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The Effect Of immune molecules in protection against COVID 19

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Abstract

project attempts to estimate the manifestation of stimulated indicators on pBL ,as the manifestation of CD45 & CD74 in patients infected with COVID19 assessment of TNF-α and IL-8 serum ranks in patients with COVID 19 , associate the consequences of pBL –stimulation indicators appearance and serological valuation of TNF-α &IL-8 using multiple infection phases.

A total of (24) positive patients for COVID19 were screened for this study .Patients attended general lab. of Al-Muthanna province , because of abdominal pain ,pulmonary distress , fever and coughing any samples expressed positive for COVID19 directly choose to show level of TNF-α & IL-8 in serum of patient and show expression of CD45 and CD74 in pBL in HCV patients. Results showed that serum samples were analyzed for IL-8 & TNF-α by ELISA , showed highly significant increases ($p<0.05$) in serum level of covid 19 patients as compared with healthy control groups , acute covid 19 revealed high as well as , increases in serum level of TNF-α significantly($p<0.05$) ,while moderate cases of disease express high increase in serum level of IL-8 significantly($p<0.05$).Activated markers study revealed high expression of CD74 & CD45 in covid 19 patients as compared with healthy normal groups ,where acute covid 19 patients were showed significantly($p<0.05$) high expression in CD74 & CD45 compared with other covid 19 patients .

Keywords: *covid 19, CD45, CD74*

I. Introduction

Main case of corona virus was advised as cold in 1960. According to the Canadian training 2001, about 500 patients were recognized as Flu-like system. 17-18 cases of them were established as infected with corona virus strain by polymerase chain reaction. Corona was treated as simple nonfatal virus till 2002(1,2).

Novel coronavirus-induced pneumonia, which was named as coronavirus disease 2019 (COVID-19) by the WHO on the February 11, 2020, has rapidly increased in epidemic scale since it first appeared inWuhan, China, in December 2019(3).

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Coronaviruses are enveloped viruses with a positive sense single-stranded RNA genome (26e32 kb) [4]. Four coronavirus genera (a, b, g, d) have been recognized up to now, with human coronaviruses (HCoVs) identified in the a coronavirus (HCoV-229E and NL63) and b coronavirus (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) genera [5].

The occurrence of vital CD8+ and CD4+ T responses in patient blood suffers from acute disease looks related with retrieval .In compare, decreasing of a response seems to expect the conclusion of chronic disease. Then Ab -mediated reduction, recalling was related with insistent disease, so authorizing the major part that CD4+ act in the get rid of serious disease(6).

Cytokines function as the molecules of defense reaction that result in numerous physiological roles and adjust the defensive, provocative and repairing patient reactions, and mostly concealed by mono and lymph cells. cytokines from T cells act essentially in the host response. Stimulated T cells classified into (2) subcategories rendering cytokines manufacturing. T helper-1 cytokines, like IL-2 & IFN-ʁ, to lead to (CMI) response while T helper -2 cytokines as IL-10 and IL-4 are concerned with AMI. Both responses have been revealed to relate in a viral disease () and the inequality among them prefer HIR and depressed adjust CMI, that is essential for immunity beside diseases (7).

II. Materials &Methods

1- Patients

The study enrolled (24) COVID 19 patient , admitted at the public health laboratory and with fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia .

This study was showing to separate and certification COVID 19 and assessment IL-8 and TNF-α by ELISA procedure, same technique used to identify the appearance of CD45and CD74 .

III. Samples Collection

(24) blood samples (5-10) ml was drained from patients(COVID 19). then the blood samples were centrifuged to get blood serum then frozen at (_ 20 °C) to estimate the COVID 19 patients then identify cytokines level in blood patients as characterized by manufacture company.

Then cytokines and CD45and CD74 biomarkers measured by ELISA technique.

Serum cytokine

Sizes of cytokines in the serum were done by ELISA test (R&D Systems). Absorbance was restrained in copies with a micro plate reader (Beckman Coulter). The last concentration was expressed in pg/ml.

Statistical analysis:

Statistical analysis was showed by using Chi-square ($\chi 2$) test to regulate the statistical changes among diverse groups by using a proposal statistical platform for social science (SPSS 2020). The possibility of ($P \leq$

0.05) was measured to be statistically significant. The examined parameters were offered in terms of means ± standard errors (S.E.), and variances between means of patients and controls were calculated by ANOVA test and the Least Significant Difference (LSD). The difference was measured significant when the possibility (P) value were ($\leq 0.05, \leq 0.01$).

IV. Results & Discussion

1-Clinical sings

Clinical sings in COVID 19 patients were , fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia, some patients showed intermediate and mild clinical sings as shown in table (1).

Table (1) Clinical signs for COVID 19 patient .

NO.	Clinical signs	Number	Percentage%
1	acute	4	16.66%
2	Intermediate	7	29.16%
3	Mild	12	50%

Our results of this study showed that 4(16.66%) cases showed signs of fever, nonproductive cough, dyspnea, myalgia, fatigue,while 7(29.16%) cases showed intermediate and 12(50%) mild disease separately. According to F- test the difference in clinical sings were significant ($p<0.05$) . Symptoms of acute phase of COVID 19 disease

As in (7) Conferring to a report published on 24 Jan 2020, corona virus infected patient have several communal types such as fever, cough, and fatigue while diarrhea and dyspnea were initiate to be as unusual chin. Numerous of them patient described bilateral abnormalities. (8)

2. Results of IL-8 in COVID 19 patients

Serum of all patients with COVID 19 and those with acute or intermediate disease action contain higher level of IL-8 than healthy control group . IL-8 concentration was particularly increased in patients with intermediate disease (1437.80±351.50) and acute patients (915.821±75.946) correspondingly than mild disease (469.20±87.849) as matched with control groups (69.40±1.390) .Nevertheless , examination of difference test exposed that there was a great arithmetically substantial variations between COVID 19 and healthy control groups ($p<0.05$).

T-test ,showed that there was an increased arithmetical substantial differences among intermediate, acute disease and mild infection group ($p<0.05$) as table (2)

Table(2) The Concentration of IL-8 in patients and controls

Group	NO.	Serum level of IL-8		
		Mean ±SE	Minimum	Maximum
Mild	12	469.20±87.849	190.00	420.00
Acute COVID 19	4	915.821±75.946	430.00	900
Intermediate	7	1437.80±351.50	1209.00	1460.00
Control	10	69.40±1.390	63.00	76.00

Chemokines apply their biological activity through linking to certain cell surface receptors. An infrequent feature of greatest chemokine receptors is their great attraction for numerous ligands [9). Additionally to conscription, IL-8 aids to stimulate the motivation of neutrophils and monocytes [10]. Neutrophils offer the principal-route of defense in contrast to attacking different pathogens as virus. These cells discharge inflammatory cytokines such as IL-8, 10 &12 ,create irritable oxygen species. IL-8 excretion effects in an elevated employment of neutrophils into lung [11]. Furthermore, the initiation of I L -8 can be augmented through linking of the (TL R2/ TL R3 & T LR7) toll-like receptors that identify constituents of the virus multiprotein , double strand RNA and anti-viral composites of host individually[12].Moreover, the discharge of responsive O2- species from granulated cells recognized modifying action previous, thus disturbing "I L – 8" appearance[13]. Lung I L -8 is noticed at less preservation grade at acute stage of COVID 19 impurity, while noticable rises in blood serum and liver grade can be detected patients with moderiate infection

3- level of serum TNF

Current study showed that all patients with COVID 19 cover higher level of TNF-α than healthy control group , T NF-α concentration was improved particularly with acute COVID patients (521.51± 15.53) ,moderate patients(81.43± 5.00) and mild patients (18.91± 0.840) correspondingly .Analysis of variance among acute H CV , moderate, mild , and control people ($p<0.001$) .T- test exhibited that there was great statistical significant alteration among acute COVID 19 , moderate and mild disease group ($p<0.001$)table(3)

Table(3) The Concentration of TNF-α in patients and controls

Group	NO.	Serum level of TNF-α		
		Mean ±SE	Minimum	Maximum
Acute COVID 19	4	521.51± 15.53	412.00	590.00
Intermediate	7	81.43± 5.00	74.00	81.5.
mild	12	18.91± 0.840	17.80	18.8

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Control	10	16.1±1.05	8.08	19.58

TNF (whole superfamily members) has been associated in not fewer than morphogenesis, inflammation, angiogenesis, apoptosis, , invasion, , proliferation , and metastasis. T NF -α is a central cytokine to the of inflammatory pathogenesis routes. The T N F -α pro- inflammatory effect is facilitated via straight initiation of other pro- inflammatory cytokines,

The cytokine storm will activate a forceful round by the immune system to the body, cause ARDS and multiple organ failure, and lastly lead to death in severe cases of SARS-CoV- 2 infection(14)

4- CD45 expression in COVID 19 patients

Results as in table (3) shown that there was highly significant differences in mean of CD 45 expression among COVID 19 patients and healthy control groups ($p<0.001$) ,the cell surface CD45 was over expressed in acute compared to intermediate COVID 19 patients, mild and healthy control groups respectively .However ,there was high statistically significant differences between intermediate , acute and mild disease actively groups ($p<0.05$) with the high expression seen in acute COVID 19 disease.

Acute determining infections are considered complete primary enlargement of "poly clonal C D 4+andCD8 + T –cell" residents that continued over allowance[15]. On other hand, prolonged infections are related with temporary hindered responses that are frail and goal a slight array of MHC class I and II limited epitopes [16]

5- Expression of CD74 in COVID 19 patients

The results demonstrated in table (4) shows there was high statistically significant difference in mean of CD74 expression among COVID 19 patients and healthy control groups ($p<0.001$),and the higher percentage of expression was found in acute patients , intermediate disease followed by mild patients and control groups respectively .

T –test results showed that there was high significant difference between acute ,intermediate and mild disease groups($p<0.05$).

To get rid of COVID 19 is related with vital multi-vague C D 4+ and C D 8+ T cell responses ,while persons that progress mild infection likely to have fragile, slimly dedicated responses [15]. CD8+ effector cells in the lung were initiate to have less serviceable proficiency, as proved by low IF N -y fabrication [16]. The determination of lung pathogens is frequently attended thru frail "CD8+ T cell response " antigens subsequent[17]. We exasperated to conclude the pathogenic status of C D74 over comparing of its expression during infection , our results make it clear that robust up-regulation of both C D45&C D74 manage a tough mark that lymphocytes in peripheral blood of COVID 19 persons within formal of immune dysregulation. C D74 is a non-polymorphic type II basic embedded membrane protein ,that was initially believed to act chiefly as an MHC class II chaperone [18]. Though, CD74 lately was establish to show an extra protagonist "assistant-signaling molecule". MQ proves great-empathy linking to the pro-inflammatory "MI F" necessary " M IF-mediated M APK activation and cell proliferation"

Table(3) The expression of CD45 in patients

Group	NO.	Serum level of CD45		
		Mean ±SE	Minimum	Maximum
Intermediate	7	8.13±0.301	4.43	11.5
Acute	4	12.17±0.225	11.5	13.5
mild	12	5.75±0.428	5.24	6.21
Control	10	2.5±0.210	0.80	3.99

Table(4) The Concentration of CD74 in patients

Group	NO.	Serum level of CD74		
		Mean ±SE	Minimum	Maximum
Mild	12	9.11±0.255	6.00	19.00
Acute	4	34.11±4.31	19.15	46.5
Intermediate	7	13.71±2.00	12.68	14.0
Control	10	5.41±0.299	4.19	7.56

6- Relationship between lymphocyte activation expression and TNF-α & IL-8 serum level in COVID 19 infection.

The possible correlations between lymphocytes markers (CD45 and CD74) , TNF-α & IL-8 serum level were examined by the value of correlation coefficient and potted in table (5) , results exhibited that a strong positive correlation was detected between CD45 with TNF-α (r=0.645).also between CD74 with TNF-α (r=0.911) and between CD74 and CD45 (r=0.611)respectively . From outcomes exposed weak correlations start between CD45 with IL-8 (r=0.151). Strong negative correlation between TNF-α with IL-8 (r= -0.90) and CD74 with IL-8 (r=-0.012) correspondingly.

Table (5) Correlation conditions of lymphocytes surface activation markers , and serum IL-8 & TNF-α concentration in COVID 19 patients.

Correlations					
CD74	**CD45**	**TNF-α**	**IL-8**		
			1	Pearson Correlation	**IL-8**
				Sig.(2-tailed)	
		1	-0.90	Pearson Correlation	**TNF-α**
			0.212	Sig.(2-tailed)	
	1	. 645**	0.151	Pearson Correlation	**CD45**
		0	0.199	Sig.(2-tailed)	
1	.611**	. 911**	-0.012	Pearson Correlation	**CD74**
	0	0	0.78	Sig.(2-tailed)	
**** Correlation (2-tailed)**					
(-) mark it mean negative correlation or reverse correlation					

Our results recognized a important fear of T – cell delicacies in COVID 19 patients, COVID 19 patients had less young T-cell than controls ,and superior memory T- cells than those of controls .The raised development of memory cells could be due to raised notable step that succeed variation of effector cells to memory cells.

Of note, the co-expression of C D45 by peripheral blood T-cells in COVID 19 patients substantially above approximates the size of naïve and memory T-cell subgroups depend only on the expression of this indicators, also , there were statistical significant variance in their mean of expression C D45 between COVID 19 patients . In addition ,they persevere strong positively correlated during the COVID 19 infection . results correlation showed that both CD45 &CD74 were expressively linked in their expression among COVID 19patients , due to the armed pBL seems to be gathered and eventually lead to liver damage when homing mechanism appropriately ascends [19].

From cytokines , CD74 and CD45 expression the results argument to the pathogenesis of COVID 19 infection is comparatively due to the immunologic response , sufficiently of interleukins & chemokines are

intricate in the development of the inflammatory progression by changing the development of the inflammatory via changing the native T h0 response to a T h1 ,T h2 or mixed T h1 & T h2 response(20).

References

[1] P. Zhou, X.L. Yang, X.G. Wang, et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature (2020), https://doi.org/ 10.1038/s41586-020-2012-7.

[2] E. de Wit, N. van Doremalen, D. Falzarano, et al., SARS and MERS: recent insights into emerging coronaviruses, Nat. Rev. Microbiol. 14 (2016) 523e534, https://doi.org/10.1038/nrmicro.2016.81.

[3] J.T. Wu, K. Leung, G.M. Leung, Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study, Lancet (2020), https://doi.org/10.1016/ S0140-6736(20)30260-9.

[4] S. Su, G. Wong, W. Shi, et al., Epidemiology, Genetic recombination, and pathogenesis of coronaviruses, Trends Microbiol. 24 (2016) 490e502, https:// doi.org/10.1016/j.tim.2016.03.003.

[5] S. Perlman, J. Netland Coronaviruses post-SARS: update on replication and pathogenesis, Nat. Rev. Microbiol. 7 (2009) 439e450, https://doi.org/10.1038/ nrmicro2147

[6] J. Liu, P. Wu, F. Gao, et al., Novel immunodominant peptide presentation strategy: a featured HLA-A*2402-restricted cytotoxic T-lymphocyte epitope stabilized by intrachain hydrogen bonds from severe acute respiratory syndrome coronavirus nucleocapsid protein, J. Virol. 84 (2010) 11849e11857, https://doi.org/10.1128/JVI.01464-10.

[7] N. Keicho, S. Itoyama, K. Kashiwase, et al., Association of human leukocyte antigen class II alleles with severe acute respiratory syndrome in the Vietnamese population, Hum. Immunol. 70 (2009) 527e531, https://doi.org/ 10.1016/j.humimm.2009.05.006.

[8] Y.M. Chen, S.Y. Liang, Y.P. Shih, et al., Epidemiological and genetic correlates of severe acute respiratory syndrome coronavirus infection in the hospital with the highest nosocomial infection rate in Taiwan in 2003, J. Clin. Microbiol. 44

(9) 359e365, https://doi.org/10.1128/JCM.44.2.359-365.2006.

[9] S.F. Wang, K.H. Chen, M. Chen, et al., Human-leukocyte antigen class I Cw1502 and class II DR 0301 genotypes are associated with resistance to severe acute respiratory syndrome (SARS) infection, Viral Immunol. 24 (2011) 421e426, https://doi.org/10.1089/vim.2011.0024.

[10] A.H. Hajeer, H. Balkhy, S. Johani, et al., Association of human leukocyte antigen class II alleles with severe Middle East respiratory syndrome-coronavirus infection, Ann. Thorac. Med. 11 (2016) 211e213, https://doi.org/10.4103/ 1817-1737.185756.

[11] X. Tu, W.P. Chong, Y. Zhai, et al., Functional polymorphisms of the CCL2 and MBL genes cumulatively increase susceptibility to severe acute respiratory syndrome coronavirus infection, J. Infect. 71 (2015) 101e109, https://doi.org/ 10.1016/j.jinf.2015.03.006.

[12] G. Li, X. Chen, A. Xu, Profile of specific antibodies to the SARS-associated coronavirus, N. Engl. J. Med. 349 (2003) 508e509, https://doi.org/10.1056/ NEJM200307313490520.

[13] Z. Xu, L. Shi, Y. Wang, et al., Pathological findings of COVID-19 associated with acute respiratory distress syndrome, Lancet Resp. Med. (2020), https:// doi.org/10.1016/S2213-2600(20)30076-X.

[14] Y.Y. Fan, Z.T. Huang, L. Li, et al., Characterization of SARS-CoV-specific memory T cells from recovered individuals 4 years after infection, Arch. Virol. 154 (2009) 1093e1099, https://doi.org/10.1007/s00705-009-0409-6.

[15] F. Tang, Y. Quan, Z.T. Xin, et al., Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow up

Followup study, J. Immunol. 186 (2011) 7264e7268, https://doi.org/10.4049/ jimmunol.0903490.

[16] J. Zhao, K. Li, C. Wohlford-Lenane, et al., Rapid generation of a mouse model for Middle East respiratory syndrome, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 4970e4975, https://doi.org/10.1073/pnas.1323279111.

[17] A.E. Williams, R.C. Chambers, The mercurial nature of neutrophils: still an enigma in ARDS? Am. J. Physiol. Lung Cell Mol. Physiol. 306 (2014) L217eL230, https://doi.org/10.1152/ajplung.00311.2013.

[18] R. Channappanavar, S. Perlman, Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology, Semin. Immunopathol. 39 (2017) 529e539, https://doi.org/10.1007/s00281-017- 0629-x.

[19] M.J. Cameron, J.F. Bermejo-Martin, A. Danesh, et al., Human immunopathogenesis of severe acute respiratory syndrome (SARS), Virus Res. 133 (2008) 13e19, https://doi.org/10.1016/j.virusres.2007.02.014.

[20] C.K. Min, S. Cheon, N.Y. Ha, et al., Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity, Sci. Rep. 6 (2016) 25359, https://doi.org/ 10.1038/srep25359.