CORRELATION BETWEEN MONOCYTIC CHEMOTACTIC PROTEIN-1 (MCP-1) AND THE SEVERITY OF COVID-19 INFECTION.

Mahmoud Salah, Lama Al-Safadi and Mariam Fathy

Department of Clinical Pathology, Faculty of Medicine, Ain Shams University
Cairo Egypt.

Corresponding author
Mahmoud Salah Abdel-saber

Mobile: + (2) 0 111 809 4494
E.mail:

ahmed.s.abdelsaber41@gmail.com
Received: 30/8/2022
Accepted: 21/9/2022

Online ISSN: 2735-3540

ABSTRACT:

Background: *The ongoing pandemic of coronavirus disease 2019(COVID-19) poses several challenges to clinicians. Serum chemokine levels such as MCP-1and IL-6 are elevated in patients with COVID-19, and they are even higher in those who required intensive-care unit (ICU) admission, suggesting a relationship between these chemokines and both lung damage and disease severity.*

Aim of the study: *To evaluate the level of monocytic chemotactic protein-1 (MCP-1) in mild, severe and post COVID-19 patients to explore the correlation between (MCP-1) and the severity of the disease. The findings of this study will add to our understanding the role of MCP-1 in diagnosis of COVID-19 patients and the relation between MCP-1 level and the severity of the disease, providing potential diagnostic, prognostic and even therapeutic strategies for COVID-19 patients using MCP-1 as a marker.*

Patients and Methods: *This case control study was conducted as a collaborate work between the Clinical Pathology Department and the Intensive Care Units at Ain Shams University Hospital between March 2021 and November 2021.The study included 87 patients diagnosed as COVID-19 positive by PCR. Patients were subdivided according to the clinical condition into severe group (n =45) (51.8%), mild group(n=22) (25.2%) and convalescent group(n=20) (23%) as well as 60 age and gender matched healthy subjects were recruited as a control group.*

Results: *Our results stated that MCP-1 can differentiate the current cases (either mild or severe) from the convalescent cases as well as the other lab markers (CRP, D-Dimer and ferritin). However, according to our results MCP-1 had even more sensitivity and specificity than other lab markers (CRP, D-Dimer and ferritin).*

Conclusion: *This study added to our understanding the role of MCP-1 in diagnosis of COVID-19 patients and the relation between MCP-1 level and the severity of the disease, providing potential diagnostic, prognostic and therapeutic strategies for COVID-19 patients using MCP-1 as a marker. However, further research is needed about the advantages of MCP-1 over the other known markers in the diagnosis and the prognosis of COVID.*

Keywords: *Monocytic Chemotactic protein-1; COVID-19 Infection.*

INTRODUCTION:

The coronavirus disease 2019 (COVID-19) pandemic has caused a public health crisis with profound long-term socioeconomic fallout. COVID-19 results from infection with the severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) virus [1].

The body's defense mechanism against viral infection involves the innate and adaptive immune responses. However, excessive immune responses after infection, also called the cytokine storm, have been found to be associated with extreme levels of pro-inflammatory cytokines [2].

Evidence suggests that high inflammation rates, oxidation and overwhelming immune response probably contribute to the pathology of COVID-19 infection. The direct cause of death from acute COVID-19 infection might be the damage to lungs and many other organs due to this cytokine storm, resulting in multi-organ failure [3].

Preliminary studies have shown that COVID-19 infection is associated with increasing the levels of a variety of cytokines and chemokines. Serum chemokine levels are elevated in patients with COVID-19, and they are even higher in those who required intensive-care unit (ICU) admission, suggesting a relationship between chemokines and both lung damage and disease severity. It is not clear whether the high expression of chemokines could be used as a marker for the diagnosis and prognosis in COVID-19 [4].

AIM OF THE WORK

This study is intended to evaluate the level of monocytic chemotactic protein-1 (MCP-1) in mild, severe and post COVID-19 patients to explore the correlation between (MCP-1) and the severity of the disease. The findings of this study will add to our understanding the role of MCP-1 in diagnosis of COVID-19 patients and the relation between MCP-1 level and the severity of the disease, providing potential diagnostic, prognostic and even therapeutic strategies for COVID-19 patients using MCP-1 as a marker.

SUBJECTS AND METHODS

This is a case control study that had been conducted on a stratified random sample of 87 patients who were admitted to Ain Shams University Hospital, Cairo, Egypt as COVID patients in the period from March 2021 to November 2021. They were 53 males (60.9%) and 34 females (39.1%) with male: female ratio 1.5:1. Their age ranged from 25 to 83 years with mean (52.52 ± 7.82). Sixty age and sex matched healthy subjects were recruited to the study as a control group.

Patients were included in the study according to the following criteria: COVID-19 positive patients confirmed by PCR. Mild cases were patients who admitted in the In-Patient department, severe cases were patients who admitted to ICU units, and convalescent cases were patients turned negative for COVID-19 by PCR after being positive. While the exclusion criteria were presence of any other auto-immune diseases at the time of sampling, patients who had any of these diseases (Alzheimer's, Parkinsonism, Multiple sclerosis, Epliepsy, Tuberclosis, Rheumatoid artheritis) and patients with flu-like symptoms, suspected COVID and not confirmed by PCR.

According to the clinical condition, patients were subdivided into: **Group 1** mild cases (n = 22) (25.2%). The mild cases were defined as patients admitted in the In-patient department and met all the following criteria: (a) history of exposure to a confirmed SARS-CoV-2 patient,(b) fever or other respiratory symptoms, and (c) typical chest computed tomography image abnormities compatible with viral pneumonia. **Group 2** severe group (n = 45) (51.8 %). The severe group included patients admitted in intensive care units and additionally met at least one of the following conditions: (a) Shortness of breath, respiration rate > 30 times/min, (b) oxygen

saturation (resting state) < 93% or (c) lung infiltrates > 50%[5]. **Group 3** convalescent group (n= 20) (23%). The convalescent group was defined as patients who tested negative for COVID by PCR after being positive for COVID.

All the patients were subjected to the following: Real-time polymerase chain reaction test for COVID-19, C-reactive protein (CRP), D-Dimer and ferritin tests, complete blood count (CBC) and MCP-1 level test by ELISA.

A written informed consent was taken from all the participants enrolled in this study according to the ethical committee regulations of Faculty of Medicine, Ain Shams University **(MS 417/2021).**

Methods:

All the participants were subjected to complete history taking and clinical examination laying stress on signs and symptoms of COVID-19, respiratory rate and oxygen saturation at rest. For each case, a sample of whole venous blood was withdrawn under complete aseptic conditions by the vacutainer. The collected blood was divided into 3 tubes: 2-3 ml. of blood were collected on tri-potassium ethylene diamine tetra-acetic acid (K3 EDTA) vacutainer for performing CBC. 2-3 ml. of blood were drawn once on a citrate tube vacutainer. Plasma was then separated by centrifugation at 2000 rpm for 20 minutes for assessment of D-Dimer. 2-3 ml. of blood were collected into a sterile gel activated vacutainer and was left to clot for 30 minutes in the vacutainer. Serum was then separated by centrifugation at 2000 rpm for 20 minutes for assessment of (MCP-1, CRP and Ferritin). Hemolysed samples were discarded.

MCP-1 level was assayed by ELISA. The principle of the assay employs the quantitative sandwich enzyme immunoassay technique. The plate has been pre-coated with MCP-1 monoclonal antibody. MCP-1 present in the sample is added and binds to antibodies pre-coated on the wells, and then biotinylated anti-human MCP-1 antibody is added and binds to human MCP-1 in the sample. Unbound biotinylated anti human MCP-1 antibody is washed away during a washing step. Then Streptavidin-HRP is added and binds to the biotinylated MCP-1 antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human MCP-1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Statistical analysis:

The collected data was revised, coded, tabulated and introduced to a PC using statistical package for social science (SPSS 25). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also qualitative variables were presented as number and percentages. Then the appropriate statistical analyses were applied. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The comparison between groups with qualitative data was done by using ***Chi-square test***. The comparison between two groups with quantitative data and parametric distribution were done by using ***Independent t-test.*** While the comparison between two groups with quantitative data and non parametric distribution was done by using ***Mann-Whitney test.*** The comparison between more than two groups with quantitative data and parametric distribution were done by using ***One Way ANOVA test***. While the comparison between more than two groups with quantitative data and non parametric distribution was done by using

Kruskall Wallis test. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

RESULTS:

The total group of patients were 87 with age range (25-83) years old and age mean ± SD (52.52 ± 7.82). The studied group of patients subdivided into Severe group n= 45 with age range (40 – 83) years old and age mean (55.11 ± 8.08). Mild group n= 22 with age range (25-60) years old and age mean ± SD (49.55 ± 7.72). Convalescent group n= 20 with age range (42-60) years old and age mean ± SD (49.95 ± 9.87). The total group of patients were 53 (60.9%) males and 34 (39.1%) females with male: female ratio 1.5:1.

Correlation between MCP-1 level as regards the age and gender of all the studied groups:

There was no statistically significant correlation between neither the age nor the gender and the level of MCP-1 in all the studied groups. P values > 0.05 as shown in **(Tables 1 & 2).**

Table (1): Correlation between age and level of MCP-1 among the patients group (sever, mild, convalescent) and the control group:

Group			Mcp1
Control (N=60)	Age (years)	r	-0.175
		p value	0.180
		Sig.	NS
Mild (N=22)	Age (years)	r	-0.089
		p value	0.695
		Sig.	NS
Severe (N=45)	Age (years)	r	-0.237
		p value	0.117
		Sig.	NS
Convalescent (N=20)	Age (years)	r	-0.102
		p value	0.670
		Sig.	NS

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS). r: Correlation coefficient.

Table (2): Comparative analysis between males and females in the cases group regarding MCP-1 level:

	Males (n=52)	Females (n=35)	Mann Whitney test		
	Median (IQR)	Median (IQR)	Test value‡	p value	sig.
Mcp1 (ng/L)	527.5 (72.5 - 600)	327.5 (70 – 397.5)	-0.26498	0.795	NS

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)‡: Mann Whitney test.

Comparison between control and cases groups regarding MCP-1 level:

The patients group showed statistically significant higher values of MCP-1 level with median (IQR) = 240 (70-470) ng/L than the control group whereas the median (IQR) = 30 (30-40) ng/L (P value < 0.001) **(Table 3).**

Table (3): Comparative analysis regarding MCP-1 level between controls and cases:

Mcp1 (ng/L)	Control group	Patients group	Test value‡	P-value	Sig.
	No. = 60	No. = 87			
Median (IQR)	30 (30 – 40)	240 (70 – 470)	-10.264	0.000	HS
Range	10 – 40	40 – 1580			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS) ‡: Mann Whitney test.

By comparing between the three sub-groups of the cases together with the control group as regards the level of MCP-1 there was a high statistically significant increase in values in relation with the severity. Severe sub-group of cases median (IQR) was 450 (360-840) ng/L. Mild sub-group of cases median (IQR) was 130 (80-220) ng/L. Convalescent sub-group of cases median (IQR) was 60 (50-60) ng/L. Control group median (IQR) was 30 (30-40) ng/L. P value = 0.000 **(Table 4)**. As per **(Table 4)**, post hoc analysis test was used to determine the statistical difference between sub-groups versus each other's. Accordingly, there was a high statistically significant difference in the level of MCP-1 between the three sub-groups versus each other's. There was a statistically significant difference between each sub-group versus the control group **(Table 4).**

Table (4): Comparative analysis between the three sub-groups of cases together with the control group regarding MCP-1 level:

Mcp1 (ng/L)	Control group	Mild	Severe	Convalescent	Test value‡	P-value	Sig.
	No. = 60	No. = 22	No. = 45	No. = 20			
Median (IQR)	30 (30 – 40)	130 (80 – 220)	450 (360 – 840)	60 (50 – 60)	130.602	0.000	HS
Range	10 – 40	50 – 240	220 – 1580	40 – 70			

Post Hoc analysis					
Control Vs Mild	Control Vs severe	Control Vs Convalescent	Mild Vs severe	Mild Vs Convalescent	Severe Vs Convalescent
0.000	0.000	< 0.05	0.000	0.000	0.000

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS) ‡: Kruskal Wallis test

Correlation between MCP-1 level in relation to CBC parameters and lab markers in the studied group of cases:

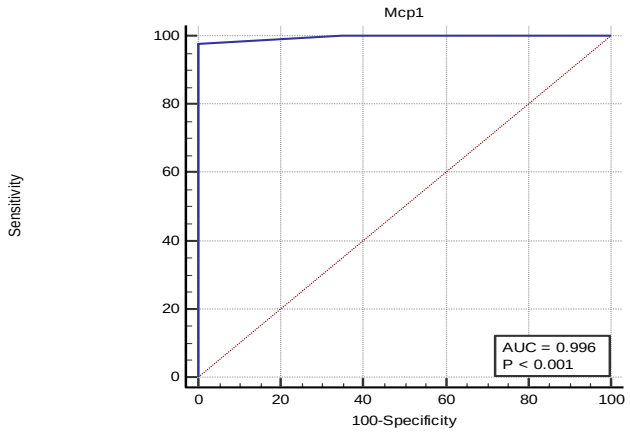
Our results showed statistically high significant negative correlation between lymphocytic absolute count and MCP-1 level (P value = 0.000). More decrease in lymphocytic absolute count (more lymphopenia) is associated with increase in MCP-1 level **(Table 5).** Monocytic absolute count as well showed statistically significant negative correlation in relation to MCP-1 level (P value = 0.012) **(Table 5).** There was high statistically significant negative correlation between both hemoglobin level and platelet count, and MCP-1 level (P value = 0.001, 0.001 respectively). Patients with more degree of Anemia and thrombo-cytopenia are associated with higher levels of MCP-1 **(Table 5).** There was high statistically significant positive correlation between both CRP, D-Dimer and ferritin in relation with MCP-1 level (P value = 0.000 in all of them). More increase in values of CRP, D-Dimer and ferritin is associated with more increase in MCP-1 level **(Table 5).** There was no correlation between both TLC and neutrophilic absolute count, and MCP-1 level (P value= 0.055, 0.188 respectively) **(Table 5).**

Table (5): Correlation between MCP-1 level in relation to CBC parameters and lab markers in the studied cases and controls:

	Mcp1 (ng/L)	
	Patients group (n=87)	
	r	P-value
TLC (x 10^9/L)	-0.142	0.188
Neutrophils (x 10^9/L)	-0.061	0.575
Lymphocytes (x 10^9/L)	-0.383**	0.000
Monocytes (x 10^9/L)	-0.267*	0.012
Hb (g/dL)	-0.357**	0.001
Platelets (x 10^9/L)	-0.340-**	0.001
CRP (mg/L)	0.528**	0.000
D-dimer (mg/L)	0.583**	0.000
Ferritin (ng/ml)	0.372**	0.000

r: Correlation coefficient; *: Significant. **: Highly significant.

In an attempt to determine the best cut-off value to discriminate between COVID-19 patients and healthy controls, a ROC curve was utillized which showed that MCP-1 level at cut-off > 40 ng/L the sensitivity was 97.7%, specificity was 100%, +PV was 100 % and -PV was 96.8 (AUC: 0.996). This makes MCP-1 statistically significant in diagnosis of new COVID cases (P value < 0.001) **(Diagram 1).**

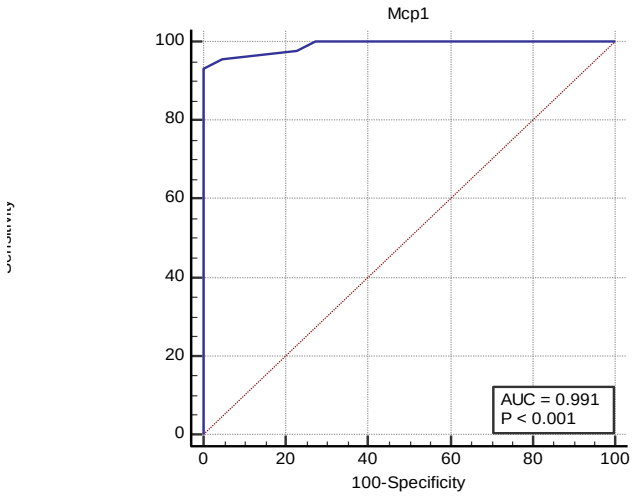


AUC	95% CI	p value	Sig.	Cut off point	Sensitivity	Specificity	+PV	-PV
0.996	0.968 to 1.000	<0.001	S	>40 ng/L	97.7 %	100 %	100 %	96.8 %

ROC Curve = Receiver Operating Characteristic curve. AUC = area under curve.

Diagram (1): ROC curve of MCP-1 to differentiate between COVID Cases and controls.

By utillizing a ROC curve for evaluation of MCP-1 as a predictor of severity, a cut-off > 240 ng/L showed 93.33 % sensitivity, 100% specificity, 100% +PV and 88% -PV (AUC: 0.991). This makes MCP-1 statistically significant in prediction of the prognosis of cases and differentiating between mild and severe cases (P value < 0.001) **(Diagram 2).**

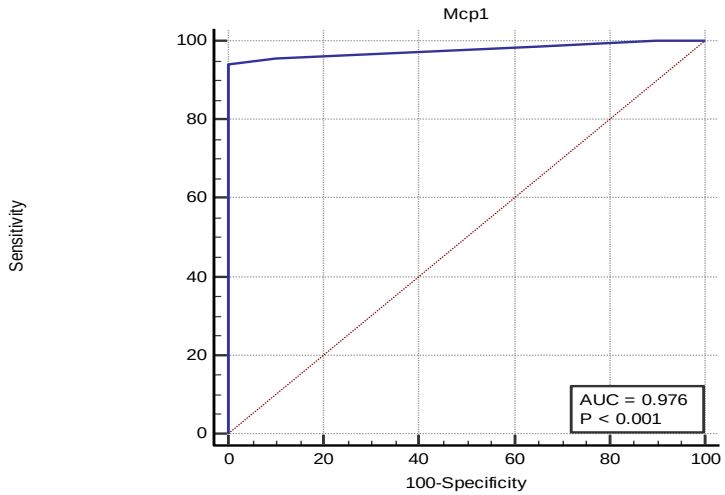


AUC	95% CI	p value	Sig.	Cut off point	Sensitivity	Specificity	+PV	-PV
0.991	0.929 to 1.000	<0.001	S	>240 ng/L	93.33 %	100 %	100 %	88 %

ROC Curve = Receiver Operating Characteristic curve. AUC = area under curve.

Diagram (2): ROC curve of MCP-1 to differentiate between mild and severe sub-groups of cases.

By utilizing a ROC curve between both mild and severe sub-groups, and the convalescent sub-group of cases to evaluate the role of MCP-1 as an indicator for recovery, a cut-off < 70 ng/L showed 94% sensitivity, 100 % specificity, 100 % +PV and 83.3 % -PV (AUC: 0.976). A drop in MCP-1 level to value less than 70 ng/L means the patient is in the way to recovery. This makes MCP-1 statistically significant as an indicator for good prognosis and convalescence (P value < 0.001) **(Diagram 3).**



AUC	95% CI	p value	Sig.	Cut off point	Sensitivity	Specificity	+PV	-PV
0.976	0.919 to 0.997	<0.001	S	< 70 ng/L	94 %	100 %	100 %	83.3 %

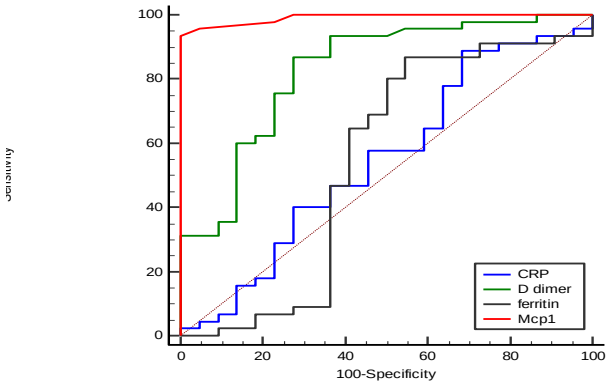
ROC Curve = Receiver Operating Characteristic curve. AUC = area under curve.

Diagram (3): ROC curve of MCP-1 to differentiate between both mild & severe sub-groups from convalescent sub-group of cases.

As per the previous data we can establish the following: Patients with MCP-1 level more than 40 ng/L are COVID cases, but they may be mild, severe or even

convalescent cases. Patients with MCP-1 level more than 70 ng/L are present cases of COVID, additionally those have MCP-1 levels more than 240 ng/L are categorized as severe cases. Patients with MCP-1 values between 40-70 ng/L mostly on the convalescence phase of the disease.

A multi-ROC curve was utilized to compare between the efficacy of both MCP-1, D-Dimer, CRP and ferritin in differentiating between mild and severe cases and find out accordingly their role in predicting the severity of the cases. Ferritin and CRP showed non statistically significant value in differentiation between mild and severe cases (P value = 0.633, 0.543 respectively). Both D-Dimer and MCP-1 were statistically significant in differentiating between mild and severe cases (P value <0.001 in both of them). A cut-off > 0.78 mg/dL in D-Dimer showed 86.67 % sensitivity, 72.73 % specificity (AUC= 0.828), while a cut-off >240 ng/L in MCP-1 showed 93.33% sensitivity and 100% specificity (AUC = 0.991) **(Diagram 4).**



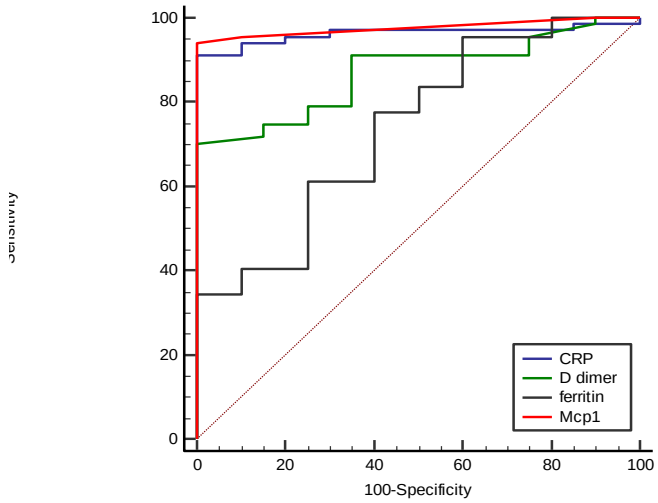
Variable	AUC	95% CI	p value	sig.	Cutoff point	sensitivity	specificity	+PV	-PV
Mcp1 ng/dL	0.991	0.929 to 1.000	<0.001	S	>240 ng/L	93.33%	100%	100%	88%
D-Dimer mg/L	0.828	0.716 to 0.909	<0.001	S	>0.78 mg/L	86.67%	72.73%	86.7%	72.7%
Ferritin ng/ml	0.542	0.416 to 0.665	0.633	NS					
CRP mg/L	0.548	0.422 to 0.670	0.542	NS					

ROC Curve = Receiver Operating Characteristic curve. AUC = area under curve.

Diagram (4): Multi-ROC curve of MCP-1 versus other lab markers (CRP, D-Dimer, Ferritin) to detect severe Cases (differentiate between mild and severe cases).

Another multi-ROC curve was utilized to compare between the efficacy of MCP-1 and other lab markers (CRP, D-Dimer and ferritin) in differentiating between patients (either mild or severe) and the convalescent sub-group. All lab markers together with MCP-1 level showed statistically significant value in differentiating between patients (mild + severe) from convalescent sub-group (P value < 0.001 in all of them). A cut-off > 70 ng/L in MCP-1 showed 94% sensitivity and 100% specificity (AUC= 0.976). A cut-off > 0.74 mg/dL in D-Dimer showed 70.15% sensitivity and 100% specificity (AUC= 0.976). A cut-off > 12 mg/dL in CRP showed 91% sensitivity and 100% specificity (AUC= 0.976). A cut-off > 269.4 ng/dL in ferritin showed 77.61% sensitivity

and 60% specificity (AUC= 0.976) **(Diagram 5).**



Variable	AUC	95% CI	p value	sig.	Cutoff point	sensitivity	specificity	+PV	-PV
Mcp1 ng/dL	0.976	0.919 to 0.997	<0.001	S	>70 ng/L	94.03	100	100	83.3
D-Dimer mg/L	0.874	0.781 to 0.932	<0.001	S	>0.74 mg/L	70.15	100	100	50
CRP mg/L	0.962	0.897 to 0.991	<0.001	S	>12 mg/L	91.04	100	100	76.9
Ferritin ng/ml	0.739	0.634 to 0.827	<0.001	S	>269.4 ng/ml	77.61	60	86.7	44.4

ROC Curve = Receiver Operating Characteristic curve. AUC = area under curve.

Diagram (5): Multi-ROC curve of MCP-1 versus other lab markers (CRP, D-Dimer, Ferritin) to differentiate between patients (mild + severe) from convalescent sub-group.

Summary for ROC curves results about MCP-1: Drop of MCP-1 level in COVID patients to < 70 ng/L and > 40 ng/L is an indication for convalescence. Further drop to < 40 ng/L suggests complete recovery. Values of MCP-1 > 240 ng/L is a sign for poor prognosis.

DISCUSSION

The present case control study was conducted on 87 patients who were admitted to Ain Shams University Hospital, Cairo, Egypt as COVID patients in the period from March 2021 to November 2021. They were 53 males (60.9%) and 34 females (39.1%) with male: female ratio 1.5:1. Their age ranged from 25 to 83 years with mean (52.52 ± 7.82). Cases were matched by age and sex to sixty controls.

It is not clear whether high expression of (MCP-1) could be used as an effective indicator marker for the diagnosis and prognosis of COVID-19. This study aimed to evaluate the role of monocytic chemotactic protein-1 in diagnosis and prognosis of COVID-19 patients.

According to our results there was no statistically significant correlation between neither the age nor the gender of the cases and the level of MCP-1 in this study. On the contrary ***Farghaly and Makboul 2021***[5] in a previous study stated that the age can be considered as a significant risk factor for the severity of COVID-19 in both sexes. However ***Statsenko et al., 2022***[6] said that one of the limitations of the previous studies about age-related features of COVID is the focus on midlife adults and elderly people. Exclusion of younger adults means it is not possible to explore the age-related features of COVID-19 across all age groups.

MCP-1 level was statistically significant higher in our group of cases than

the control group. In agreement with our results ***Guo et al., 2021***[7] assumed that the role of cytokines in the pathogenesis of COVID-19 is the reason. ***Singh et al., 2021***[8] also explained that the cytokine storm which is represented by the increase in MCP-1 level and other cytokines has a crucial role in COVID-19 pathogenesis.

Our results also revealed statistically significant difference in the level of MCP-1 between mild, severe and convalescent sub-groups of cases. Severe cases showed higher levels of MCP-1 than mild cases. Mild cases showed higher levels of MCP-1 than convalescent cases. Our results came in agreement with results of ***Guo et al., 2021***[7] who stated that Serum chemokine levels are elevated in mildly ill patients with COVID-19, and they are even higher in those who required intensive-care unit admission (severe cases), suggesting a relationship between chemokines and both lung damage and disease severity.

However, the results of a study conducted by ***Xi et al., 2021***[9] revealed that mild and severe disease are both associated with increased serum levels of MCP-1, but only severe cases is characterized by increased IL-8 and IP-10.

Interestingly, in this study there were three patients from the sub-group of severe cases turned negative for COVID by PCR and were subjected in the sub-group of convalescent cases, level of MCP-1 was measured during the illness and after the convalescence and it decreased obviously in all of them, This findings strengthen our claim about the prognostic value of MCP-1 in the COVID infection.

On the other hand, ***Chen et al., 2020***[10] stated that MCP-1 could be related to the risk of death in COVID-19 patients. However, he said that since the selected patients for the study were already severe or critically ill, the results did not show any difference between survival and death. The diversity of the selected sample together with the strict follow up of the cases by serial measurments of MCP-1 is required for more emphasis on its prognostic role.

Our work revealed a statistically significant correlation between MCP-1 level and the absolute count of lymphocytes and monocytes in the studied group of cases. Lymphopenia and moncoytosis are seen with high serum levels of MCP-1. In agreement with ***Bhaskar et al., 2020***[11] who stated that high serum levels of cytokines are inversely related to the total lymphocyte count.

Carr et al., 1994[12] also said that MCP-1 is the major lymphocyte chemoattractant secreted by mitogen-stimulated peripheral blood mononuclear cells and is capable of acting as a potent T-lymphocyte, as well as monocyte, chemoattractant.

Our study also elicited a statistically significant negative correlation between MCP-1 level and platelets count in the studied group of cases. High serum levels of MCP-1 are inversely related to the platelets count. ***Alberca et al., 2021***[13] agreed with our results and claimed that platelets are destroyed during the process of production of cytokines and chemokines. He added that recently the platelet count has been proposed as a severity biomarker in COVID-19, and thrombocytopenia at the first day of hospitalization has been proposed as a biomarker for a higher mortality rate.

Moreover, our results found a statistically significant positive correlation between MCP-1 level and both CRP and ferritin levels in the studied group of cases. High serum levels of MCP-1 are associated with high serum levels of both CRP and ferritin. Results by ***Bhaskar et al., 2020***[11] and ***Bozkurt et al., 2021***[14] matched ours, they concluded that cytokines drive an acute phase response that elevates serum ferritin, complement, CRP, and pro-coagulant factors.

MCP-1 level as well was found to have a statistically significant positive correlation with D-Dimer in the studied group of cases. ***Chen et al., 2020***[10] concluded from his study that patients with high D-Dimer have higher levels of MCP-1. Since the increase of MCP-1 level (one of cytokines in cytokine storm) is associated with endothelial cell damage which induce microvasculer thrombosis, vigorous fibrinolysis happens leading to the production of D-Dimer which increase in the blood.

According to our results MCP-1 was significant in detecting new COVID-19 cases, with 97.7% sensitivity and 100% specificity levels more than 40 ng/dL were diagnosed as cases. However, this cut-off point can not state exactly the category of the case either mild, severe or convalescent.

Moreover, our results revealed a privilege of the MCP-1 over the other lab markers (CRP, D-Dimer and ferritin) in detecting severe cases. With sensitivity 93.33 % and specificity 100%, MCP-1 preceded D-Dimer (sensitivity 86.67% & specificity 72.73%) in differentiating the severe cases from the mild cases and predicting bad prognosis of the cases. CRP and ferritin were statistically non significant in detecting the severe cases according to our results.

Our results matched with the results of previous study by ***Idiz et al., 2022***[15] which revealed that MCP-1 and D-Dimer was significantly higher in the more severe group than mild COVID-19 patients but not ferritin. However, his results didn't match ours regarding the CRP. He said that CRP values increased significantly in COVID-19 cases and showed a positive correlation with the severity of the disease.

Our results stated that MCP-1 can differentiate the current cases (either mild or severe) from the convalescent cases as well as the other lab markers (CRP, D-Dimer and ferritin). However, according to our results, MCP-1 had even more sensitivity and specificity than other lab markers (CRP, D-Dimer and ferritin).

The results of previous study by ***Chi et al., 2020***[16] in China matched ours. It showed that the serum level of cytokines and chemokines such as MCP-1 in convalescent cases were significantly lower than those in symptomatic cases.

Of course we can`t establish the prognostic value of MCP-1 unless the level of MCP-1 is serially measured in the patients with follow up to their clinical condition starting from being COVID-19 positive by PCR till the complete recovery. However we still claim –according to our results- that our marker will play a great role in COVID-19 diagnosis, prognosis and even in treatment but further research is still needed.

Ozger et al., 2021[17] conducted a study in Ankara, Turkey on a serial measurments of cytokines in COVID-19 patients. His study results suggested that IL-6, IP-10 and MCP-1 might be used to predict mortality in COVID-19 patients. According to his results, serial sampling of some cytokines seem to be more predictive for the outcome in comparison to a single measurement, as frequently reported even without a definite sampling time. However, since the measurement of cytokines/chemokines is time-consuming and expensive, serial measurements can be made only for certain cytokines such as IL-6 and MCP-1.

The costs of sample collection, diagnosis, prognosis and contact tracing for COVID-19 are high specially in developing countries. The need for minimizing this cost when faced with pandemic diseases is a must. The findings of this study can help in replacing three lab markers (CRP, D-Dimer and ferritin) with only one (MCP-1). The cost effectiveness of this claim have to be investigated especially when MCP-1

showed more sensitivity and specificity than the others, because despite one lab marker versus three lab markers seems to be better, but the MCP-1 is measured by ELISA which is more expensive than commercially available tests such as CRP, D-Dimer and ferritin.

Focusing on the potential lines of treatment, and since the COVID-19 pathogensis is based on the cytokine storm with MCP-1 as one of those cytokines, immunomodulatory therapies targeting cytokine storm show a chance for improving outcomes and reducing mortality due to COVID-19 in patients. Future studies are required for further evaluation of the efficacy of immunomodulatory therapies in preventing cytokine storm induced severe illness in COVID-19 patients.

Bhaskar et al., 2020[11] said that various stages of the cytokine storm pathway can be targeted for therapeutic effects. He suggested that blocking cytokine storm mediators such as IL-6 and MCP-1 is a potential therapeutic strategies.

Alfarouk et al., 2021[18] at the journal of xenobiotics said that MCP-1 is associated with COVID-19 infection and pathogensis. Therefore, he suggested that targeting MCP-1 could also be considered in managing SARS-CoV-2 infection. He said that melatonin inhibits MCP-1 expression. Bindarit (a selective inhibitor of the monocyte chemotactic proteins MCP-1) decreases MCP-1 synthesis. Spiegelmer (L-ribonucleic acid aptamer) inhibits MCP-1 activity. Furthermore, MCP-1 could be blocked at the receptor level (CCR2 blockage) using compounds such as 747 (a natural combination related in structure to kaempferol) and 15a (an orthostatic CCR2, a small molecule antagonist).

Conclusion:

This study added to our understanding the role of MCP-1 in diagnosis of COVID-19 patients and the relation between MCP-1 level and the severity of the disease, providing potential diagnostic, prognostic and therapeutic strategies for COVID-19 patients using MCP-1 as a marker. However, further research is needed about the advantages of MCP-1 over the other known markers in the diagnosis and the prognosis of COVID.

Conflicts of Interest: The authors state that the publishing of this paper is free of any conflicts of interest.

REFERENCES

1. Mehta P, Qing Ye, Bili W (2020): COVID-19: consider cytokine storm syndromes and immunosuppression. The Lancet 395, 1033–1034.
2. Wang D, Yin Y, Hu C (2020): Clinical course and outcome of 107 patients infected with the novel coronavirus, SARS-CoV-2, discharged from two hospitals in Wuhan, China. Critical Care. 24:188.
3. Kavianpour M, Saleh M, Verdi J (2020): The role of mesenchymal stromal cells in immune modulation of COVID-19: focus on cytokine storm. Stem Cell Research & Therapy 11, 404.
4. Huang C, Wang Y, Zhao J (2020): Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet 395, 497–506.
5. Farghaly S, Makboul M (2021): Correlation between age, sex, and severity of Coronavirus disease-19 based on chest computed tomography severity scoring system. Egyptian Journal of Radiology and Nuclear Medicine;52(1):23.
6. Statsenko Y, Al Zahmi F, Habuza T (2022): Impact of Age and Sex on COVID-19 Severity Assessed From Radiologic and Clinical Findings. Frontiers in Cellular and Infection Microbiology 25;11:777070.
7. Guo Y, Xi X, Zhu M (2021): Higher expression of monocyte chemotactic protein 1 in mild COVID-19 patients might be correlated with inhibition of Type I IFN signaling. Virology Journal 18: 12-16.

8. Singh S, Anshita D, Ravichandiran V (2021): MCP-1: Function, regulation, and involvement in disease. International Immunopharmacology. 5(3): 17-23.
9. Xi X, Guo Y, Zhu M, Wei Y, Li G, Du B, Wang Y (2021): Higher expression of monocyte chemotactic protein 1 in mild COVID-19 patients might be correlated with inhibition of Type I IFN signaling. Virology Journal; 18(1):1-7.
10. Chen Y, Wang J, Liu C, Su L (2020): IP-10 and MCP-1 as biomarkers associated with disease severity of COVID-19. Moleculer Medicine, BioMed Central 26:97.
11. Bhaskar S, Sinha A, Banach M, Mittoo S, Weissert R, Kass JS, Rajagopal S, Pai AR and Kutty S (2020): Cytokine Storm in COVID-19—Immunopathological Mechanisms, Clinical Considerations, and Therapeutic Approaches: The REPROGRAM Consortium Position Paper. Frontiers in Immunology 11:1648.
12. Carr MW, Stephen J, Roth S (1994): Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. Proceedings of the National Academy of Sciences of the United States of America,Vol. 91, pp. 3652-3656.
13. Alberca RW (2021): Asthma endotypes and COVID-19. Journal of Asthma.; 58(11):1424-5.
14. Bozkurt FT, Tercan M, Patmano G, Bingol Tanrıverdi T, Demir HA, Yurekli UF., (2021): Can Ferritin Levels Predict the Severity of Illness in Patients With COVID-19?, Cureus. 21;13(1):e12832.
15. Idiz UO, Yurttas TT, Degirmencioglu S (2022): Immunophenotyping of lymphocytes and monocytes and the status of cytokines in the clinical course of Covid-19 patients. Journal of medical virology 20;105-109.
16. Chi Y, Ge Y, Wu B (2020): Serum Cytokine and Chemokine Profile in Relation to the Severity of Coronavirus Disease 2019 in China. Journal of Infectious Diseases. 4;222(5):746-754.
17. Ozger HS, Karakus R, Kuscu EN, Bagriacik UE, Oruklu N (2021): Serial measurement of cytokines strongly predict COVID-19 outcome. PLOS ONE 17(7): e0271141. 2021
18. Alfarouk KO, AlHoufie STS, Ahmed SBM (2021): Pathogenesis and Management of COVID-19. The Journal of Xenobiotics 5(3): 12-19.

العلاقة بين البروتين الكيميائي أحادي الخلية-١ و شدة العدوى بفيروس كورونا المستجد

محمود صلاح عبد الصابر و لما الصفدى و مريم فتحى

قسم الباثولوجيا الإكلينيكية ، كلية الطب ، جامعة عين شمس

للمراسلة محمود صلاح الهاتف : ٠١١١٨٠٩٤٤٩٤ الإيميل :

ahmed.s.abdelsaber41@gmail.com

المقدمة: تشكل جائحة فيروس **كوفيد-١٩** العديد من التحديات أمام الأطباء. أشارت الدراسات إلي أن مستويات كيموكين المصل مثل MCP-1 وIL-6 مرتفعة في المرضى الذين يعانون من الإصابة بفيروس **كوفيد-١٩**، وهي أعلى في أولئك الذين احتاجوا إلى دخول وحدة العناية المركزة (ICU)، مما يشير إلى وجود علاقة بين هذه الكيماويات وكل من تلف الرئة وشدة المرض.

الهدف من الدراسة: تقييم مستوى البروتين الكيميائي أحادي الخلية ١ (MCP-1) في المرضى سواء حالات خفيفة أو شديدة أو تم شفائها من فيروس **كوفيد-١٩** لاستكشاف العلاقة بين البروتين الكيميائي أحادي الخلية-١ (MCP-1) وشدة المرض. ستضيف نتائج هذه الدراسة إلى فهمنا دور البروتين الكيميائي أحادي الخلية-١ (MCP-1) في تشخيص مرضى **كوفيد-١٩**والعلاقة بين مستوى البروتين الكيميائي أحادي الخلية-١ MCP-1 وشدة المرض، مما يوفر استراتيجيات تشخيصية وإنذارية وحتى علاجية محتملة لفيروس كورونا COVID- 19 بإستخدام البروتين الكيميائي أحادي الخلية-١ MCP-1 كإختبار.

المرضى والطرق: أجريت دراسة الحالة هذه كعمل تعاوني بين قسم علم الأمراض الإكلينيكي ووحدات العناية المركزة في مستشفى جامعة عين شمس بين مارس ٢٠٢١ ونوفمبر ٢٠٢١. وشملت الدراسة ٨٧ مريضًا تم تشخيص إصابتهم بفيروس **كوفيد-١٩** بواسطة تفاعل البوليميريز المتسلسل (PCR). تم تقسيم المرضى حسب الحالة السريرية إلى مجموعة شديدة (ن = ٤٥) (٥١,٨٪)، مجموعة خفيفة (ن = ٢٢) (٢٥,٢٪) ومجموعة نقاهة (ن = ٢٠) (٢٣٪) وكذلك ٦٠ شخص سليم متماثلين في العمر والجنس مع المرضي كمجموعة ضابطة.

النتائج: أشارت نتائجنا إلى أن مستوى البروتين الكيميائي أحادي الخلية ١ (MCP-1) يمكن أن يفرق بين الحالات الحالية (سواء كانت خفيفة أو شديدة) من حالات النقاهة وكذلك علامات المختبر الأخرى (CRP وD-Dimer وferritin). ومع ذلك، وفقًا لنتائجنا، كان لدى البروتين الكيميائي أحادي الخلية ١ MCP-1 حساسية وخصوصية أكثر من علامات المعمل الأخرى (CRP وD-Dimer وferritin).

الخلاصة: أضافت هذه الدراسة إلى فهمنا لدور البروتين الكيميائي أحادي الخلية-١ MCP-1 في تشخيص مرضى كورونا COVID-19 والعلاقة بين مستوى البروتين الكيميائي أحادي الخلية-١ MCP-1 وشدة المرض، مما يوفر استراتيجيات تشخيصية وإنذارية وعلاجية محتملة لمرضى COVID-19 باستخدام البروتين الكيميائي أحادي الخلية-١ MCP-1 كإختبار. ومع ذلك، هناك حاجة إلى مزيد من البحث حول مزايا البروتين الكيميائي أحادي الخلية-١ MCP-1 على العلامات الأخرى المعروفة في تشخيص كورونا COVID والتنبؤ به.