



ROLE OF METFORMIN IN COVID-19

Pharmacology

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ABSTRACT

Cytokine storm an uncontrolled over-production of soluble markers of inflammation which is systemic inflammatory response, is a major responsible for the occurrence of ARDS. Animal studies that have implicated ACE2 in the acute lung injury caused due to SARS-CoV. It has been hypothesized that ACE2 causes ALI by bringing about autophagy through the AMPK/mTOR pathway. Metformin works through AMPK activation, which leads to phosphorylation of ACE2 and there is a structural change which unables SARS CoV2 to bind with it also it is known that metformin can reduce CRP level. This study aim to find the role of metformin in COVID 19 on disease regression by both clinical assessment and laboratory values. Single blinded randomised controlled trial, parallel group, placebo controlled study for 1 month duration at tertiary health care centre. There is significant clinical improvement in 69% of the subjects at day 6 and 31% at day 7 in metformin group. Also there is effective reduction in CRP level whereas Sr.Ferritin levels are moderately reduced. There is no adverse effect during study period.

KEYWORDS

COVID 19, Metformin, mTOR, ACE2.

Introduction:

COVID-19 caused by SARS-CoV-2 has spread rapidly and widely all around the world with more than 26,415,380 confirmed cases and more than 870,286 deaths reported as of 5 september 2020(1). Patients present with fever, cough, fatigue, dyspnea and diarrhea and a few patients have some other symptoms such as headache and hemoptysis. There is still no specific drug, and several numbers of trials are undergoing around the world provide relevant insights for clinical treatment. Since COVID-19 belongs to the same genus of CoV as SARS-COV and MERS-COV both of which are beta-cov. Whole genome sequencing showed that COVID-19 shared 79.5% of sequence identity with SARS-CoV based on the with the treatment experience of SARS-COV and MERS-COV(2). Several drugs have been used in treatment of SARS-CoV-2 including Arbidol, Hydroxychloroquine, and Lopinavir, Ritonavir, new nucleoside analogues Remdesivir and convalescent plasma. COVID 19 triggers an innate immunity mediated by type 1 which is usually followed by increased IL6 secretion from alveolar macrophage (3)(4). The major cause of mortality is Acute respiratory distress syndrome (ARDS) which is due to cytokine storm. ARDS is driven by overproduction of proinflammatory cytokines and hyperinflammation(5). Thromboembolism occurring in COVID 19 also aggravates the hyperinflammatory response.(6)(7). Budinger group on Invitro cell and Invivo mouse model found that Metformin inhibits mitochondrial complex 1, this suppress the Mitochondrial Reactive oxygen species(ROS) which is necessary for opening of CRAC(Calcium release activated Ca²⁺) channels in generation of IL6 from macrophage(8)(9). This prevents the cytokine storm. Also another mechanism has been described that ACE2 causes Acute lung injury by bringing about autophagy through the AMPK/mTOR pathway. (10) AMPK is activated as an adaptive response to decrease in ATP to AMP ratio (11). AMPK increases the expression of ACE2 and increase its stability by phosphorylating ACE2 Ser680 in human umbilical vein endothelial cells (HUVECs) and human embryonic kidney 293 (HEK293T) cells (12)(13). COVID 19 enters the human body through interaction between its spike proteins (S1) and the N-terminal region of ACE2(14)(15). Metformin activates the AMPK thereby stabilise ACE2. Other mechanisms are it also acts like chloroquine. COVID 19 viral membrane fusion in endocytosis phases happens only in acidic pH(16). Chloroquine and hydroxychloroquine increases pH thereby inhibit endocytosis, maturation of endosome and transport of virion(17). Metformin acts on Vacuolar ATPase (V-ATPase) and endosomal Na⁺/H⁺ exchangers (eNHEs) through guanidine scaffold are major alkalising component in endosome(18). Hence this study focuses on decreasing the progression of COVID19 from mild and moderate to severe cases. Objectives of this study are to find role of Metformin in COVID 19 on disease regression by both clinical assessment and laboratory values.

Materials and methods:

Study design: A Single Blinded, Parallel group, Placebo controlled, Randomised controlled trial was carried out for a period of 3 months in a Tertiary Health Care centre. Ethical approval was obtained from

institutional ethics committee prior to initiation of the study according to the institution regulations.

Study population:

Non diabetic patients with age between 18-75 years of Mild and Moderate COVID19(based on WHO classification) with NEWS score 0-4 are included. Excluded population were Pregnant and lactating women, asymptomatic cases, severe COVID 19 cases, Known hypersensitivity to Metformin, diabetic patient, patients with end organ damage, patients receiving Metformin for any other indications and patients with any other active infections or other inflammatory diseases.

Data collection:

Demographic details, habit history (alcohol and tobacco), diagnosis, general examination with lab parameters, medication chart, length of hospital stay were recorded in case report form.

METHODOLOGY:

Patients diagnosed by RT-PCR confirmed COVID19 had been included in this study. Written informed consent form was obtained from all subjects before starting the study. Clinical examination and lab investigations were done to all. Clinical assessment done by National Early warning score(NEWS) which consists of 7 components Respiratory rate, saturation, systolic and Heart rate, temperature, any oxygen support, level of consciousness. Subjects were randomised and included in study are considered as an Intention to treat population. The following investigations were done to all patients before initiation of therapy, 5th day and at the end of 10 days for all subjects – CRP, Sr.Ferritin, NL ratio. Supportive care treatment is given to all the subjects. Study population were randomised by Computer generated randomisation into 2 groups. Both groups were clinically assessed for improvement based on NEWS with the help of Physician daily and lab parameters were assessed on 5th and 10th day. Both the groups received standard and supportive treatment. During the study period if the subjects become severe they were transferred to Intensive care unit and study drug stopped. Patients who became asymptomatic were transferred to the corona care centre.

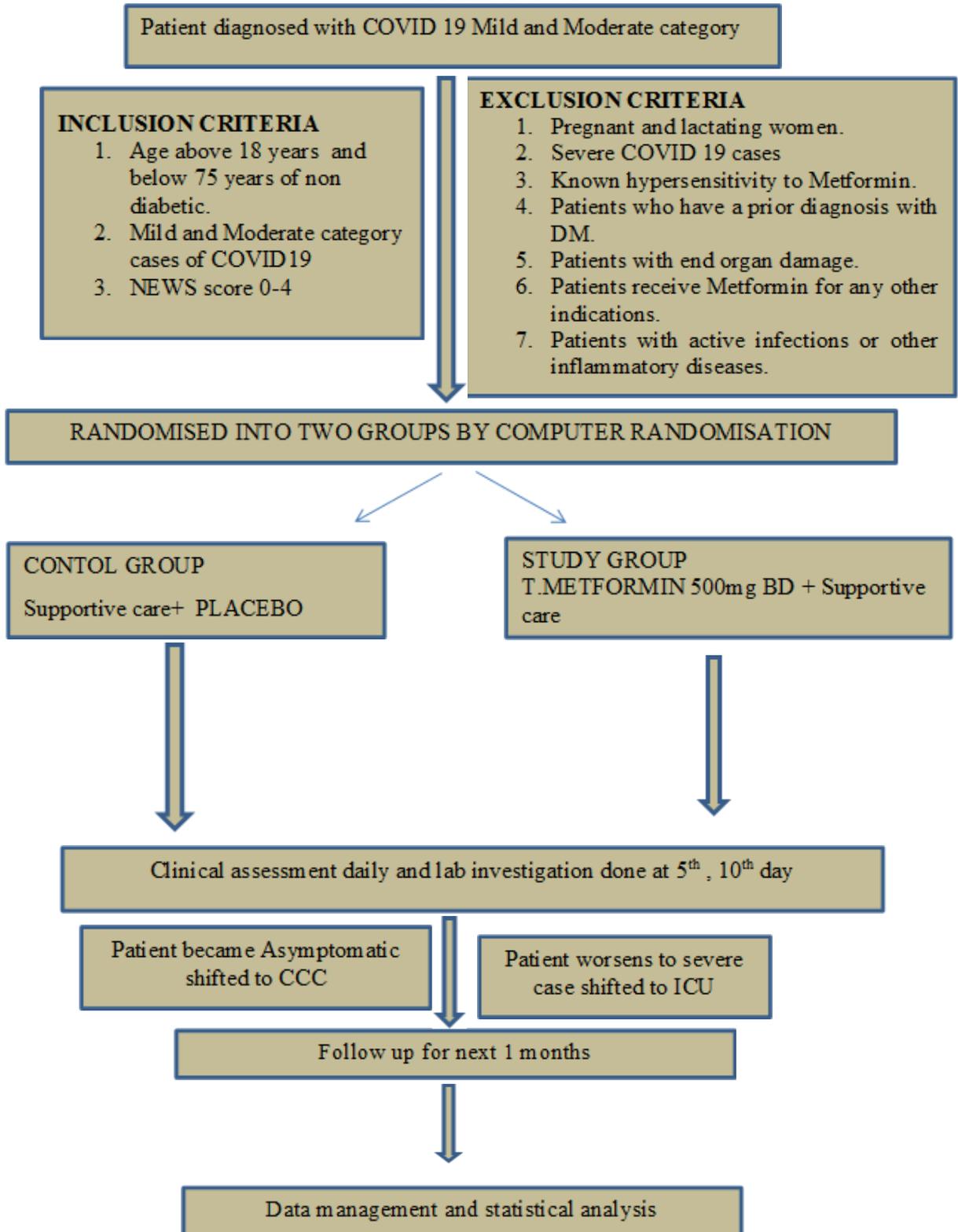
STATISTICAL ANALYSIS:

Data were collected and entered in Microsoft Excel 2010 spreadsheet, and analysis was done by SPSS software 23.0. Frequencies and percentages were used to represent gender, age and number of cases worsened. Lab parameters between test group and placebo group are compared by unpaired t test and improvement among same group after treatment is compared by paired t test.

Ethical consideration:

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

STUDY METHODOLOGY



RESULTS:

Demographic profile: Total sample size is 33 in each group. Male and female ratio is maintained, in Metformin group number of males are 18(54%) and females are 15(46%) whereas in placebo group number of males are 17 (51%) and females are 16(49%). Age group almost equally distributed between age groups 18-30, 30-40, 40-50 in both arms.

	Metformin group	Placebo group
Total population	33	33
Sex	M 18 F 15	M 17 F 16

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At the start of the study among the age group of 18 to 30 years had no abnormality of CRP, NL ratio, Sr. Ferritin in both arms. In Metformin group total of 17 (51.1%) cases had elevated CRP of which 10 (59%) cases are from 40-60 age group and 7 (41%) is from 30-40 age group and regarding elevated Sr.Ferritin level 10 (30.3%) cases elevated of which 7 (70%) from 40-60 age group and 3 (30%) from 30-40 age group. In placebo group total of 16(48.5%) cases had elevated CRP

level of which 10 (62.5%) from 40-60 age group and 6 cases from 30-40 (37.5%) age group and regarding elevated Sr. Ferritin level 11(33.33%) cases had abnormal elevation in which 8(72.7%) from 40-60 age group and 3(27.3%) from the 30-40 age group(Figure1&2) . There is no elevation in NLratio.

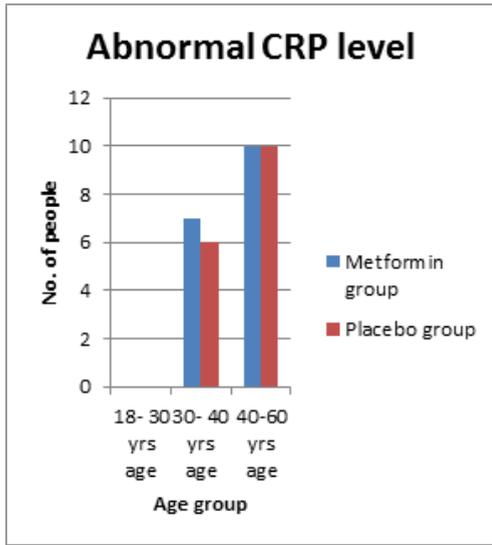


Figure 1

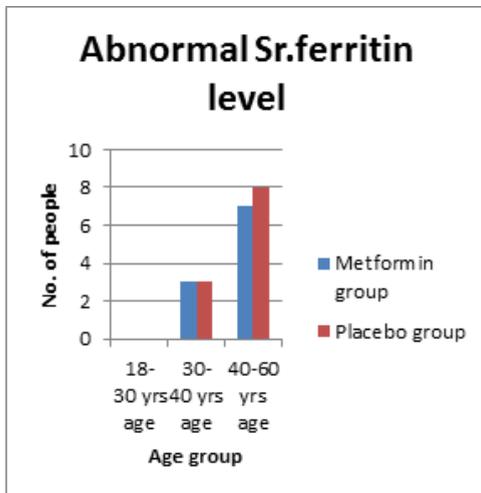


Figure 2

Clinical improvement: It was assessed by National early warning score (NEWS). The score consist of 7 components all are assessed in patients. The mean NEWS in Metformin group at start of the study is 2.84 and in placebo is 2.72. At the end of 10 days when two arms compared p value is 0.0197(table2) which is statistically significant. On comparing within the Metformin group at 5th day and at 10th day p value < 0.001 which is very highly significant (Table3). The clinical improvement on daily basis is represented in line diagram(figure 3). At day 7 itself NEWS become zero in Metformin group whereas in placebo on day 10 too the mean score is 0.15.

	Metformin	Placebo	p value
5th day	0.72	0.87	0.415
10th day	0	0.15	0.0197

Table 2 Comparing NEWS Metformin vs Placebo at day 5 and 10

	Metformin	p value
1st day	2.848	
5th day	0.72	<0.0001
10th day	0	<0.0001

Table 3: Comparing NEWS within Metformin group at day 1st, 5th, 10th day

Lab parameter (Mean)	Metformin	Placebo	p value	Metformin	Placebo	p value
	5th day	5th day	5th day	10th day	10th day	10th day
CRP (mg/L)	10.35	12.07	0.374	6.42	9.78	<0.01
NLR	1.97	1.96	0.919	1.84	1.96	0.249
Sr.Ferritin (ng/ml)	187.68	183.54	0.846	164.93	179.8	0.395

Table 4: Metformin vs placebo p value

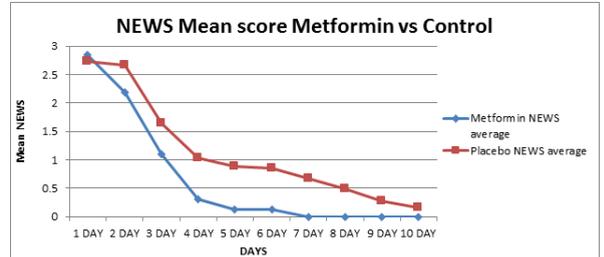


Figure 3

Lab parameters: CRP , Sr.Ferritin and NL ratio is assessed at 5th and 10th day. At 5th day, the parameters between two groups are compared but found to be statistically insignificant but at 10th day there is significant change is absorbed in CRP level (Table4). There is no statistically significant change absorbed in NLR and Sr.Ferritin level at 10th day (Table4). But on comparing within the Metformin group itself after treatment all the lab parameters at both day 5 and 10 are statistically significant compared to the day 1 findings (Table 5).

p value	Within Metformin group	
	5th day	10th day
CRP	<0.001	<0.001
NLR	0.066	<0.01
Sr.Ferritin	<0.01	<0.01

Table 5 : Changes within Metformin group

Length of hospital stay:

In Metformin group 23 cases shifted to Corona care centre(ccc) at day 6 and 10 cases at day 7. In control group 27 cases shifted to ccc at day 10 , 2 to Intensive unit at day 6 so total of 6 subjects remains at hospital at the end of day 10. At followup none of the subjects have reinfection.

Discussion:

COVID 19 is a dreadful pandemic humankind is facing. Day by day there is increase in incidence and mortality. Though the mortality rate is maintained at approximately 2% worldwide but the actual number of deaths are 775,893 because of increased incidence. The severity mainly depends on age group that it is more in elderly compared to the young adults. It also depends on the comorbidity. The worsening of disease is mainly due to the acute inflammatory reaction and the major cause of death is pneumonia with cytokine storm. There are several drugs used to control these like Tocilizumab and Steroids but failed to decrease the severity and progression of disease from mild to moderate. Due to unavailability of standard treatment the main focus turns towards "Drug repurposing".

Based on these findings we compare the clinical improvement and lab parameters with patients taking Metformin and placebo with supportive treatment. During the treatment period at 5th and 10th day using NEWS we found that significant clinical improvement in Metformin group compared to the control group moreover at day 7 itself subjects in Metformin showed complete clinical improvement. As far as lab parameters compared there is significant change in CRP level at day 10 in Metformin. Although the levels of NL ratio and Sr.Ferritin is not statistically significant , there were exponentially improvement in values. Also there is statistically significant change absorbed within the Metformin group on day 5 and day 10 in CRP and Sr.Ferritin level but NLR shows significance only in day 10. From this study we found Metformin will act as an anti-inflammatory in COVID 19 mild and moderate cases and it is not a treatment option for severe cases because of risk of lactic acidosis.

Limitation of the study:

The sample size of the study is less. The high risk cases requiring oxygen was not included. The CT scan findings of the lung was not

taken into consideration. Since the risk of developing lactic acidosis and severe cases will already be in acidosis state we cannot prescribe in severe cases. Repeat swab was not taken in this study.

Conclusion:

From the above findings we concluded that there is significant clinical improvement in 69% of the subjects at day 6 and 31% at day 7 in metformin group. Also there is effective reduction in CRP level whereas Sr.Ferritin levels are moderately reduced. There is no adverse effect during study period. Due to less side effect and cost effective Metformin can be used as an add on therapy to control the inflammation along with supportive or standard treatment. Further studies are needed to prove the use of metformin in COVID 19 in large number of samples.

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