morbidity and mortality in diabetic patients. Cardiovascular risk factors such as obesity, hypertension and dyslipidemia are more common in patients with DM, placing them at increased risk for cardiac events. In addition, they have found biological mechanisms associated with DM that independently increases the risk of CVD in diabetic patients.Metformin is the most commonly used antidiabetic agent derived from Gallegaofficinalis. Metformin provided greater protection against macro vascular complications than would be expected from its effects upon glycemic control alone. Hence this study evaluated the anti-inflammatory effect of metformin on C Reactive Protein (CRP) in patients. In this study fifty type 2 diabetes patients were enrolled in the study including 23 males and 27 females with mean age 40±4.33. FBS and PPBS baseline values expressed as Mean ± SD were 138.06±17.12 mg/dl and 223.12±30.63 mg/dl respectively. After 6 months of metformin therapy, FBS and PPBS were 91.64±10.55 mg/dl and 133.88±7.99 mg/dl respectively. HBA1C baseline value expressed as Mean ± SD was 7.966±0.85 %. After 6 months of metformin therapy, HBA1C improved to 6.8±0.93.hs-CRP baseline value expressed as Mean±SD was 3.4±1.16 mg/L. After 6 months of metformin therapy, hs - CRP effectively reduced to 1.7±0.81

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mg/L. Prompt treatment intensification in such cases may thus be sensible. Further studies are needed to identify predictors of metformin treatment response, especially focusing on hs-CRP levels, lipid levels and genetic factors.

Keywords: Diabetes mellitus; metformin; C Reactive Protein (CRP); HBA1C.

1. INTRODUCTION

Diabetes mellitus representssignificantglobal health problem [1].Type 2 diabetes is the most common form of diabetes identified by Insulin deficiency, Insulinresistanceand increased hepatic gluconeogenesis leading to hyperglycemia, beta cell debilitation and finally beta cell failure [2]. The main aim of treating diabetes is to prevent morbidity and mortality and to prevent complications by keeping the blood glucose levels within normallimit.

Metformin is the most commonly used antidiabetic agent in the management of diabetes mellitus, discovery of metformin was initially startedwith the synthesis of galegine-like compounds derived from *Gallegaofficinalis*, a traditional plant in Europeand later it was used in the manangement of diabetes for centuries. Metformin was relatively found to have effective glucose lowering capacity and also a better safety profile and displayed a wide safety margin [2,3].

Metformin, apart fromitshypoglycemicactivity, when prescribed along with diet and life style modifications to prevent diabetes in patients with high risk category. Metformin – a iguanid group of anti diabetic agent, mainly acts by reducing the blood glucose level and insulin resistance and thereby decreasing the complications of diabetes. Metformin mainly decreases the hepatic gluconeogenesis and intestinal glucose absorptionandincreasesthe peripheral glucose utilization and actsasinsulin sensitisersinthe muscle and adipose tissue, and reduces hyperinsulinemia [3,4].

Recent evidence suggeststhatpersistent hyperglycemia in patients of diabetesisthe principle cause of Microvascular and macrovascular complications [5]. The probable mechanisms in the development of micro and macrovascular complications are overproduction of reactive oxygen species whichplays aimportantrole inactivationof pathways like polyol pathway activation, non enzymatic glycation and elevated (PKC) levels.

Prolonged chronic inflammation, results in increased levels of serum C-reactive protein, which has been recently linked to obesity, in sulin resistancesyn dromesan devidence of increased risk of cardiovascular diseases. Many studies have identified that C-reactive protein which is a marker of systemic inflammationplays an majorkeyrole and important independent risk factor for development of cardiovascular complications [5,6].

Elevated CRP levels are at increasedriskofthromboticevents like myocardial infarction leading to ischemic heart disease [6]. Increased CRP levels and obesityinnon diabeticsaremore prone for developing diabetes at later stages of life [7]. Furthermore, there is increased CRP levels in diabetics than the non diabeticpatients [7,8].Treatment modalities that target the insulin resistance will eventually decreases the inflammatory markers and thereby reducing the macrovascular complications. Oral antidiabeticagentsare the most important agents employed inachievingblood glucose levels within normal limits in patients with diabetes. Studies suggest that OH As a part from its hypoglycemic activity also decreases CRPlevel in diabetic patients. Considering the above fact relationship among oral hypoglycemic agents and CRP level is complex [9].

Metformin monotherapyfor newly diagnosed Type-2 diabetas mellitus has shown a significant decrease in high- sensitivity-CRP level in Type 2 diabetes. This positiveeffect may be because of decrease in the expression of pro inflammatory cytokines and other mediators, including adhesion molecules, and thereby decreasing the incidence of macrovascularcomplictions. However, this effect is probably dependent on improving glycemiccontrol [10].

Many studies suggested Metformin maydirectlyimpactthe pathophysiology of athero-thrombosis, because in T2DM subjects its short-term use improved markers of endothelial dysfunction and inflammatoryactivity.Thus this study was designed to know the anti inflammatory effect of metformin on CRP level in newly diagnosed Type-2 diabetes mellitus patients.

2. MATERIALS AND METHODS

This study was conductedin SreeBalajiMedical College and Hospital, Chennai during the period from November 2017 to April 2018 inaccordance withdeclaration of Helsinki and ICH GCP guidelines.

All the 50 patients who fulfilled the Eligibilitycriteria was advised to take the study drug Tablet Metformin 500mg twice daily one in the morning and one in night. The study Drug was given free of cost to the patients and they were given assurance that any withdrawal from the study would notaffect their future treatment in the same hospital.

Baseline laboratory investigations were done before study and repeated after 3 months and6months of treatment. The baseline features like demographic data, general, systemic and local examination were carefully noted in the case reportform.Contact numbers of the investigators and emergency physician were provided to all the study participants for any queries during the study period and for reporting of any adverse events. There were three scheduled visits during the study- baseline visit, 3rd month and 6th month (end of the study visit).

2.1 Study Population

A total of 50 patients with newly diagnosed type 2DM were included in the study. Both sexes were included in study. Study data was documented and patients were assessed periodically.

2.2 Drug Dosage

Tablet Metformin 500 mg twice daily, one in the morning and one in the night were advised to the study participants. A baseline investigation protocol was followed before starting the study, the following investigations were performed at the beginning of the studies Complete BloodCount, Fasting Blood Sugar, Post Prandial BloodSugar, High-Sensitivity (Hs),C- Reactive Protein Level, Hemoglobin A1c(Hba1c), Urea and Creatinine. and the following blood parameters were repeated.

2.3 Statistical Analysis

All values are expressedas mean ± standard deviation. Comparison of FBS, PPBS, Hs-CRP, Hba1c valuebeforeand after the study was performed by paired t -test using SPSS software 16.0 version. Comparsionofbloodparameters at baseline, 3month and 6 month were found to be statistically significant. And also comparsion of blood parameters at 3 month and 6 month were found to be statistically significant ($p<0.05$value).

3. RESULTS AND DISCUSSION

A total of 70 patients were screened and 50 patientswithnewly diagnosed Type 2 Diabetes mellitus who met the eligibility criteria were included in this study. The current study was doneto measure the anti inflammatory effect of Metformin on C-Reactive protein in patients with newly diagnosed type 2 Diabetes mellitus patients -a prospective, open label study. The demographic features of the subjects who took part in this study and their statistical analyses are given.

3.1 Gender and Age Distribution

Fifty newly diagnosed Diabetes mellitus patients were enrolled in the study. Among these 50 patients (mean age 40±4.33, 23 males and 27 females) received the study drug metformin .Major age group included in the study was between 41-50 years.

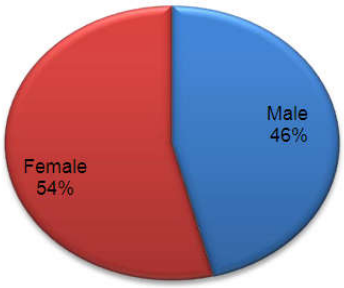

Fig. 1. Gender distribution in the study group

3.2 Effect of Metformin on Hs-CRP

Hs-CRP baseline value expressed as Mean±SDwas3.4±1.16 mg/L . After 3 months of metformin therapy, hs-CRP reduced to 2.93±0.9 mg/L. After 6 months of metformin therapy, hs-CRP effectively reduced to 1.7±0.81 mg/L. CRP levels werereducedafter 6 months of treatment. Treatment with Metformin showed significant reduction in hs-CRP values comparatively between hs-CRP baseline values and hs-CRP values at sixth month were significant ($P<0.05$).

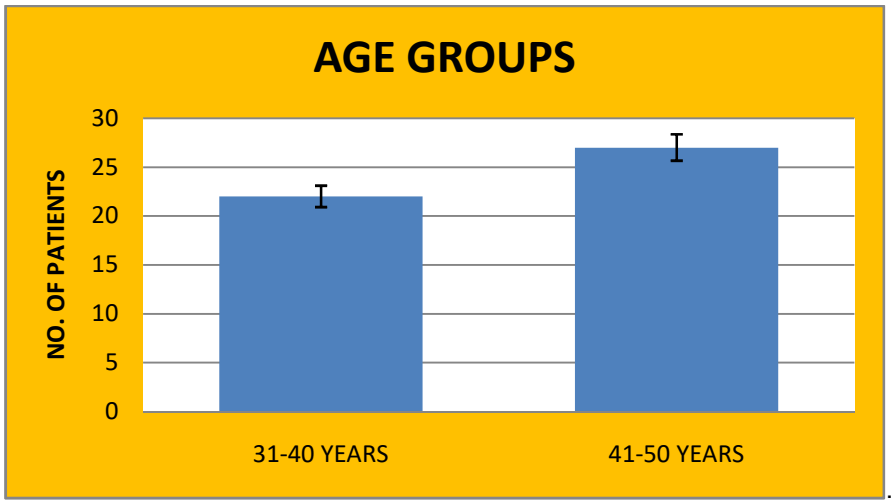

Fig. 2. Summary of age groups included in the study

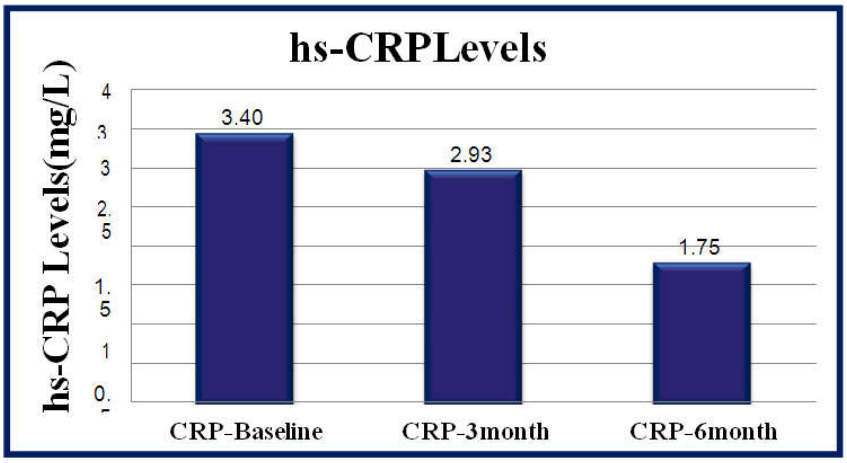

Fig. 3. Comparison of hs-CRP levels between Baseline and at the end of the study

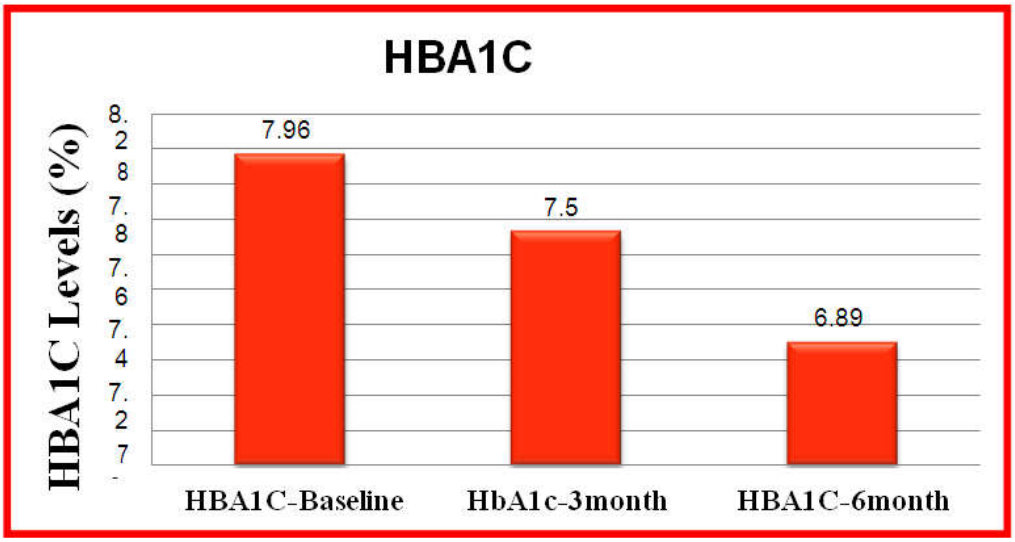

Fig. 4. Comparison of HBA1C levels during study period

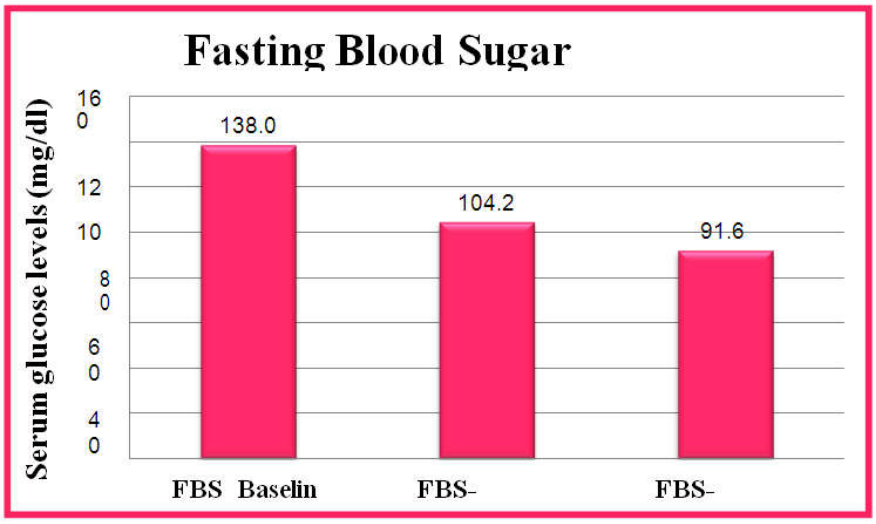

Fig. 5. Comparison of FBS between Base line and Post - treatment (6 month)

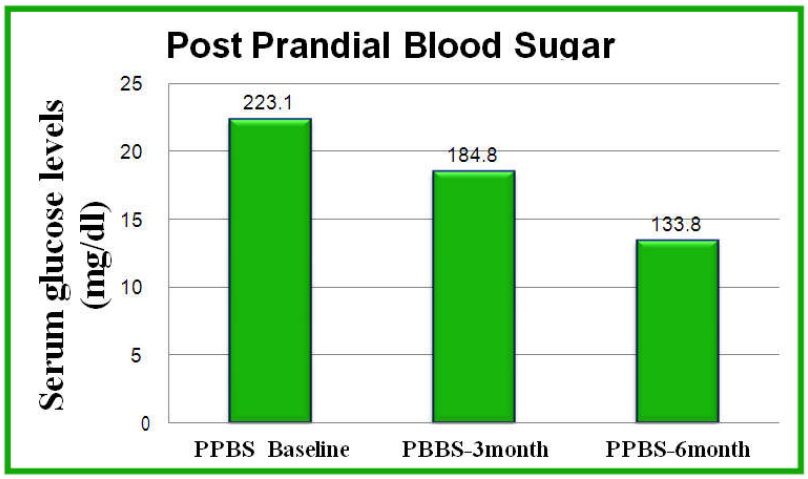

Fig. 6. Comparison of PPBS between Baseline and Post - treatment (6 month)

Table 1. Comparison of lab parameters between baseline and aftertreatment

S.No	Parameter	N	Metformin (n=50)	
			Baseline (1 month)	End ofThe Study(6 month)
1	FBS	50	138.06 ± 17.12	91.64 ± 10.55 *
2	PPBS	50	223.12 ± 30.63	133.88 ± 7.99 *
3	HBA1C	50	7.966 ± 0.85	6.898 ± 0.94 *
4	CRP	50	3.406 ± 1.16	1.754 ± 0.81 *

*Values expressed in mean ± std. Deviation, * represents p<0.05*

3.3 Effect of Met Form in on HBA1C

HBA1C baseline value expressed as Mean±SDwas7.966±0.85 %. After 3 months ofmetformintherapy, HBA1C mean obtained was 7.53±0.83 %.After 6 months of metformin therapy, HBA1C improved to 6.8±0.93. HBA1C baseline values and HBA1C values at the end of

the study were compared and was found tobesignificant (P<0.05).

3.4 Effect of FBS and PPBS on Hs-CRP

FBS and PPBS baseline values expressed as Mean±SDwere138.06±17.12 mg/dl and 223.12±30.63 mg/dl respectively. After 3 months of metformin therapy, FBS and PPBS were 104.24±16.63 mg/dl and 184.84±26.9 mg/dl respectively. After 6 months of metformin therapy, FBS and PPBS were 91.64±10.55 mg/dl and 133.88±7.99 mg/dl respectively.Treatment with Metformin showed effective reduction in FBS and PPBS values comparatively between FBS and PPBS baseline values and FBS and PPBS values at 6thmonth were statistically significant (P<0.05).

3.5 Effect of Metorminon FBS, PPBS, HbA1Candonhs-CRP

The below table explains the controlof metformin over fasting and postprandial blood glucose that is implied in 6 months glycemic control of HbA1C which in turn reduces the CRPlevel.

Diabetes mellitus is characterized by metabolic alterations that correlate elevated blood glucose levels to other risk factorsthat contribute tomajormicrovascularandmacrovascularcomplications which results in higher incidence of morbidity and mortality [11]. Experimental studiesdemonstrated that elevated blood glucose levels stimulates the release of the inflammatory mediators and results in the induction and release of acute-phase reactants byadipocytes.

CRP is an acute-phase reactant produced primarily inthe liver under the stimulation of adipocyte-derived proinflammatory cytokines, including IL-6 and TNF-α. Increased levels of hs-CRP emerged as a reliable biomarker ofinflammation, in patients of diabetes mellitus [12].

This study evaluated the effect of Metformin on C-Reactive protein level to prove its anti-inflammatory effect in patients with newly diagnosed Diabetes Mellitus. Among oral Anti diabetic agents, many studies shows evidence that metformin reduces CRP concentrations in patients of diabetes mellitus. However, this effect is probably dependent on improving glycemic control [13].

Current evidence suggests that measurementofhs-CRP for assessing vascular risk and treatment efficacy in insulin-resistant diabetic patients is a reliableprognostictool.

Treatment modalities that target insulinresistancemay benefit individuals by reducing various inflammatory makers and thereby preventing the development of macrovascular complications. Treatment was targeted at improving the insulin-resistant state, whether by non pharmacologicalmeasuressuch as life style modifications (promoting exercise and weight reduction) or pharmacological, such as metformin and other oral hypoglycemic agents that result in decreasing the CRP levels beyond mere glucose lowering effect.

CRP has numerous adverse cardiovascular effects that can contribute to the pathophysiology of cardiovascular disease. In experimental animals, metformin significantly improves survival rate in treated mice compared with untreated one [14,15]. Diabetic patients mainly die of cardiovascular complications such as, coronary artery disease and myocardial infarction and microvascular complications such as retinopthy, nephropthyandneuropathy, of which approximately 70% of diabetic patients mainly die of heart and brain macrovascular diseases [15]. It has been found out that diabetic patients without a known past history of myocardial infarction have the same risk of coronary artery disease as patients without diabetes with a known past history of myocardial infarction [16].

This has eventually made the National Cholesterol Education Program to consider diabetes mellitus asacoronary heart disease risk factor [17]. Although it is evidenced that there is an high risk of developing CADeventsin patients with diabetes mellitus, there is still some unreliability as to whether the cardiovascular risk conferred by diabetes is truly identical to that of a previous history of myocardial infarction [18]. And Scambatoetal.evidencedthat, in a 3-year observational study patients treated with metformin had reduced rates of re-infarction, occurrence of angina pectoris, acute coronary events other than acute myocardial infarction, and death in patients [19].

A number of clinical studies have found out that metformin has cardiovascular protective effects and it effectively decreases the incidence and mortality of cardiovascular events. The HOME

trial reported a decreased risk of developing macrovasculardisease [20]. And in UKPDS study, which was the first trial to determine that metformin showed effective reduction in risk of all-cause mortality and acute myocardial infarction in obese patients with diabetes mellitus [21]. In addition, to that anotherUKPDSsurvivor cohort further evaluated that treatment with metformin had a long-term benefit oncardiovascularriskin obese patients. Comparisionofsulfonylurea and insulin treatment, with metformin signficantly decrease the risk of myocardial infarction [22]. Roumieetalalsofound out that comparison of metformin treatment withsulfonylureatherapy, had effective reduction in decreased hazard of cardiovascular disease events in diabetes mellitus [22,23].

Moreover, decreased incidence of AtherothrombosisforContinued Health Registry indicated that the use of metformin in secondary prevention was associated with a 24% reduction in all-cause mortality among patients with atherothrombosis. Thus, metformin has greater cardiovascular protection role which is independent of glucose-loweringeffects [24,25]. Hong et al found out that in diabetic patients with CAD, comparison of glipizide with metformin treatment reduced major cardiovascular events which indicated the potential benefit of metformin cardiovascular outcomes in diabetic patients [24]. Metformin is theonlyAntidiabeticdrugto be recommended for its cardiovascular benefit (according to AACE guidelines 2013).

The risk factors of cardiovascular disease include dyslipidemia, obesity, hypertension, and insulin resistance. Metformin may improve lipometabolism and the level of cholesterol by activation of AMPK [25]. And metformin was associated with weight loss or less weight gain [26], this is probablydue to suppression of satiety centre [26]. Metformin could effectively reducestheblood pressure levels in non- diabetic patients [27]; probable mechanisms of blood pressure lowering effects are reduction of insulin resistanceandhyper insulinemiaandadrenergic receptor deactivation, and thereby reduction of intra cytoplasmic calcium,andinhibition of sympathetic drive inconditionsassociated with high dietary salt intake and increase of glomerular filtration rate and sodium excretion. Apart from that, metformin can alleviate oxidative stress and inflammatory markers as well as improve endothelial dysfunction [27,28].

Dragan Micic et al. study shows that metformin has an effect on CRP and insulin sensitivity in type 2 diabetes patients after 12 weeks of therapy, associatedwithsignificant decrease in HbA1c levels and insulin sensitivity independent of changes in CRP levels [29]. King et al., foundout that for each level ofincreasein HbA1c level, the percentage of CRP was increased as follows: <7, 48.9%; 7 – 8.9, 45.4%; 9 –10.9, 60.7%;and>11, 70.6%. Overall, 51.5% of participants hadelevated CRP. Freeman et al. [7] showed thattherewas a strong and graded relation of CRP level with the incidence of diabetes independent of established risk factors.

The present study showed that a higher HbA1c values was associated with a higher CRP levels among patients with diabetes, and the results of this study wasHbA1cvalues when compared with baseline and sixth month, the HbA1c values at sixth month were reduced effectively after treatment with metformin. Our study findings demonstrated the decreasing pattern of HbA1c with metformin treatment. And FBS and PPBS values at baseline and sixth month were compared and FBS and PPBS values at six months was reduced significantly which shows that metformin is an effective antidiabetic agent. And hencethis study proves that metformin provided greater protection against the development of macro vascular complications than would be expected from its effects upon glycemic control alone. In light of current findings that there is a strong association of inflammatory proteins, endothelial dysfunction, and insulin resistance, and the results of this study was to provide additional support for arelation between blood glucose control andsystemic inflammation in people with newly diabetes. Our study findings demonstrated the decreasing pattern of HbA1candCRP with metformin treatment in newly diagnosed diabetes mellitus and this proves the additional pleiotropic effects of metormin.

To conclude, despite including more variables that are clinicalthis study confirmed that only few demographic or clinical factors canpredict HbA1c response in diabetes patients initiating metformin treatment. In addition to previous studies, our study showed that diabetes duration was in particular associated with HbA1c levels in patients treated with low dose metformin in the first 6 months of treatmentand also its association with CRP levels. Prompt treatment intensification in such cases may thus be sensible. Further studies are neededto identify predictors of metformin treatment response, especially focusing on hs-CRP levels, lipid levels and geneticfactors.

4. CONCLUSION

The results of the present study proves that metormininnewly diagnosed type 2 diabetes mellitus patients significantly improves the HbA1c levels and CRP level (inflammatory marker) after six months of treatment. So this study emphasizes the intitaionofmetformin as mono therapy in diabetic patients that apart from its effective gylcemic control it also reduces CRP level which prove its additional pleotropic effect (anti inflammatoryeffect), and hence therebyindiabetespatientsbydecreasing the long term macrovascular complications.

CONSENT AND ETHICAL APPROVAL

The protocol was reviewed and was approved by the Institutional Ethics Committee and all the participants have been informed about the study procedures and written informed consent was obtained.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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