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
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 represents a significant global health problem [1]. Type 2 diabetes is the most common form of diabetes identified by Insulin deficiency, Insulin resistance and 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 normal limit.

Metformin is the most commonly used antidiabetic agent in the management of diabetes mellitus, discovery of metformin was initially started with the synthesis of galegine-like compounds derived from Gallega officinalis, a traditional plant in Europe and later it was used in the management 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 from its hypoglycemic activity, when prescribed along with diet and life style modifications to prevent diabetes in patients with high risk category. Metformin – a biguanide 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 absorption and increases the peripheral glucose utilization and acts as insulin sensitiser in muscle and adipose tissue, and reduces hyperinsulinemia [3,4].

Recent evidence suggest that persistent hyperglycemia in patients of diabetes is the 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 which plays a key role in activation of 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, inulin resistance syn dromes an deidence of increased risk of cardiovascular diseases. Many studies have identified that C-reactive protein which is a marker of systemic inflammation plays a major role and important independent risk factor for development of cardiovascular complications [5,6].

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

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

Many studies suggested Metformin may directly impact the pathophysiology of atherothrombosis, because in T2DM subjects its short-term use improved markers of endothelial dysfunction and inflammatory activity. 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 conducted in Sree Balaji Medical College and Hospital, Chennai during the period from November 2017 to April 2018 in accordance with declaration of Helsinki and ICH GCP guidelines.

All the 50 patients who fulfilled the Eligibility criteria 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 not affect their future treatment in the same hospital.

Baseline laboratory investigations were done before study and repeated after 3 months and 6 months of treatment. The baseline features like demographic data, general, systemic and local examination were carefully noted in the case report form. 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 2 DM 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 Blood Count, Fasting Blood Sugar, Post Prandial Blood Sugar, 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 expressed as mean ± standard deviation. Comparison of FBS, PPBS, Hs-CRP, Hba1c value before and after the study was performed by paired t-test using SPSS software 16.0 version. Comparison of blood parameters at baseline, 3 month and 6 month were found to be statistically significant. And also comparison 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 patients with newly diagnosed Type 2 Diabetes mellitus who met the eligibility criteria were included in this study. The current study was done to 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±SD was 3.4±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 were reduced after 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 of The 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±SD was 7.966±0.85 %. After 3 months of metformin therapy, 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 to be significant (P<0.05).

3.4 Effect of FBS and PPBS on Hs-CRP

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 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 6th month were statistically significant (P<0.05).

3.5 Effect of Metformin on FBS, PPBS, HbA1C and hs-CRP

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

Diabetes mellitus is characterized by metabolic alterations that correlate elevated blood glucose levels to other risk factors that contribute to major microvascular and macrovascular complications which results in higher incidence of morbidity and mortality [11]. Experimental studies demonstrated that elevated blood glucose levels stimulates the release of the inflammatory mediators and results in the induction and release of acute-phase reactants by adipocytes.

CRP is an acute-phase reactant produced primarily in the 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 of inflammation, 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 glycemc control [13].

Current evidence suggests that measurement of hs-CRP for assessing vascular risk and treatment efficacy in insulin-resistant diabetic patients is a reliable prognostic tool.

Treatment modalities that target insulin resistance may 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 pharmacological measures such as lifestyle 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 retinopathy, nephropathy and neuropathy, 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 as a coronary heart disease risk factor [17]. Although it is evidenced that there is an high risk of developing CAD events in 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 Scambato etal. evidenced that, in a 3-year observational study patients treated with metformin had reduced rates of re-infraction, 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 macrovascular disease [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 another UKPDS survivor cohort further evaluated that treatment with metformin had a long-term benefit on cardiovascular risk in obese patients. Comparison of sulfonylurea and insulin treatment, with metformin significantly decrease the risk of myocardial infarction [22]. Roumieetal also found out that comparison of metformin treatment with sulfonylurea therapy, 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-lowering effects [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 theoreticallyAntidiabeticdrug to 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 probably due to suppression of satiety centre [26]. Metformin could effectively reduce the blood pressure levels in non-diabetic patients [27]; probable mechanisms of blood pressure lowering effects are reduction of insulin resistance and hyperinsulinemia and adrenergic receptor deactivation, and thereby reduction of intracellular calcium, and inhibition of sympathetic drive in conditions associated 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, associated with significant decrease in HbA1c levels and insulin sensitivity independent of changes in CRP levels [29]. King et al., found that for each level of increase in 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 had elevated CRP. Freeman et al. [7] showed that there was 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 was the HbA1c values 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 hence this study proves that metformin provided greater protection against the development of macrovascular 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 and systemic inflammation in people with newly diabetes. Our study findings demonstrated the decreasing pattern of HbA1c and CRP with metformin treatment in newly diagnosed diabetes mellitus and this proves the additional pleiotropic effects of metformin.

To conclude, despite including more variables that are clinical, this study confirmed that only few demographic or clinical factors can predict 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 treatment and also its association with CRP levels. 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.
4. CONCLUSION

The results of the present study prove that metformin in newly 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 initiation of metformin as mono therapy in diabetic patients that apart from its effective glycemic control it also reduces CRP level which prove its additional pleotropic effect (anti-inflammatory effect), and hence thereby diabetes patients by decreasing 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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