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Pattern of serum protein capillary electrophoretogram in SARS-CoV-2 infection

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ABSTRACT

Background and aims: Monoclonal/biclonalgammopathy of unknown significance (MGUS/BGUS) is observed in COVID-19. This study was conducted to determine the changes in serum protein electrophoresis (SPEP) in COVID-19.

Materials and methods: In this descriptive (cross-sectional) study, serum inflammatory markers (CRP, IL-6 and ferritin) were measured and SPEP was carried out by capillary electrophoresis method in 35 controls and 30 moderate & 58 severe COVID-19 cases.

Results: Serum inflammatory markers were increased in COVID-19 cases with severity. M−band(s), β-γ bridging and pre-albumin band(s) on SPEP were observed in 15.5, 11 & 12% of severe cases and 3, 4 & 0% moderate COVID-19 cases respectively. Area under curve (AUC) of α 1 and α 2 bands of SPEP increased significantly in severe COVID-19.

Conclusions: We conclude that SPEP changes like the appearance of M−band(s) indicating MGUS(BGUS), β- γ bridging indicating the presence of fast-moving immunoglobulins, pre-albumin band indicating the rise in serum transthyretin level and the increase in AUC of α 1 and α 2 bands indicating the rise in positive acute phase reactants occur in COVID-19. The occurrence and magnitude of these changes are higher in severe COVID-19 than that in moderate COVID-19. The diagnostic and prognostic significance of these SPEP changes are worth exploring.

1. Introduction

Corona Virus Disease-19 (COVID-19) is caused by SARS-CoV-2 that originated from Wuhan, China. It caused a global pandemic [1]. The majority (80–90%) of SARS-CoV-2 infected subjects have mild disease with fever, sore throat, head & body ache, cough, diarrhea, anosmia and ageusia [2,3]. A few (5–10%) develop the moderate disease. As per the Ministry of Health and Family Welfare (MOHFW), Government of India classification [4], they also develop pneumonia associated with the respiratory rate (RR) between 24 and 29/min &/or SpO2 between 90 and 93%. Less than 5% of SARS-CoV-2 infected subjects develop the severe disease and have RR greater than 29/min &/or SpO2 level < 90%. Laboratory monitoring of COVID-19 cases is commonly done by assessing various markers (e.g., C-reactive protein, ferritin, procalcitonin, total leukocyte count, neutrophil lymphocyte ratio, proBNP, D-dimer, prothrombin time, activated partial thromboplastin time, IL-6, TNF- α etc.) [5]. The treatment is mainly symptomatic with antipyretics, antihistaminics, multivitamins and prophylactic antibiotics [6,7]. The major game-changers in decreasing COVID-19 related morbidity and mortality are the therapeutic use of steroids and low molecular weight heparin (LMH) [6,8].

Serum protein electrophoresis (SPEP) resolves serum proteins into

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six bands [9]. The albumin band is constituted by albumin and retinol-binding protein. Sometimes, a pre-albumin band is observed and it is produced by transthyretin, a protein that has only 2 days half-life in human blood vascular system. α 1 band is made up of α1-antitrypsin, α1-acid-glycoprotein and α-fetoprotein. Hepatoglobulin, α2-macroglobulin and ceruloplasmin produce α2 band. β1 band has transferrin, low-density lipoprotein and C4. β2 band is made up of C3 and β2 micro-globulin. γ-band has IgG, IgM, IgA and C-reactive protein (CRP). Resolution of these bands is sharper in capillary electrophoresis (CE) and recent advances in CE instrumentation have made automated analysis of protein content & percentage distribution of each band. Although changes in SPEP are observed in many diseases, its diagnostic use is restricted to multiple myeloma and other gammopathies [9]. Some of the serum proteins are acute phase reactants (APRs) and are altered in inflammatory conditions.

COVID-19 causes inflammation and alters serum levels of APRs [10]. Monoclonal gammopathy of unknown significance (MGUS) and biclonalgammopathy of unknown significance (BGUS) are also reported in COVID-19 [11–14]. However, the changes in SPEP in COVID-19 disease have not yet been well characterized and their occurrences in COVID-19 cases have not yet been explored.

2. Materials and methods

The study was conducted between August 2020 to December 2020 in the Department of Biochemistry and Multidisciplinary Research Unit in collaboration with the Department of Medicine and Department of Anaesthesia, Maulana Azad Medical College (MAMC) and LN Hospital, New Delhi. LN Hospital acted as a dedicated COVID hospital during this period.

The study design was descriptive (cross-sectional) employing a convenient sampling method from the COVID-19 cases and healthy controls. Ethical approval (F.1/IEC/MAMC/(79/07/2020/No198)) and subsequent written informed consent from all controls and patients were obtained. For the patients who were on (invasive) ventilator and unable to consent by themselves, consent was obtained from patient's relative (legally authorized person). Controls (n = 35) were adult healthy volunteers (age between 34 and 64 years) of either sex. They were recruited from staff of MAMC and LN Hospital after obtaining written consent. Cases were the adult patients (age between 21 and 85 years) of either sex with moderate (n = 30) and severe (n = 58) COVID-19 who required hospitalization for the treatment to medicine ward and ICU respectively with confirmed RT-PCR report. This severity classification was based on MOH&FW, India criteria as detailed in the introduction section [4]. The cases were recruited within three weeks of onset of symptoms. The moderate cases were on oxygen therapy through nasal cannula or normal mask and the severe cases through non-rebreathing musk or were on non-invasive or invasive ventilator. The cases who reported within seven days of onset of symptoms received Ramdesivir. All the patients received low molecular weight heparin, steroid, antibiotics and other symptomatic therapy as per LN Hospital's protocol. Subjects with known hematological (including plasma cell dyscrasias and MGUS) and other cancers, alcoholic liver disease, viral hepatitis, chronic kidney disease and protein-energy malnutrition were excluded. The patient who received convalescent plasma therapy or tocilizumab/etulizumab were also excluded.

Available clinical data and investigation reports were noted from the case sheet on the day of sample collection. Four milliliters of venous blood was collected in red-capped vial from all the study subjects. Serum was analyzed immediately.

2.1. Sample analysis

1) Serum total protein, albumin and CRP were estimated by Biuret, BCG and immunoturbidometric method respectively by using Ortho Clinical Diagnostic (OCD) kit adopted to Vitros 5600 (OCD, Markham,Canada).
2) Serum Ferritin and IL-6 were measured by using the kit from Roche (Basel, Switzerland) by electrochemiluminescence-based immunoassay method adopted to Cobas e411 analyzer (Basel, Switzerland).
3) Capillary SPEP was done by using minicapSebia flex peircing (91090 Lisses,France). All the serum that showed M−band(s) were subjected to capillary electrophoresis after immunosubstraction by adding anti-sera directed to heavy chains (γ, μ and ∞) and light chains (λ and κ). Automatic overlay of the SPEP on the electrophoretogram of immunosubstracted sample by above-mentioned equipment revealed the light chain and isotype based on heavy chain of immunoglobulin present in the M−band(s).

2.2. Statistical analysis

Qualitative data were presented as a percentage. Normal distribution of quantitative data was checked by Kolmogorov–Smirnov test. Parametric data were presented as mean & standard deviation and non-parametric data as median & interquartile range (IQR)/range. Comparison of parametric data was done by one-way ANOVA followed by post-hoc Tukey's HSD test. and non-parametric data by Mann Whitney *U* test (for comparison of two groups) or by Mann Whitney *U* test with Bonferroni correction following Kruskal-Wallis test (for comparison of more than two groups). P-value<0.05 was considered significant for all statistical tests.

3. Results

Total thirty five controls (M:F = 19:16), 30 moderate COVID-19 (M:F = 20:10) and 58 severe COVID- 19 (M:F = 38:20) had age 50.77 ± 7.98, 53.53 ± 14.92 and 59.41 ± 12.9 years respectively. Age of severe COVID-19 group was not statistically different from that of moderate group but was higher ($p < 0.05$) than that of control group. Study was conducted before the COVID-19 vaccines were made available in India. So none of the study population was vaccinated. Seventeen (56.6%) moderate and 51 (87.9%) severe COVID-19 cases had serum CRP level above 5 mg/L i.e., its upper limit of reference range (URR). Fourteen (46.6%) moderate and 42 (72%) severe COVID-19 cases had serum ferritin levels above its URR (RR for male: 30–400 ng/ml and for female: 13–150 ng/ml). Twenty-one (70%) moderate and 54 (93%) severe patients had serum IL-6 levels higher than its URR (i.e., 7 pg/ml). The median of serum CRP, ferritin and IL-6 levels were significantly ($p < 0.05$) higher in severe cases [Median and (IQR) of CRP in mg/L: 118.85 (38.87–270.58); Median and (IQR) of ferritin in ng/ml: 601.9 (281.9–110.9); Median and (IQR) of IL-6 in pg/ml: 201.7 (82.94–378)] in comparison to moderate COVID-19 patients [Median and (IQR) of CRP in mg/L: 12.51 (1.67–36.05); Median and (IQR) of ferritin in ng/ml: 319.6 (148.2–496.5); Median and (IQR) of IL-6 in pg/ml: 13.55 (6.4–318.57)] (Table 1). The treatment received by the patients was as shown in the same table.

As shown in Table 2, the pre-albumin band was present in 7 (12.07%) severe COVID-19 cases and one of them had duplicate pre-albumin bands. The pre-albumin band was not found in any controls and moderate cases. The amount of proteins in the albumin band was significantly lower and that in the α1 band was significantly higher in severe COVID-19 cases than those of controls and moderate cases. The amount of proteins in the α1 band was significantly higher but that in the β1 band was significantly lower in severe cases than those of controls. In the moderate group, 9 (30%) had<3 g/dl (i.e., less than lower reference range or LRR) proteins in the albumin band, 5 (16.7%) had more than 0.4 g/dl (i.e., more than URR) proteins in α1 band, 5 (16.7%) had more than 0.9 g/dl (i.e., more than URR) proteins in α2 band, 6 (20%) had<0.3 g/dl (i.e., less than LRR) proteins in β1 band, 2 (6%) had more than 0.5 g/dl (i.e., more than URR) proteins in β2 band and 5 (16.6%) had more than 1.4 g/dl (i.e., more than URR) proteins in γ -band. In

Table 1
Age, gender distribution, vaccination status, level of pro-inflammatory markers and treatment profile of the study subjects.

S. No.	Parameters		Controls (n = 35)	Moderate COVID-19 (n = 30)	Severe COVID-19 (n = 58)
1.	Median (range) of age (Years)		52 (34–64)	57 (21–83)	61 (24–85)[#]
2.	Gander distributions[Ψ]	Male	19	20	38
		Female	16	10	20
3.	Vaccine received		None	None	None
4.	Median (interquartile range) of pro-inflammatory markers level	CRP (0–5 mg/L)	–	12.51 (1.67–36.05)	118.85[*] (38.87–270.58)
		IL-6 (<7pg/ml)	–	319.6 (148.2–496.5)	601.9[*] (281.9–110.9)
		Ferritins (30–400 ng/ml (male) 13–150 ng/ml (female))	–	13.55 (6.4–318.57)	201.7[*] (82.94–378)
5.	Treatment received	Paracetamol	None	Everyone	Everyone
		Antibiotics	None	Everyone	Everyone
		Others e.g., Vit D, E, Zinc, B-Complex.	None	Everyone	Everyone
		Remdesivir	None	13	28
		Steroid Low dose (<60 Mg /day of Methyl prednisolone or < 12 mg of Dexamethasone)	None	28	05
		High dose (greater than60 Mg /day of Methyl prednisolone or greater than 12 mg of Dexamethasone)	None	02	53
		Low molecular weight Heparin	None	Everyone	Everyone

[*] $p < 0.05$ in comparison to moderate COVID-19 group by Mann-Whiteney *U* test.
[#] $p < 0.05$ in comparison to controls by Kruskal-Wallis test followed by Bonferroni corrected Mann-Whiteney *U* test.
[Ψ] Gender distribution of three groups was not significantly different by Chi-square test.

Table 2
Occurrence of Pre-albumin band(s), β-γ bridging & M−band(s) and the amount of protein present in different bands of serum protein electrophoretogram of control subjects, moderate COVID-19 and severe COVID-19 cases.

S. No.	Parameters (Reference range)	Control Subjects (n = 35)	Moderate COVID- 19 cases (n = 30)	Severe COVID-19 cases (n = 58)
1.	Occurrence of Pre-albumin	0	0	7 (12.07%)
2.	Albumin (gm/dl) (4.0–4.8gm/dl)	3.79 ± 0.57	3.30 ± 0.88	2.78 ± 0.72[*#]
3.	α1 band (gm/dl) (0.2–0.4gm/dl)	0.4 (0.3–0.45)	0.3(0.3–0.4)	0.5(0.4–0.6)[Ψ♣]
4.	α2 band (gm/dl) (0.5–0.9gm/dl)	0.77 ± 0.19	0.74 ± 0.30	0.89 ± 0.32[*]
5.	β1 band (gm/dl) (0.3–0.5gm/dl)	0.43 ± 0.14	0.37 ± 0.13	0.32 ± 0.10[*]
6.	β2 band (gm/dl) (0.2–0.5gm/dl)	0.3(0.3–0.4)	0.3(0.2–0.4)	0.3(0.2–0.4)
7.	Occurrenceof β-γ bridge	0	4 (13.3%)	11 (19%)
8.	γ band (gm/dl) (0.8–1.4gm/dl)	1.31 ± 0.43	1.08 ± 0.43	1.10 ± 0.40
9.	Occurrence of M−band i.e., MGUS & BGUS	0	1 (3.3%) MGUS	9 (15.5%) [7 MGUS & 2 BGUS]
10.	Total Protein (gm/dl) (6.4–8.3gm/dl)	7.08 ± 0.99	6.17 ± 1.51[*]	5.94 ± 1.25[*]
11.	Albumin : Globulin	1.20 ± 0.27	1.21 ± 0.40	0.94 ± 0.33[*#]

Occurrence of a new band or a pattern is presented as number (%), parametric data as mean ± SD and non-parametric data as median (interquartile range).
[*] $p < 0.05$ in comparison to control subjects and
[#] $p < 0.05$ in comparison to moderate COVID-19 cases by one-way ANOVA followed by Tukey's Post-hoc test.
[Ψ] $p < 0.016$ in comparison to control subjects and
[♣] $p < 0.016$ in comparison to moderate COVID-19 cases by Mann-Whitney *U* test with Bonferroni correction following Kruskal-Wallis test. MGUS = Monoclonal gammopathy of unknown significance and BGUS = Biclonalgammopathy of unknown significance.

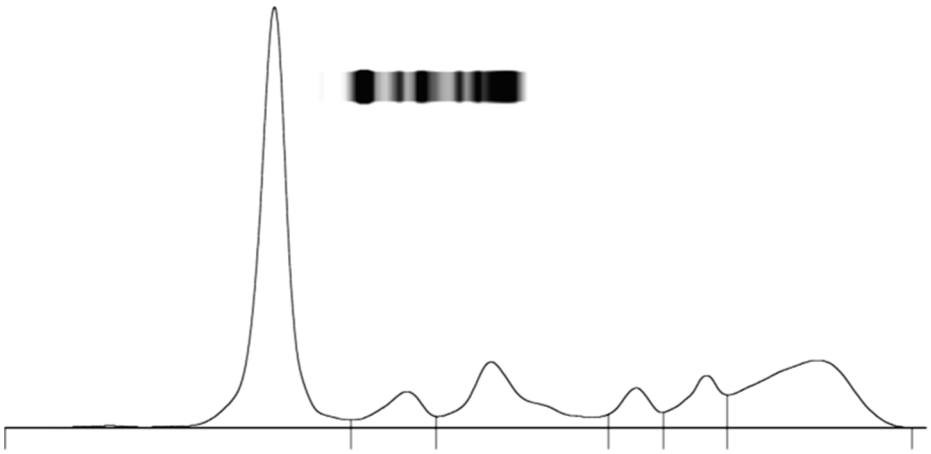
severe COVID-19 group, the above- mentioned distributions were 35 (60.3%), 34 (58.6%), 22 (37.9%), 13 (22.4%), 2 (3.5%) and5 (8.6%) respectively. β-γ bridging was observed in 11 (19%) of severe and 4 (13.3%) of moderate COVID-19 cases. Nine (15.5%) of severe cases and one (3.3%) of moderate cases had monoclonal or biclonal M−band(s) indicating the presence of MGUS or BGUS. Two severe cases but none of the moderate cases showed BGUS. Immunosubstraction revealed that Isotype and light chain of immunoglobulin in M−band of single MGUS in moderate COVID group were IgA and kappa respectively. In severe group, those of five MGUS were IgG and kappa respectively and those of one MGUS were IgG and lambda respectively. One of the BGUS had IgG and IgM *iso*-types with lambda and kappa light chain respectively. Both the M−bands of the other BGUS were constituted by IgG, one had kappa light chain and the other lambda. Total serum protein level was decreased in moderate and severe cases but albumin and albumin to globulin (A:G) ratio was found to be decreased in severe cases only.

Fig. 1 shows the SPEP of a moderate COVID-19 case who had decreased AUC of albumin band and increased AUC of α2 and γ bands. Fig. 2A shows the electrophoretogram of a severe COVID- 19 patient who had a pre-albumin band, low albumin band, increased α 1, α 2 & γ bands and a β- γ bridging. Fig. 2B shows SPEP of another severe COVID-19 case showing duplicate pre-albumin bands, low albumin and one M−band (at β 1 region). Fig. 2C shows SPEP of another severe case showing pre-albumin band, low albumin, decreased β 1, M−band (MGUS) and a β - γ bridging and Fig. 2D shows SPEP of an another severe COVID-19 who had pre-albumin band, low albumin, increased α 1 & α 2 bands, two M−bands (BGUS) and a β - γ bridging.

Out of nine patients with M−band in severe COVID-19 group, two had age less than 60 and seven patients had age above 60. Median and (IQR) of age and IL-6 levels of the patients with severe COVID-19 having M−band [Age in yrs: 65, (45–70); IL-6 in pg/ml: 189, (157.1–417.3)] was not significantly different from that of rest of the patient in that group [Age: 64, (54–70); IL-6: 209.5, (50.5–367.1)] respectively.

4. Discussion

Age and gender distribution of moderate and severe COVID-19



Fractions	%		Ref. %	Conc.	Ref. Conc.
Albumin	45.7	<	55.8 - 66.1	3.2	4.0 - 4.8
Alpha 1	5.6	>	2.9 - 4.9	0.4	0.2 - 0.4
Alpha 2	14.5	>	7.1 - 11.8	1.0	0.5 - 0.9
Beta 1	4.6	<	4.7 - 7.2	0.3	0.3 - 0.5
Beta 2	6.8	>	3.2 - 6.5	0.5	0.2 - 0.5
Gamma	22.8	>	11.1 - 18.8	1.6	0.8 - 1.4
A/G Ratio:	0.84			T. P.: 7.1	g/dL

Fig. 1. Serum protein electrophoretogram of a moderate COVID-19 patient showing increased protein content of α 2 & γ bands and decreased protein content in albumin band. > indicates that the protein content of the band in electrophoresis is greater than its upper reference limit and < indicates that the protein content of the band in electrophoresis is lesser than its lower reference limit.

groups were not statistically different. So, the difference in the results between these two groups is not influenced by age and gender bias. The purpose of controls was to verify the reference range of the parameters. We surmise that the age difference of controls might not affect the interpretations of our test results. None of the study subjects had any COVID-19 vaccines. So the changes in the SPEP observed in the study are not due to COVID-19 vaccine(s). The pro-inflammatory markers in serum were raised in moderate and severe COVID-19 cases. Their levels were higher in severe COVID-19 in comparison to those in moderate cases (Table 1). This indicates that inflammation prevails in COVID-19 and the degree of inflammation is dependent on the severity of COVID-19 [15,16].

As shown in Table 2, 12.07 % of severe COVID-19 patients but none from controls or moderate cases had the pre-albumin band in their electrophoretegram (Fig. 2A, B, C and D). The transthyretin level that constitutes the pre-albumin band in SPEP is increased on providing nutritional therapy to malnourished children and used for monitoring therapy in malnutrition [17,18].

None of the recruited controls or patients had any sign of malnutrition. Transthyretin being a negative acute phase reactant [10] is expected to be decreased in COVID-19 as it imparts an inflammatory state [19]. But it was increased in the present study. Hepatocyte nuclear factors (especially, HNF-4) and glucocorticoids increase the expression of the transthyretin gene [20,21]. HNF-4 measurement from liver tissue was not possible in these severely sick patients receiving LMH therapy. But these cases were receiving methyl-prednisolone (60 mg twice daily through intravenous route). We presume that steroid therapy may be responsible for high expression of the transthyretin gene and thereby, the appearance of the pre-albumin band in SPEP. However, all severe COVID-19 cases receiving similar doses of methyl-prednisolone did not develop the pre-albumin band indicating an individual variability. One of the severe COVID-19 patients had a duplicate pre-albumin band (Fig. 2B) that has been reported in renal and other clinical conditions [22]. But its cause and implications are not yet clearly defined.

The amount of protein in the albumin band in EPSP decreased gradually from moderate to severe COVID-19 (Fig. 1 and Fig. 2) and this decrease was statistically significant in severe COVID-19 (Table 2). Nearly 60% of severe but only 30% of moderate COVID-19 cases had this concentration less than 3 g/dl in the albumin band. Albumin is a negative APR [9]. As both moderate and severe COVID-19 patients had raised levels of serum pro-inflammatory markers (Table 1), we hypothesize that inflammation and pro-inflammatory cytokines might have contributed to hypoalbuminemic condition in COVID-19.

As shown in Table 2, the AUC of α1 and α2 bands were increased and that of the β1 band was decreased significantly in severe COVID-19 cases (Fig. 2) but not in moderate cases. β2 band remained unaltered in both moderate and severe COVID-19. Proteins in the α1 band crossed its upper reference limit in 16.7% of moderate and 58.6% of severe cases. Similarly, for the α2, it was 16.6 % and 37.9 % and for the β2 band, it was 6% and 3.4 % respectively. Proteins in the β1 band were below the LRR in 20% of moderate and 22% of severe cases. Two proteins (α1 antitrypsin and α1 acid glycoprotein) out of three that constitute α1 band and all the three proteins that constitute α2 and β2 bands are positive acute phase reactants. Transferrin that is present in the β1 band is a negative acute-phase reactant. The raised inflammatory markers indicate that the changes in α1, α2 and β1 bands in SPEP are probably the reflections of acute phase response due to inflammation in COVID-19.

AUC of γ -band in SPEP did not differ in moderate or severe COVID-19 cases from that in controls (Table 2). However, 16.6% of moderate cases and 8.6% of severe cases had their protein content in γ-band more than its URR. Polyclonal immunoglobulin level rise is a known phenomenon in the post-viral infection phase [9]. We observed such polyclonal rise in γ -band in a few COVID-19 cases, (Figs. 1 and 2), but not in all, probably because the blood samples were collected in this study at an early stage of the disease. Hence we did not find a change in the AUC of γ -band in most of the COVID-19 cases. One interesting observation in SPEP was that 3.3% of moderate COVID-19 cases and 15.5% of severe COVID-19 cases had M−band (Table 2). Out of these nine severe cases with M−band, seven had one i.e., MGUS (Fig. 2C) and two patients had two M−bands i.e., BGUS (Fig. 2D). These are probably MGUS or BGUS as there was no history that was suggestive of the presence of other gammopathies in these cases before the onset of COVID-19. Our results of

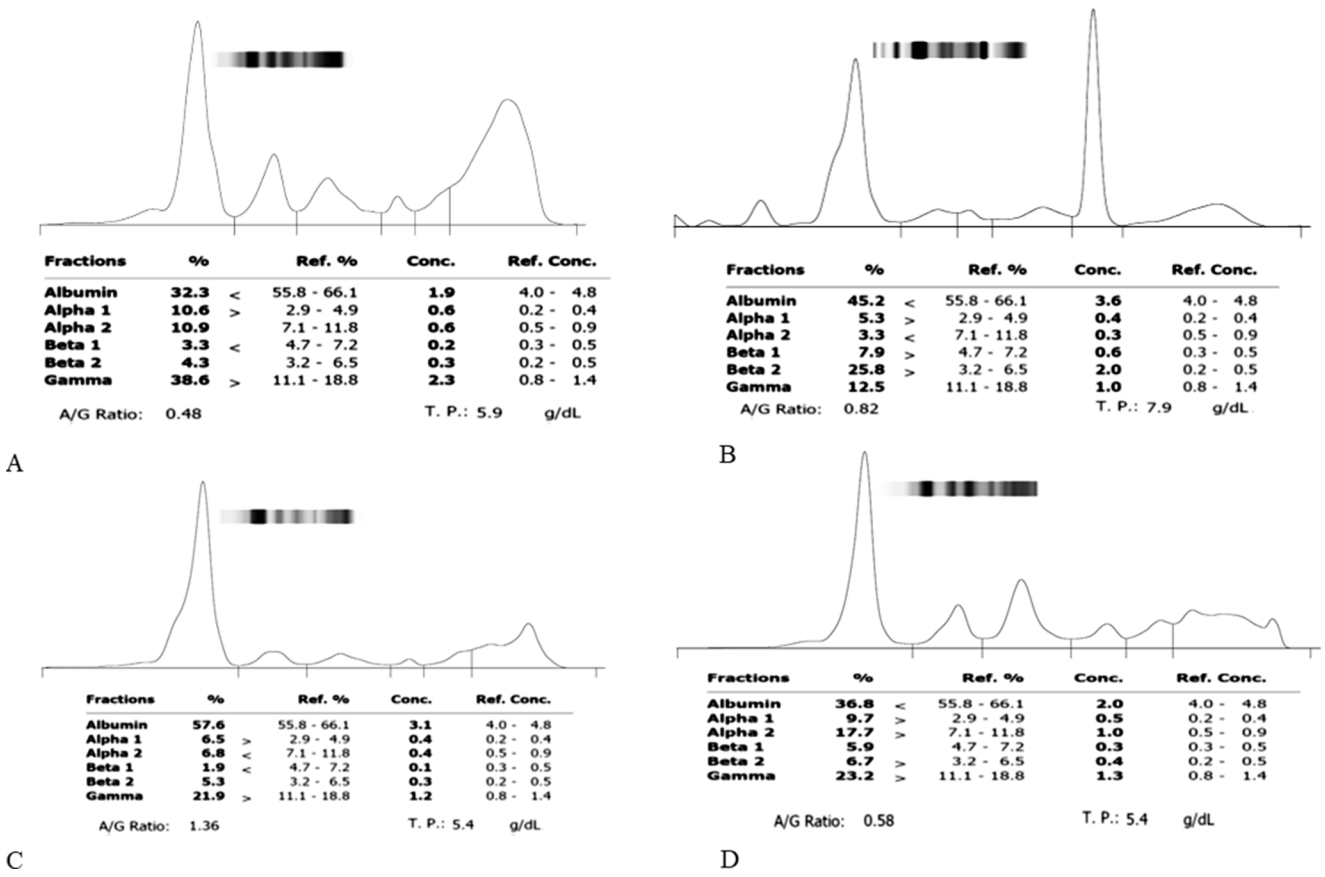


Fig. 2. (A) Serum protein electrophoretogram of a patient with severe COVID-19 showing pre- albumin band, low albumin, increased alpha 1, alpha 2 & gamma band and beta-gamma bridging, (B) Serum protein electrophoretogram of a patient with severe COVID-19 showing duplicate pre- albumin bands, low albumin and one M band (at beta 1 region), (C) Serum protein electrophoretogram of a patient with severe COVID-19 showing pre-albumin band, low albumin, decreased beta 1, M−band (monoclonal gammopathy of unkown significance) and beta-gamma bridging, (D) Serum protein electrophoretogram of a patient with severe COVID-19 showing pre- albumin band, low albumin, increased alpha 1 & alpha 2, two M−bands (biclonalgammopathy of unkown significance) and beta-gamma bridging. > indicates that the protein content of the band in electrophoresis is greater than its upper reference limit and < indicates that the protein content of the band in electrophoresis is lesser than its lower reference limit.

determinations of *iso*-types of Immunoglobulin by Immunosubstraction methods substantiate the presence of M−band(s). Such MGUS in COVID-19 cases is reported by others also (11,12,14) and we had previously reported a BGUS in COVID-19 [13]. As claimed earlier, we surmise that MGUS or BGUS is due to activation of dormant dyscratic plasma cells by COVID-induced rise in IL-6 level that is a known activator of plasma cells [13,23]. Most of the COVID-19 patients with M−band were elderly (greater than 60 years). Although we did not find any statistical difference in age and IL-6 levels of the patients with severe COVID-19 having MGUS/BGUS from that of the rest of the patients in that group, we feel age and IL-6 are their determinants. The chances of developing dormant dyscratic plasma cells increase with age, but only a few develop such dyscratic plasma cells in their body with age. Thus, those elderly patients who had such dormant dyscratic plasma cells, probably, developed MGUS or BGUS. In a study from USA, prevalence of MGUS is found to be 3.2% in persons with age above 50 and 5.3% in persons with age above 70 [24]. Our laboratory record also found 5 (3.7%) MGUS out of 135 SPEP carried out during 2017–2019 from patients with age above 60. Although this is not the right way to determine the prevalence of a condition in a population, we presume it to be a rough estimate of MGUS in this population with some degree of error. Hence, higher age alone probably cannot explain 15% MGUS/BGUS in severe COVID group. We think that 15% prevalence of MGUS/BGUS is very unlikely unless severe COVID plays a role in the pathogenesis of MGUS/BGUS. High IL-6 levels in COVID-19 cases might play a permissive role in the development of the M−band(s). These cases need long follow up to check how many of them develop multiple myeloma in the subsequent periods of their life.

β- γ bridging on SPEP was observed in 13.3% of moderate and 19% of severe COVID-19 cases (Table 2; Fig. 2). The β- γ bridge is observed due to fast-moving immunoglobulins and is considered as pathognomic of alcoholic liver disease (ALD) [9]. The present study reveals that it is not limited to ALD, it prevails in COVID-19 also. Another study also demonstrated β- γ bridging in infectious diseases in animals [25]. The β- γ bridging in SPEP of COVID-19 subjects indicates that some of the antibodies produced in COVID-19 are fast moving immunoglobulins but its significance needs further exploration.

As it was a cross-sectional study, we could not evaluate if mortality can be predicted from the SPEP changes.

We conclude that several changes in SPEP occur in many moderate and severe COVID-19 cases that include appearance of M−band(s), pre-albumin band(s), β- γ bridging, rise in α1 and α2 bands, attenuation of β1 band and polyclonal rise in γ band. Many of these changes depend on severity and a few of these might have potential diagnostic or prognostic significance in COVID-19 which is worth exploring.

CRedit authorship contribution statement

Surbhi Garg: Investigation, Methodology and Writing -original draft. **Vijay Kumar Singh:** Data curation, Formal analysis, Validation. **Subash Chandra Sonkar:** Data curation, Formal analysis, Validation. **Harshit Kelkar:** Investigation, Methodology, Writing -original draft. **Shlesh Singh:** Investigation, Methodology, Writing -original draft.

Sandeep Garg: Conceptualization, Methodology, Project administration, Writting review and editing. **Mona Arya:** Conceptualization, Methodology, Project administration, Writting review and editing. **Farah Husain:** Conceptualization, Methodology, Project administration, Writting review and editing. **Lal Chandra:** Conceptualization, Methodology, Project administration, Writting review and editing. **Anubhuti Chitkara:** Conceptualization, Methodology, Project administration, Writting review and editing. **Tanmaya Talukdar:** Conceptualization, Methodology, Project administration, Writting review and editing. **Binita Goswami:** Conceptualization, Methodology, Project administration, Writting review and editing. **Bidhan Chandra Koner:** Conceptualization, Methodology, Project administration, Writting review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2022.01.003.

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