Research article

A cross sectional study to assess the elevation in glucose regulated protein 78 levels in severe acute respiratory syndrome Coronavirus 2 infected patients and its correlation with metabolic conditions, severity, complications

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ABSTRACT

Introduction and Aim: COVID-19 pandemic has posed an exceptional challenge worldwide. Research encompassing newer diagnostic tests, targeted therapy has become the need of the hour. The objective of this work is to evaluate the levels of Glucose regulated protein 78 (GRP78) an Endoplasmic Reticulum stress (ERS) protein in severe acute respiratory syndrome coronavirus -2 (SARS -CoV-2) infected patients.

Materials and Methods: We carried out a cross-sectional investigation at the hospital, with three distinct groups: SARS-CoV-2-positive patients with metabolic syndrome (Group A), SARS-CoV-2-positive patients without metabolic abnormalities (Group B), and healthy volunteers (Group C). Enzyme linked immunosorbent assay (ELISA) method was used to estimate the serum levels of GRP78.

Results: Our results showed that serum GRP78 levels were elevated in patients with SARS-CoV-2 infections. Furthermore, it could be demonstrated that Group A had considerably higher serum GRP78 levels than Groups B and C suggesting an elevated GRP78 levels in COVID-19 patients with metabolic syndrome These findings highlight the clinical importance of GRP78 in COVID-19 and its utility as a marker for severity of the illness.

Conclusion: This work compares the trend of serum GRP78 levels in SARS-CoV-2 patients with and without metabolic conditions as compared to the control group. This finding encourages more research on GRP78 to aid the development of targeted therapeutic and prophylactic interventions to combat COVID-19 infection.

Keywords: Glucose regulated protein 78; SARS-CoV-2; metabolic syndrome.

INTRODUCTION

In the year 2020, WHO declared a global pandemic of COVID-19. The novel Coronavirus belonging to the group *Betacoronaviridae* was subsequently named as severe acute respiratory syndrome coronavirus -2 (SARS -Cov-2; 1, 2).

Viral protein and host receptor interaction plays a pivotal role in viral entry and infection. Spike proteins of SARS-CoV-2 play a major role in pathogenesis by recognition and binding to the target site in the host cells. This binding protein specifically recognizes Angiotensin converting enzyme -2 (ACE-2; 2).

Studies have demonstrated that in addition to ACE-2, it can also bind to glucose regulated protein 78 (GRP78), a heat shock protein present on cell membranes (3, 4). GRP78 is a chaperone belonging to the Heat Shock Protein 70 (HSP 70) family. It is present ubiquitously in all host cells and plays a central role in cell survival. GRP 78 binds to the hydrophobic areas of unfolded protein and prevents its aggregation. It is also an initial component of signalling cascade that results in unfolded protein response (UPR). Cell surface GRP 78 acts as a receptor and also helps in transmitting signals from the extracellular environment into cells. Endoplasmic reticulum stress (ERS) in various inflammatory and infective conditions leads to an elevation of GRP-78(5,6). Studies have demonstrated the possible role of GRP78 as a receptor and antigen presenting cell in viral infectious diseases (6, 7).

Replication and assembly of coronavirus transmembrane protein occurs in ER, leading to endoplasmic reticulum stress (ERS) and increased expression of GRP78 on cell surface (8). Cellular stress could have an impact on inflammation and cytokine storm syndrome. GRP 78 which is released early in stress conditions could be the initial measurable condition to predict the possibility of hyper inflammation and cytokine storm (9).

Spore coat protein homolog (Cot H) of Rhizopus oryzae binds to GRP 78, viral infection or conditions which can cause an increase in GRP78 could lead to a higher risk of mucormycosis (10). Studies have demonstrated the high levels of GRP78 in metabolic syndrome (11). In our knowledge the studies done till date to correlate the levels of GRP-78 in SARS-CoV-2 infected patients have not taken into consideration the elevation in subjects with metabolic syndrome. Hence, in this study we aim to evaluate the GRP78 levels in SARS-CoV-2 infected patients with and without metabolic syndrome. Our study also aims to compare

the GRP78 levels in mild, moderate and severe infections.

MATERIALS AND METHODS

A cross sectional study was carried out in the teaching hospital after approval from the institutional ethics committee. Ethics approval has been obtained with letter number (DCGI reg. No. EC/NEW/INST/2020/ 741), dated 14/07/2022.

Study population

Based on the metabolic syndrome and SARS-CoV-2 active infection status, the study population was divided into three groups.

Group A

Patients diagnosed positive for SARS-CoV-2 by Real-time PCR in the past 14 days with pre-existing metabolic syndrome as defined by the International Diabetes Federation (IDF) criteria.

Group B

Patients diagnosed positive for SARS-CoV-2 by Real-time PCR in the past 14 days, with no metabolic abnormalities or co-morbid conditions.

Group C

Healthy volunteers who did not suffer from any infection in the past 14 days, who had no acute, sub-acute or chronic diseases. Not on any medications.

Informed consent from patients were taken before sample collection. Paediatric age group and patients with diagnosis of sepsis, renal failure, hepatic failure, autoimmune disorders, pregnancy, malignancy were excluded from the study.

Samples were collected in duplicates for all patients. Haematological and biochemical analysis were carried out to determine the levels of D-dimer, C reactive protein (CRP) and ferritin. Samples stored at 2˚ to 8˚ celsius were processed within 5 days of collection. In the event of delayed testing, aliquoted samples were stored at -20˚ C for a duration of 30 days.

Measurement of GRP78 levels

Samples were processed as mentioned in the instruction manual of the kit [Human Glucose regulated protein 78 ELISA kit, Bioassay Technology Laboratory, Zhejiang, China.] A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis. A best fit curve was plotted on the graph. The standard curve range of the kit is 0.5 – 100 ng/ml and sensitivity is 0.23ng/ml.

Clinical evaluation

Patients were classified into mild, moderate, and severe based on the National Clinical Management protocol for COVID-19 issued by the Government of India, Ministry of Health and Family Welfare (Version 24.05.2021).

Sampling technique

Since a similar type of study does not exist, sample size was assessed based on a closely related study conducted by Sabirli *et al.*, (13). The sample size estimated for the present study was 109.8 approximately equal to 110 patients in each group.

Statistical analysis

The baseline data is represented using percentage. Diagrams and graphs are used to present the data. Quantitative variables are depicted using mean and Standard deviation. The Z test has been used to compare and to test the significant differences in the parameters such as GRP78 between the two groups.

RESULTS

The demographic characteristics of the cases (Group A and Group B) as defined are tabulated in Table 1. In both Group A and Group B, the majority were males (66.2% and 60%) respectively. The mean age of cases in Group A is 62.66 ±11.95 and Group B is 35.71 ±12.405.

Table 1: Demographic characteristics of the cases

Variables	Group A	Group B
Male	66.2%	60%
Female	33.3%	40%
Age (Mean ± SD)	62.66 ±11.95	35.71 ±12.405

Type II diabetes mellitus is the commonly associated metabolic condition with a frequency of 58.33% in cases of Group A followed by ischemic heart disease, hyperlipidaemia and cerebrovascular accident as shown in Table 2.

Table 2: Frequency of comorbid conditions in Group A

Metabolic conditions	Percentage (%)
Type II diabetes mellitus	58.33%
Hyperlipidemia	16.66%
Ischemic heart disease	25%
Cerebrovascular accident	8.33%

The mean of CRP (mg/ml) in Group A is 103.41±68.53 and Group B is 29.21±27.81. The mean Ferritin levels (ng/ml) in Group A is 837.47 ±949.98 and Group B is 209±86.17. The Mean ± SD values of Laboratory indicators are depicted in Table 3.

Table 3: Inflammatory markers in Group A and Group B

Laboratory parameters	Group A (Mean ± SD)	Group B (Mean ± SD)
C Reactive protein (mg/ml)	103.41±68.53	29.21±27.81
Serum Ferritin (ng/ml)	837.47 ±949.98	209±86.17

Based on the clinical and laboratory parameters cases classified into Mild, Moderate and Severe The mean of GRP 78 levels (ng/ml; Fig. 1) in mild, moderate and severe infection is 2.275 ng/ml, 2.45 ng/ml and 7.3 ng/ml respectively.

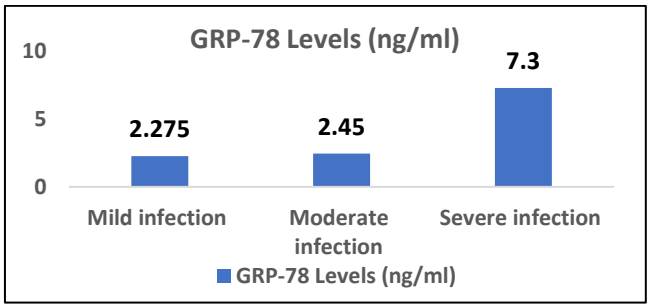

Fig. 1: Association of GRP-78 levels with severity of infection

The median GRP 78 level was 13.250 (Interquartile range 10.598) ng/mL in Group A cases, which was higher than that in Group B cases 4.250 (Interquartile range 2.500) ng/mL ($p < 0.001$) and Group C controls 1.225 (Interquartile range 0.800) ($p < 0.001$) as shown in Table 4.

Table 4: Median and interquartile range of GRP-78 levels in Group A, B, C

Group		**Statistics**
Group A	Median	13.250
	Interquartile Range	10.598
Group B	Median	4.250
	Interquartile Range	2.500
Group C	Median	1.225
	Interquartile Range	.800

Table 5: Test of significance between GRP 78 levels of the Cases (Group A, Group B) and controls (Group C)

Inter comparison	**Mean difference**	**Standard error**	**z**	**P**
Group A vs. Group B	7.497	0.496	8.81	<0.001*
Group A vs. Group C	11.127	0.496	12.13	<0.001*
Group B vs. Group C	3.629	0.496	11.79	<0.001*

Z=Mann – Whitney U test, p<0.001 *(highly significant)

The mean value of GRP 78 levels in Group A is 12.149 ng/ml, Group B is 4.651 ng/ml and Group C is 1.022 ng/ml as depicted in Fig 2.

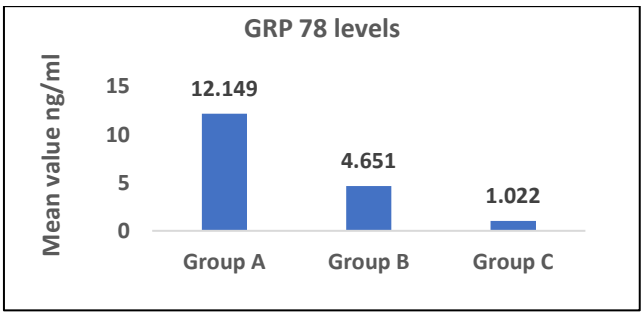

Fig. 2: Mean GRP 78 levels (ng/ml) in the cases (Group A, Group B) and controls (Group C)

DISCUSSION

Newer therapeutic targets against SARS-CoV-2 are the need of hour to tackle and prevent the re-emergence of the pandemic (14). GRP78 levels are elevated in organ dysfunctions, malignancies, autoimmune conditions, and infections. (15-17). ERS leads to increased expression and movement of cytoplasmic GRP78 to the cell surface. Studies have demonstrated that some viral infections use GRP78 as a receptor to enter host cells. SARS-CoV-2 infection tends to upregulate GRP78 expression in hosts (12,8).

The study conducted by Sabirili *et al.*, confirmed that the GRP 78 levels are elevated in SARS-CoV-2 infections, furthermore corroborated the theory that ER stress causes damage to the endothelial barrier in the pulmonary tissue of COVID-19 pneumonia patients (13). GRP78 is one of the binding targets for SARS-CoV-2 spike protein; this ligand-target binding site can be utilized for therapeutic and preventive research (18). Studies have revealed that during COVID-19 infection, there is a proportional increase in the blood levels of GRP78 protein and SARS-CoV-2 mRNA levels. In a similar vein, Palmeira et al. demonstrated a significantly higher level of GRP78 in patients with SARS-CoV-2 pneumonia as compared to the control group (19,20).

In the present study, we could demonstrate that GRP78 levels were significantly higher in SARS-CoV-2 positive patients with preexisting or newly diagnosed metabolic syndrome as compared to SARS-CoV-2 infected patients with no metabolic syndrome and control group. GRP78 levels were elevated in severe COVID-19 infections when compared to moderate and mild infections. It can be inferred that elevation in GRP78 levels is directly proportional to metabolic syndrome and the severity of infection. SARS-Cov-2 patients with no metabolic syndrome had an elevated GRP78 level as compared to the control group. Our research demonstrates that determining the serum GRP78 levels could help in identifying and evaluating patients at a higher risk for severe COVID-19 infection. This is in concordance with studies that emphasize the role of GRP78 as a key marker in Coronavirus infection and the need to explore the possibilities of this chaperone protein in therapeutic and preventive interventions (21,22).

Limitation of the study

This study did not take into consideration the duration of onset of symptoms and the vaccination status of the cases. The analysis of these parameters would help to understand the variation of GRP78 levels at different stages of infection and establish its role in breakthrough infections.

CONCLUSION

This work compares the trend of serum GRP78 levels in SARS-CoV-2 patients with and without metabolic

syndrome as compared with the control group. It sheds further light on the steady increase in GRP78 levels associated with mild, moderate, and severe COVID-19 infections. This finding encourages more research on GRP78 to utilize its potential in diagnosis and therapy.

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CONFLICT OF INTEREST

None declared.

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