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Analysis of GM-CSF in SARS-CoV-2 patients in Hilla City

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***Abstract*---**Objectives**:** 1.Using COVID-19, examine the demographic data (obese and smoking) on Pentian. 2. To determine the serm level of GM-CSF. Methods: In this study, 170 patients with COVID-19 and AHC were included .The value of the GM-CSF and demographic data was collected and their correlation with disease severity was analyzed. Results: the Patients group contained 36 (51.4%) patients with COVID-19, 19 of whom were obese (BMI 30 kg/m2), and 34 (48.6%) were non-obese. Only 27 (27.0%) of the participants in the control group were fat, while 73 (73.0%) of the participants in the COVID-19 group were not obese. In terms of smoking status, the current study found that 28 (40.0 percent) of the patients in the COVID-19 group were smokers, whereas 42 (60.0 percent) were non-smokers. Only 13 (13.0%) of the participants in the control group were smokers, with 87 (87.0%) having no COVID-19. the case (with COVID19) group had significantly greater levels of (IL33) than the control group.

***Keywords*---**GM-CSF, COVID-19, SARS-CoV-2 patients.

Introduction

The family of viruses that are particularly prevalent in mammals includes the coronavirus. There have been reports of seven different coronaviruses, including the new SARS CoV-2, coronaviruses 229E and NL63, OC43, HKU1, MERS, and

SARS (Berger, 2020). The most prevalent method of transmission is close proximity contact (such as 15 minutes face-to-face and within 2 meters), and the most efficient environments for spread are homes and social groups like family and friends (Cevik et al., 2020).This virus harbours pleomorphic RNA or spike proteins (S-proteins) peplomers of 80–160 nM in size with 27–32 kb positive polarity (Sahin, *et al.*,2020). The most prominent feature of Coronaviruses is the club-shaped spike projections emanating from the surface of the virion. The coronavirus got its name because of these spikes, which are specific to the virion and resemble a solar corona. Spike protein (S), membrane protein (M), 16s nucleo-capsid protein (N), and envelope protein (E), which together make up the key structural components of these viruses, are all encoded at the 30 end of the viral genome (Mousavizadeh and Ghasemi,2021). Particles smaller than 5 m can be produced during some clinical procedures involving the upper airway, such as taking a nose or throat sample, endotracheal intubation, manual breathing, or nebulization, allowing for airborne transmission in medical facilities (WHO, 2020). Intensive care units (ICUs) in particular have been linked to an increased risk of infection (Guo et al, 2020).In addition to other pro-inflammatory cytokines and chemokines, GM-CSF is thought to be a major initiator of lung inflammation in severe and critical COVID-19 pneumonia (Mehta et al.,2020; Zhou et al.,2020). The complicated functions of GM-CSF in lung homeostasis and inflammation in COVID-19 illness. Alveolar macrophage development, function, and surfactant metabolism are all maintained by GM-CSF in a healthy lung (Lang et al., 2020).

Materials and Methods

Group research

The case group consisted of 70 patients with covid-19 who were all admitted to the ICU and identified by a specialist physician with extreme acute respiratory syndrome due to covid-19 documented by RT-PCR along with other clinical and laboratory criteria in Marjan Teaching Hospital in Babylon province. The study's control group included of 100 seemingly healthy people who were all found to be free of covid-19 using the Rapid Test (covid-19 antibody test negative).

Collection of Specimens

All patients' blood specimens and AHC 3ml venous blood were collected aseptically using gel tubes and EDTA tubes for gating blood serum at Babylon University's Virology Research Unit, and then refrigerated at (-20°C). The remaining 3ml of blood was kept in the jelly tube without anticoagulant and allowed to coagulate at 37°C for up to 1 hour on the laboratory table. The serum was centrifuged at 2500 rpm for 15 minutes before being collected and stored at -20°C until usage to prevent loss of bioactive human GM-CSF.

Research methodology

All participants were asked to accept to participate in the study after a detailed explanation of the study's objectives and techniques. A detailed questionnaire was filled out, including information such as name, smoking habit, and BMI (BMI). All

patients were subjected to GM-CSF serological testing. The medical and family histories of the control subjects were taken into consideration.

All anthropometric measurements were acquired with a focus on body height and weight, which were assessed using a portable stadiometer while wearing light clothing With patients standing in an erect barefoot position, arms by side, and feet together,height was measured using a wall-mounted, non-extendable measuring tape Each person was weighed while standing in light clothing, without shoes orsocks, in the center of the weighing scale. BMI was computed using the formula BMI = weight (kg)/ height2 (m)2 and classified as overweight (BMI 25-29.9), obesity (BMI 30-39.9), and morbid obesity (BMI > 40). (Sturm,2007).

ELISA Kit for Human GM-CSF (Granulocyte-macrophage Colony-stimulating Factor)

To quantify total GM-CSF in the serum, an enzyme linked immunosorbent assay (ELISA) was used to measure the concentration of GM-CSF in the serum of patients with respiratory infections. The Elabscience ELISA kit, which includes the solutions and components listed in the table below

Table (1): GM-CSF EILSA Kit ELISA Reagents Preparation

Item	Company	Origin
1. Diluent for Biotinylated Detection Ab 2. Analysis Certificate 3. -Biotinylated Detection Ab Concentrate (100 x) 4. HRP Conjugate Concentrate (100x) 5. Wash Buffer Concentrate (25 mL) 6. Diluent for HRP Conjugate 7. Micro ELISA Plate (Dismountable) 8. 5 pcs of plate sealer 9. Product Information 10. tenth standard of reference 11. Sample Diluent & Reference Standard 12. Step Procedure 13. Substrate Reagent	Elabscience	China

Washing buffer solution

This solution was made by diluting the standard wash buffer supplied with the kit in a ratio of 1:25, with the total volume of 30ml of wash buffer diluted in 750 ml of deionized or distilled water D.W and gently mixed. The wells in the microtiter plates were washed with wash buffe

Preparation of a standard solution

These solutions were utilized to create the standard curve, which was then used to compute the concentration of the above-mentioned factors. These solutions

must be made in 15 minutes. The preparation was completed by adding one ml of the standard and sample diluent solution provided with the kits, which resulted in a high concentration, and then transferring 0.5 ml of it into tube number 2, which contained 0.5 ml of standard and sample diluent, to obtain a concentration half that of the previous concentration, and so on to obtain seven serial dilutions in the wells from (B-H), while zero concentration was placed in well A. (diluted only).

Biotinylated detection antibody: This solution is included in the kit in a quantity of 120L. It was made by diluting a specific solution in a 1:100 ratio.

HRP (conjugated horseradish peroxidase): It was made by diluting it in a 1:100 ratio with the kit's solution

Assaying Principles

The enzyme-linked immunosorbent assay (ELISA) is a medical diagnostic technique that employs antibodies/antigens and color change to identify a drug. The Sandwich-ELISA principle is used in the TNF-a test, according to the Elabscience® made corporation in the United States. An antibody specific for Human TNF-a has been pre-coated on the micro ELISA plate. Standards or samples are mixed with the specific antibody in the micro ELISA plate wells. After that, each microplate well is treated with a Biotinylated detection antibody specific for Human TNF-a and an Avidin-Horseradish Peroxidase (HRP) conjugate. The addition of stop solution stops the enzyme-substrate reaction, and the color changes to yellow. At a wavelength of 450 nm 2 nm, the optical density (OD) is determined spectrophotometrically. The OD value is proportional to the amount of Human TNF-a in the sample. The concentration of Human TNF-a in the samples was determined by comparing the OD of the samples to the standard curve using the trend line equation in the Excel Microsoft Office 2016 application for all samples

According to the Elabscience ELISA methods manual, perform the assay

1. To the first two columns of wells, 100µl of standard working solution was added. Each concentration of the solution was added in two wells, one on top of the other.
2. In the remaining wells, 100µl of sample serum was added.
3. The plate was sealed with the kit's sealer and incubated at 37°C for 90 minutes.
4. Without washing, the liquid was removed from each well, and 100 µl of Biotinylated Detection Ab working solution was immediately added to each well.
5. The plates were covered with the plate sealer and gently mixed before being incubated at 37°C for 1 hour.
6. After removing the solution from each well, 350µl of wash buffer was added to each well. The solution from each well was removed and the plate dried against the clean absorbent paper after being soaked for 12 minutes. This step was repeated three times.
7. Each well was filled with 100µ l of HRP Conjugate working solution.

8. The plate was sealed and incubated for 30 minutes at 37°C using the plate sealer.
9. The solution was withdrawn from each well and washed five times with 350µl of wash buffer, as in the previous step.
10. Substrate Reagent was added to each well in a volume of 90µl.
11. To shield the plate from light, it was immediately sealed with a new plate sealer and incubated for about 15 minutes at 37°C.
12. The Stop Solution was applied to each well at a volume of 50µl.
13. Using a microplate reader set to 450 nm, measure the optical density (OD value) of each well at the same time, and compute the results using the Excel Microsoft Office 2016 program. (y = 0.7931x - 1.4867)

Table 2: In the plate, the GM-CSF standards are arranged

tube	1	2	3	4	5	6	7
Pg/ml	500	250	125	62.5	31.15	15.63	7.81
O.D	3.143	2.537	2.084	1.122	0.573	0.267	0.128

Results

Patients receiving covid-19 are distributed according to their BMI and smoking status

Table 3: The BMI categories and smoking status of patients with covid-19 and control participants were compared.

			Control (N=100)	Patients (N=70)
BMI Categories	Non-Obesity	No	73	34
		%	73.0%	48.6%
	Obesity	No	27	36
		%	27.0%	51.4%
Smoking	No	No	87	42
		%	87.0%	60.0%
	Yes	No	13	28
		%	13.0%	40.0%

Table 4: The BMI categories and gender distribution of patients with covid-19 and control subjects

				Control	Patients
Male	BMI	Non- Obesity	No	26	17
			%	78.8%	44.7%
		Obesity	No	7	21
			%	21.2%	55.3%
Female	BMI	Non- Obesity	No	47	17
			%	70.1%	53.1%
		Obesity	No	20	15
			%	29.9%	46.9%

Table 5: To find characteristics independently linked with COVID 19 hospitalization, researchers used univariate logistic regression analysis

		B	P. value	OR	95% C.I. for OR	
					Lower	Upper
BMI Categories	Non- obesity	Reference				
	Obesity	1.052	0.001*	2.863	1.504	5.450
Smoking	No	Reference				
	Yes	1.495	<0.001*	4.462	2.099	9.482

Table 6: Comparison of the mean values of GM-CSF between case and control groups

		Median (IQR)	Mean Rank	Sum of Ranks	Mann-Whitney U	P. value
GM-CSF	Control	2.52 (0.42)	70.55	7054.50	2004.500	<0.001*
	Patients	3.02 (3.45)	106.86	7480.50		

Discussion

Between February and December 2020, 170 persons were involved in this study: 70 covid-19 patients admitted to intensive care units in Merjan and Emam Alsadiq hospitals in Babylon governorate, Iraq, and 100 healthy adults. The table shows the demographic characteristics of patients and controls (3).

According to Table 4, the Patients group contained 36 (51.4%) patients with Covid 19, 19 of whom were obese (BMI 30 kg/m2), and 34 (48.6%) were non-obese. Only 27 (27.0%) of the participants in the control group were fat, while 73 (73.0%) of the participants in the Covid 19 group were not obese. These findings matched those of a Mexican study (Hernández-Garduo, 2020), which indicated that 38.8% of patients have obesity compared to 33.3 percent of controls, and that 66.7 percent of controls (SARS-CoV-2) have no obesity compared to 61.2 percent of patients (positive SARS-CoV-2).

In terms of smoking status, the current study found that 28 (40.0 percent) of the patients in the Covid 19 group were smokers, whereas 42 (60.0 percent) were non-smokers. Only 13 (13.0%) of the participants in the control group were smokers, with 87 (87.0%) having no Covid 19. These findings matched those of Berumen et al., (2020), who discovered that non-smokers made up 69 percent of non-confirm cases with covid 19.

Obese males had a higher incidence of covid19 infection than females, as shown in Table4. This matched the findings of a study conducted in Shenzhen, China (Cai et al., 2020), which indicated that a higher percentage of patients (males) have obesity than females (78.1%). Another study (Busetto et al., 2020) in Italy found that 72.4 percent of patients with covid 19 (males) are obese. The greater fatality in men could be explained by differences in fat distribution patterns between men and women. To put it another way, overweight men carry the

majority of their fat in their abdomen, which has a negative impact on lung function, which is exacerbated when combating serious lung illnesses as COVID-19 pneumonia (Mundell, 2021).

A Univariate Logistic regression analysis was used to identify characteristics that were independently linked with COVID 19 hospitalization in Table5. Obese people were found to be substantially more likely than non-obese people to be admitted to the hospital for COVID-19 (OR 2.863; 95 percent CI, 1.504-5.450, p=0.001). When compared to non-smokers, smokers were substantially more likely to be hospitalized for COVID-19 (OR 4.462; 95 percent CI, 2.099-9.482, p=0.001).

The adjusted odds ratios in overweight and obese people were 1.13 (95 percent confidence interval [CI], 1.03-1.25) and 1.26 (95 percent confidence interval [CI], 1.03-1.25), respectively, as compared to normal-weight people (Jung et al., 2021). (95 percent CI, 1.15-1.39). Another study by Hernández-Garduo (2020) found that obesity was associated with a higher risk of COVID-19 infection than non-obese people (OR 1.31; 95 percent CI, 1.25–1.37, p=0.0001), and smokers were associated with a higher risk of COVID-19 infection than non-smokers (OR 0.8; 95 percent CI, 0.74–0.86, p=0.0001). Those who smoked were more likely to become infected with COVID-19 than those who did not (OR 0.8; 95 percent CI, 0.74–0.86, p=0.0001). Furthermore, the current findings were consistent with those of Ji et al., (2021), who found that overweight (BMI 23 to 24.9 kg/m2; adjusted odds ratio [aOR], 1.16; 95 percent confidence interval [CI], 1.1.03 to 1.30) and class 1 obesity (BMI 25 to 29.9 kg/m2; aOR, 1.27; 95 percent confidence interval [CI], 1.14 to 1.42) had significantly increased COVID-19 risk, while classes 2 and 3 (Ji et al., 2021). Smokers have 1.91 times the odds of progressing to severe forms of COVID-19 than never smokers, according to a recent meta-analysis by Patanavanich and Glantz (2020), which included 11,590 COVID-19 patients. This is exactly congruent with the current findings.

Many studies have proven that obesity, as defined by a body mass index (BMI) greater than 30 kg/m2, is a risk factor for bacterial and viral pneumonia, ARDS acute respiratory distress syndrome, and acute respiratory failure after lung transplantation (Lederer et al.,2011; Mertz et al.,2013), and this was observed by (Peng et al.,2020; Wu et al.,2020; Docherty et al.,2020; Obesity was identified as a clinically significant risk factor for severe disease during the COVID-19 pandemic, according to Docherty et al.,2020; Petrilli et al.,2020. The observed link between obesity and acute respiratory failure and death from SARS-CoV-2 infection could be due to a variety of causes. First, obesity causes immunological activation, which results in higher levels of inflammatory chemicals such as interleukin-6, tumor necrosis factor-, and monocyte chemoattractant protein-1 in the bloodstream (Nishimura et al.,2009).

We showed in this study that because COVID-19 has a severe stage of pneumonia or respiratory failure, smokers are significantly more susceptible to severe disease due to their immunocompromised lungs (Pötschke-Langer et al., 2015). In a pulmonology article, Brake et al explained the biological process of coronavirus attacking the human body, stating that smoking is one factor that speeds up the production of the angiotensin-converting enzyme-2 (ACE2) receptor, which is known to be the coronavirus receptor, and thus smokers are more vulnerable to

this disease than non-smokers (Brake et al.,2020). Zhao et al. reviewed seven studies and found that, while the link between smoking and COVID-19 is not statistically significant, active smoking raises the chance of severe COVID-19, with smokers having a 1.98 times higher risk of reaching the severe stage (Zhao et al.,2020). Nargis et al discovered that 23 percent of adults over 15 years of age are smokers in an evidence-based study done in Bangladesh from 2009 to 2012, while another study indicated that 53.5 percent of second-hand smokers are exposed to smoking at home (Fischer et al.,2011; Nargis et al.,2015).

Conclusion

1. Mostly patients with Covid 19 [36 (51.4%)] were obese having BMI ≥ 30 kg/m2. Likewise, Patients group included 28 (40.0%) patients with Covid 19 were smokers. Obese males were more at risk of covid19 infection than females and significantly more likely to be hospitalized for COVID-19 compared with those who were non-obese and non-smokers.
2. The immunological parameters (GM-CSF) levels for the case (with Covid 19) group was significantly higher than that for the control group.

References

1. Berger, J. R. (2020). COVID-19 and the nervous system. *Journal of neurovirology*, *26*(2), 143-148.
2. Cevik, M., Marcus, J. L., Buckee, C., & Smith, T. C. (2021). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission dynamics should inform policy. Clinical Infectious Diseases, 73(Supplement_2), S170-S176.
3. Sahin, A. R., Erdogan, A., Agaoglu, P. M., Dineri, Y., Cakirci, A. Y., Senel, M. E., ... & Tasdogan, A. M. (2020). 2019 novel coronavirus (COVID-19) outbreak: a review of the current literature. EJMO, 4(1), 1-7.
4. Mousavizadeh, L., & Ghasemi, S. (2021). Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of Microbiology, Immunology and Infection, 54(2), 159-163.
5. WHO, (2020). Modes of Transmission of Virus Causing COVID-19: Implications for IPC Precaution Recommendations. Geneva, Switzerland: World Health Organization.
6. Guo, Z. D., Wang, Z. Y., Zhang, S. F., Li, X., Li, L., Li, C., ... & Chen, W. (2020). Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, Wuhan, China, 2020. Emerging infectious diseases, 26(7), 1586.
7. Mehta, P., Porter, J. C., Manson, J. J., Isaacs, J. D., Openshaw, P. J., McInnes, I. B., ... & Chambers, R. C. (2020). Therapeutic blockade of granulocyte macrophage colony-stimulating factor in COVID-19-associated hyperinflammation: challenges and opportunities. The Lancet Respiratory Medicine, 8(8), 822-830.
8. Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Sun, R., ... & Wei, H. (2020). Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+ CD16+ monocytes in severe pulmonary syndrome patients of a new coronavirus. biorxiv.

9. Lang, F. M., Lee, K. M. C., Teijaro, J. R., Becher, B., & Hamilton, J. A. (2020). GM-CSF-based treatments in COVID-19: reconciling opposing therapeutic approaches. Nature Reviews Immunology, 20(8), 507-514.
10. Hernández-Garduño, E. (2020). Obesity is the comorbidity more strongly associated for Covid-19 in Mexico. A case-control study. Obesity research & clinical practice, 14(4), 375-379.
11. Berumen, J., Schmulson, M., Alegre-Díaz, J., Guerrero, G., Larriva-Sahd, J., Olaiz, G., ... & Chiquete, E. (2020). Risk of infection and hospitalization by Covid-19 in Mexico: a case-control study. MedRxiv
12. Cai, Q., Chen, F., Wang, T., Luo, F., Liu, X., Wu, Q., ... & Xu, L. (2020). Obesity and COVID-19 severity in a designated hospital in Shenzhen, China. Diabetes care, 43(7), 1392-1398.
13. Mundell, E. (2021). Obesity More Deadly for Men Than Women When COVID Strikes. In U.S. News & World Report.
14. Jung, C. Y., Park, H., Kim, D. W., Lim, H., Chang, J. H., Choi, Y. J., ... & Chang, T. I. (2021). Association between body mass index and risk of coronavirus disease 2019 (COVID-19): a nationwide case-control study in South Korea. Clinical Infectious Diseases, 73(7), e1855-e1862.
15. Ji, W., Lee, R., Huh, K., Kang, M., Hwang, I. C., Radnaabaatar, M., ... & Jung, J. (2021). Overweight and obesity are risk factors for coronavirus disease 2019: a propensity score-matched case-control study. Endocrinology and Metabolism, 36(1), 196.
16. Patanavanich, R., & Glantz, S. A. (2020). Smoking is associated with COVID-19 progression: a meta-analysis. Nicotine and Tobacco Research, 22(9), 1653-1656.
17. Lederer, D. J., Kawut, S. M., Wickersham, N., Winterbottom, C., Bhorade, S., Palmer, S. M., ... & Christie, J. D. (2011). Obesity and primary graft dysfunction after lung transplantation: the Lung Transplant Outcomes Group Obesity Study. American journal of respiratory and critical care medicine, 184(9), 1055-1061.
18. Mertz, D., Kim, T. H., Johnstone, J., & Lam, P. P. Science, M., Kuster, SP,... & Loeb, M.(2013). Populations at risk for severe or complicated influenza illness: systematic review and meta-analysis. BMJ: British Medical Journal, 347.
19. Peng, Y. D., Meng, K., Guan, H. Q., Leng, L., Zhu, R. R., Wang, B. Y., ... & Zeng, Q. T. (2020). Clinical characteristics and outcomes of 112 cardiovascular disease patients infected by 2019-nCoV. Zhonghua xin xue guan bing za zhi, 48(6), 450-455.
20. Wu, C., Chen, X., Cai, Y., Zhou, X., Xu, S., Huang, H., ... & Song, Y. (2020). Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA internal medicine, 180(7), 934-943.
21. Docherty, A. B., Harrison, E. M., Green, C. A., Hardwick, H. E., Pius, R., Norman, L., ... & Semple, M. G. (2020). Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. bmj, 369.
22. Petrilli, C. M., Jones, S. A., Yang, J., Rajagopalan, H., O'Donnell, L., Chernyak, Y., ... & Horwitz, L. I. (2020). Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. bmj, 369.

23. Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., ... & Nagai, R. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nature medicine, 15(8), 914-920.
24. Pötschke-Langer, M., Schotte, K., & Szilagyi, T. (2015). The WHO framework convention on tobacco control. In The Tobacco Epidemic (Vol. 42, pp. 149-157). Karger Publishers.
25. Akbarov, A. N., & Xabilov, D. N. U. (2021). The condition of the oral cavity in patients who have had a viral infection COVID-19. International Journal of Health & Medical Sciences, 4(4), 381-383. https://doi.org/10.21744/ijhms.v4n4.1796
26. Brake, S. J., Barnsley, K., Lu, W., McAlinden, K. D., Eapen, M. S., & Sohal, S. S. (2020). Smoking upregulates angiotensin-converting enzyme-2 receptor: a potential adhesion site for novel coronavirus SARS-CoV-2 (Covid-19). Journal of clinical medicine, 9(3), 841.
27. Zhao, Q., Meng, M., Kumar, R., Wu, Y., Huang, J., Lian, N., ... & Lin, S. (2020). The impact of COPD and smoking history on the severity of COVID-19: A systemic review and meta-analysis. Journal of medical virology, 92(10), 1915-1921.
28. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. International Journal of Health Sciences, 5(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937
29. Fischer, F., Minnwegen, M., Kaneider, U., Kraemer, A., & Khan, M. M. H. (2015). Prevalence and determinants of secondhand smoke exposure among women in Bangladesh, 2011. Nicotine & Tobacco Research, 17(1), 58-65.
30. Nargis, N., Thompson, M. E., Fong, G. T., Driezen, P., Hussain, A. G., Ruthbah, U. H., ... & Abdullah, A. S. (2015). Prevalence and patterns of tobacco use in Bangladesh from 2009 to 2012: evidence from International Tobacco Control (ITC) Study. PloS one, 10(11), e0141135.