Perspective of methemoglobin, hemoglobin, lactate dehydrogenase and C reactive protein levels in covid-19 patients with diabetes mellitus

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Abstract

Objectives: Dynamics of COVID-19 disease are changing with the emergence of the new variant of the COVID virus. Still, the severity of this disease is associated with comorbid conditions like diabetes mellitus (DM), hypertension, etc. and several biomarkers are studied. The objectives of the study were to estimate methemoglobin (Met-Hb), hemoglobin(Hb), lactate dehydrogenase (LDH) and C reactive protein(CRP) levels in COVID-19 patients with DM and without DM and then to compare between two groups.

Materials and methods: This observational study was conducted in 40 COVID-19 patients with DM and 40 COVID-19 patients without DM from June 2021 to October 2021 in the biochemistry department of a tertiary care hospital. For all patients, estimation of Met-Hb, Hb, LDH and CRP levels were estimated on the 2nd-3rd day of hospital admission.

Results: Met-Hb, LDH and CRP levels were significantly high and Hb levels were significantly low in elderly COVID-19 patients with DM than in those without DM ($P<0.05$). There was a significant positive correlation between Met-Hb with LDH and Met-Hb with CRP in both groups and a significant negative correlation was found between Met-Hb with Hb in the diabetic group.

Conclusion: In elderly patients, diabetes is one of the important and independent risk factors for the severity of COVID-19 disease. Derangement of Met-Hb along with LDH and CRP shows the need for routine monitoring of Met-Hb. This may open new options in the treatment of COVID-19 disease with DM and improve outcomes in the future.

Keywords- COVID-19, methemoglobin (Met-Hb), hemoglobin(Hb), lactate dehydrogenase (LDH), C reactive protein(CRP)

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Introduction:

As of July 2022, over 567 million confirmed cases and over 6.3 million deaths due to COVID-19 have been reported globally. In India, till the end of July 2022, more than 44 million confirmedcases with over5 lakh deaths from COVID-19 have been reported.[1]

COVID-19 is not a localized respiratory infection but a multisystem disease caused by a diffuse systemic process involving a complex interplay of the immunological, inflammatory and coagulative cascades. Serious illness is most likely to occur in old persons and people having previous as well as unrevealed medical conditions like diabetes mellitus, cardiovascular disease, chronic respiratory disease or cancer, etc. [2]

Methemoglobin(Met-Hb) is one of the important derivatives of hemoglobin. Increased Met-Hb levels were found in COVID-19 patients as compared to healthy individuals [3] and were also shown to be related to the severity of sepsis.[4] C Reactive Protein (CRP) is a marker of inflammation.In tissue injury, necrosis, hemolysis, hypoxia, or malignancies elevated levels of serum LDH has been seen.[5]In severe infections, cytokine-mediated tissue damage may occur with the release of LDH.[6]

Several studies showed that in COVID-19 infected patients, diabetes was the most common morbidity. [7,8]Diabetic patients are more susceptible to infections, so the prognosis of the patients may worsen with hyperglycemia, viremia and inflammation in COVID-19 disease. [8,9]The combined effect of the high incidence of diabetes and the progression of the COVID-19 pandemic may be problematic.

To our knowledge, no previous studies are done with methemoglobin in COVID-19 patients with diabetes mellitus (DM). In this study, we estimated methemoglobin along with hemoglobin, C reactive protein and lactate dehydrogenase in COVID-19 patients with and without diabetes to find out a perspective view on their association.

Materials and methods

This observational study was conducted on COVID-19 patients admitted to Krishna Hospital and Medical Research Center, Karad, Maharashtra from June 2021 to October 2021. Ethical clearance was taken from Institutional Ethics Committee, KIMSDU, Karad, Maharashtra, India.

The sample sizewas calculated by using formula:n= $(SD_1^2 + SD_2^2)$ $(Z_{1-\alpha/2} + Z_{1-\beta})^2$ $/d^2$.By taking CRP values from a study by Pei Zhang et al.[10] among COVID-19 patients with and without DM with 10% permissible error, 95% confidence interval and80% power;the sample size was calculated as a minimum of 39 in each group. Group A - 40 COVID-19 patients with DM and group B - 40 COVID-19 patients without DM.

The COVID-19 patients were diagnosed by reverse transcription-polymerase chain reaction (RT-PCR) or clinical and high- resolution computed tomography (HR-CT) findings. Patients admitted tothe hospital with DM and without DM, age >30 years were included in the study.

Following patients were excluded:COVID-19 patients on drugs causing methemoglobinemia such as hydroxychloroquine, chloroquine,isobutyl nitrite, sodium nitrate, sodium valproate, etc, patients on treatment with immunosuppressive agents and previously known case of glucose 6 phosphate dehydrogenase (G6PD) deficiency, severe renal failure, cirrhosis, hepatitis, COPD, active smokers, pregnant and lactating females, etc. For the selection of patients, a purposive sampling method was used. According to inclusion & exclusion criteria, patients were selected. After explaining the study to every patient, informed written consent was taken. Demographic data, medical history, clinical data,random blood glucose levels on admission and glycohemoglobin (Hemoglobin A1c/ HbA1c) levels from the patient's file were filledin a proforma for every patient.Duration of hospitalizationand outcomes of patients were extracted from medical records afterward.

From each patient 4 ml venous blood sample was collected. In the EDTA vacutainer,

1ml blood was collected and in the plain vacutainer,3ml blood was collected on the 2nd or 3rd day of admission to the hospital. Blood processing was done in the Biochemistry laboratory of KIMSU, Karad. Serum LDH in Units per liter (U/L)estimation was done on Erba 360 auto analyser by Deutsche Gesellschaft Fur Klinische Chemie (DGKCH) method (reference range- 200-400U/L).[11,12] The kit contains LDH-P reagent which uses pyruvate. Met-Hb in percentage (%)estimated by the method described by Sato on Spectronic 20 spectrophotometer (reference range- 0-1.5%). [13] Hb in grams per deciliter (gm/dl) was measured by an automated hematology analyzer by spectrophotometry (reference range-12-15gm/dl). CRP in mg/L was estimated by turbidimetric immunoassay on an autoanalyzer. [14]

Statistical Analysis -

Variables were expressed as numbers (percentages) or as the mean ± standard deviation (SD). With the Kolmogorov Smirnov test,the normal distribution of the data was assessed. Categorical variables were analyzed by the chi-square significance test.Continuous variables were evaluated by Mann Whitney U test. Correlation analysiswas done with the Spearman correlation test and was expressed as Spearman's correlation coefficient. IBM SPSS Statistics, version 20 was used for data analysis. P-value ≤ 0.05 was considered statistically significant.

Results-

Total 80 patients diagnosed with COVID-19 patients admitted to Krishna hospital were studied from June 2021 to October 2021.

General characteristics

On analyzing the general characteristics of the COVID-19 patients (Table 1), gender-wise two groups were similar without significant difference. The diabetic patients were significantly older (61.85 ± 12.37years), had more HRCT scores (12 ± 5), longer duration of hospitalization (20 ± 12days) andhad low survival (57.5%) compared to nondiabetic patients.

Table1: General characteristics of patients

		Group A	Group B	P value
Gender	Male	23(57.5%)	28(70%)	0.176
	Female	17(42.5%)	12(30%)	
Severity of disease	Moderate	3(7.5%)	17(42.5%)	0.000*
	Severe	14(35 %)	16(40 %)	
	Critical	23(57.5%)	7(17.5%)	
Survived	Yes	23(57.5%)	35(87.5%)	0.003*
	No	17(42.5%)	5(12.5%)	
Age(years) (mean ± SD)		61.85 ± 12.37	53.55 ± 11.65	0.002*
Duration of hospitalization(days) (mean ± SD)		20 ± 12	14 ± 5	0.010*
HRCT score(mean ± SD)		12 ± 5	9 ± 4	0.005*

COVID-19: Coronavirus disease-19
Group A:COVID patients with DM
Group B:COVID patients without DM
*P<0.05.

Laboratory findings

As per the laboratory findings (Table 2), patients in the diabetic group had significantly higher Met-Hb (1.63 ± 0.44 %), LDH (933.4 ± 308.4 U/L), CRP (26 ± 16.5 mg/L), random blood glucose (14.45 ± 3.95 mmol/L) and HbA1c(7.9±1.96%) levels and statistically significantly lower Hb (9.40 ± 3.13 gm/dl) levels as compared to non-diabetic group (P=0.000).

Table 2: Laboratory findings in patients

	Group A(mean ± SD)	**Group B(mean ± SD)**	**P value**
Random blood glucose level (mmol/L)	14.45 ± 3.95	5.83 ± 0.92	0.000*
Hb(g/dl)	9.40 ± 3.13	11.42 ± 2.77	0.003*
Met-Hb(%)	1.63 ± 0.44	1.26 ± 0.32	0.000*
LDH (IU/L)	933.4 ± 308.4	630.2 ± 266.2	0.000*
CRP (mg/L)	26 ± 16.5	15.9 ± 12.3	0.003*
HbA1c(%)	7.9±1.96	4.47±0.8	0.000*

COVID-19: Coronavirus disease-19
Group A:COVID patients with DM
Group B:COVID patients without DM
*P<0.05.

Correlation analysis:

Correlation analysis in COVID-19 patients is detailed in Table 3. In both groups, a significant strong positive correlation was found between Met-Hb and LDH (ρ = 0.471) (P=0.000) & (ρ =0.547)(P=0.002), between Met-Hb and CRP (ρ =0.710) (P=0.000)& (ρ =0.527)(P=0.000) and between CRP and LDH (ρ =0.445) (P=0.007)& (ρ =0.704)(P=0.000) respectively. A significant negative correlation was found between Met-Hb and Hb (ρ = -0.419) ((P=0.007) in only diabetic groups.

Table 3: Correlation analysis

Parameters	**Correlation Coefficient**	**Group A**	**Group B**
Met-Hb with LDH	Spearman's rho	0.471	0.547
	P value	0.000*	0.000*
Met-Hb with CRP	Spearman's rho	0.710	0.527
	P value	0.000*	0.000*
CRP with LDH	Spearman's rho	0.445	0.704
	P value	0.007*	0.000*
Met-Hb with Hb	Spearman's rho	-0.419	-0.204
	P value	0.007*	0.206

COVID-19: Coronavirus disease-19
Group A:COVID patients with DM
Group B:COVID patients without DM
*P<0.05.

Discussion

In India,the prevalence of DM ranged from 5 to 17 % according to theepidemiological study by Anjana RM et al.[15] With this high prevalence, India is facing a big challenge in managing COVID-19 patients as diabetes has been shown as one of the major comorbidities for COVID-19 disease progression and mortality.[7]In this observational study, a total of 80 COVID-19 patients were studied with and without DM. The general characteristics and laboratory findings were compared within the groups.

Our finding about significantly older age in diabetic COVID-19 patients wassupported by other studies. [16,17,18]This may be related to low immunity in older age that further declines with associated diabetes. Also, almost 57.5% of diabetic patients in our study faced critical illness, had a longer hospital stay and had low survival. A similarfinding with 69% of critically ill diabetic patients was shown in the study by Reem Al Argan et al.[19]

The present study showed higher levels of CRP and LDH in the diabetic group. These results were similar to previous studies by Wan Zhou et al.[16], Pei Zhang et al.[10],and Reem Al Argan et al.[19]One of the mechanisms associated with low survival in diabetic patients may be high levels of markers of inflammation such as CRP which is strongly associated with critical infection and mortality. [20]

The following mechanisms may suggestan association between diabetes and COVID-19 disease severity: i) malfunction of immunity with increased susceptibility to bacterial infection, ii) diminished viral removal and iii) hyper-inflammatory state. [17, 21]Our finding of raised CRP in diabetic patients with the majority having severe to critical COVID-19 infection (92%)is in line with above observations.

Higher values of LDH in diabetic COVID-19 patients can be explained as follows:isozyme LDH 3 present in lung tissue may bereleasedi) after developing a severe form of interstitial pneumonia due to cytokine storm, [22] or ii) due to multiple organ injuries [23] which possibly exacerbated with pre-existing diabetes. Some studies showed no significant difference in LDH levels in the two groups. [17, 18]The non-diabetic group also showed LDH values higher than the normal range which suggests other mechanisms also play role in disease pathology.

While CRP is an acute phase reactant, LDH is a biomarker that indicates the degree of tissue damage.[22] This may suggest a role of CRP as an inflammatory biomarker and LDH as a severity-related marker. [17]

HbA1c values were higher in diabetic patients but it may not predict the severity of the disease. Factors like duration of diabetes, presence of macrovascular complications, hypoglycemic events, associated anemia, etc. may present difficulty in interpreting random blood glucose levels and HbA1c values.[17]

The meta-analysis by Taneri PE et al. showed that SARS-CoV-2 causes hemolysis resulting in decreased hemoglobin and ultimately oxygen transport.[24] In diabetic patients, anemia is a common clinical feature.[25] This supports our finding of lower Hb levels in diabetic COVID-19 patients. Similar findings were seen in the study by Noha M. et al [6].On the other hand, Reem Al Argan et al[4] showed no significant difference in Hb in both groups.

Met-Hb which is a derivative of hemoglobin was also higher in DM COVID-19 patients. Higher Met-Hb levels were seen in COVID-19 patients as compared to healthy individuals.[3]Alunno A et al. suggested that activation of macrophages was caused by the SARS-CoV-2 infection generating a large number of inflammatory molecules in COVID-19 patients leading to a cytokine storm.[26]Oxidative stress due to macrophage activation [27] causes increased oxidation of iron in hemoglobin from the ferrous (Fe2+) to the ferric form (Fe3+) i.e., increased methemoglobin formation[28] with a decrease in oxygen transport ultimately leading to tissue hypoxia.[3] So, one of the probable reasons for hypoxia in COVID-19 patients is increased methemoglobin.[3] Glucose is a reducing agent. Ideally, Met-Hb should be low with the increase in glucose as it reduces Met-Hb to Hb. But in diabetic

COVID-19 patients oxidative stress is increased so the net effect of glucose is possibly nullified with an increase in Met-Hb levels.

In acute anemia, there is an increase in nitric oxide (NO) signalling along with an increase in MetHb as a by-product. It is due to the increased NO-based oxidation of Hb to Met-Hb. [29] This can explain an increase in Met-Hb with a decrease in Hb in the diabetic group as shown in our study.

From the positive correlations between Met-Hb and CRP, MET-Hb and LDH and CRP & LDH in both the groups, it demonstrates that irrespective of diabetes Met-Hb is increased along with CRP and LDH. This suggests thatalong with diabetes other mechanisms also play role in COVID-19 disease pathology.

Limitations of the study:

This study evaluated parameters in the blood drawn on the 2nd -3rd day of hospitalization, we did not evaluate the effect of serial levels over the period and how alterations in levels of parameters could forecast the outcomes. The sample size was relatively small and this was a single-center study which maynot show all the characteristics of the patients. There were no type 1 diabetes mellitus and gestational diabetes mellitus patients in our study.

Conclusion

In elderly patients, diabetes is one of the important and independent risk factors for the severity of COVID-19 disease. An increase in Met-Hb in presence of glucose suggests high oxidative stress in COVID-19 disease due to other mechanisms along with diabetes. Although Met-Hb showed a minimal increase it has a definite positive correlation with LDH and CRP. This shows the need for routine monitoring of Met-Hb. Other reducing agents can be added in treating increased Met-Hb in diabetic COVID-19 patients. This may open new options in the treatment of COVID-19 disease with DM and improve outcomes in the future.

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