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Possible Role of IL-6 And IL-17 Among COVID-19 Patients

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Abstract. Cytokine storm has been reported in a number of patients with severe acute respiratory syndrome - corona virus-2 (SARS-CoV-2) due to elevated level of pro-inflammatory cytokine such as interleukin-6 and interleukin-17. The aim of this study was to evaluate the level of interleukin-6 and interleukin-17 among patients with coronavirus disease 2019 (COVID-19). This case control study included 78 confirmed cases with COVID-19 (58 males and 30 females; their age ranged between 15- 83 years old) and compared with 10 apparently healthy individuals (8 males and 2 females), were admitted to Epidemiological Monitoring Unit, Emergency Department in Baqubah Teaching Hospital during the period from 1st of October to the 1st of December 2020. Nasopharyngeal swabs were collected from all patients who were included in this study and directly placed in tube contain 2ml of viral transport medium then stored at -20 ºC until the time of RNA extraction and real time polymerase chain reaction processing as well as blood sample were collected from patients then the sera were separated, labelled and sored until the time of test for quantitative detection of interleukin-6 and interleukin-17. There is significant difference in level of interleukin-6 and interleukin-17 between patients and control using ELISA assay, 0.0398 and 0,0046 respectively, and the mean serum level of IL-6 were 136.25±12.55µg and 72.93±15.02µg respectively and IL-17 was 37.87±4.83µg and 13.48±2.23µg respectively. Most infections were noticed among males and age group 15-35 years old.

Keywords. Coronavirus disease 2019, Interleukin-6 and interleukin-17, ELISA assay.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an exceptionally contagious respiratory illness coming about because of a dangerous novel coronavirus, sever acute respiratory syndrome - corona virus-2 (SARS-CoV-2). As of February 20, 2021, there have been 110,743,440 confirmed cases and 2,452,437 deaths by the SARS-CoV-2 disease worldwide [1].

Sever acute respiratory syndrome - corona virus-2 genome consists of 29 903 base pair (bp) single-stranded positive sense RNA and containing several open reading frames (ORFs) coding various proteins [2]. It can be transmitted thought respiratory droplets and direct contact; while the main transmission route of SARS-CoV-2 is aerosols the virus was also found in stool of recover's patient [3].

Sever acute respiratory syndrome - corona virus-2 infection is characterized by flu-like symptoms such as cough, fever, weakness, and myalgia. Patients may experience diarrhea and nausea for a few days before developing a fever, meaning that fever is the most frequent but not the most severe symptom of infection. A small percentage of patients can experience headaches or hemoptysis [4]. The clinical signs show after 5 days and side effect beginning is inside 11 days in 97.5% of patients [5].

Inflammatory cytokine storm is closely related to the development and progression of acute respiratory distress syndrome (ARDS) during COVID-19 infection. The serum levels of cytokines are significantly increased in patients with ARDS, and the degree of increase is positively correlated with mortality rate [6]. The IL-6 levels were higher in

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the patients with severe infection than those with moderate infection [7]. Several studies showed high level of pro-inflammatory cytokine in patients with SARS COV-2 and these include IL-1, IL-2, IL-6, IL-8**,** IL-17**,** chemokines. Interferon gamma may assume a critical part in the improvement of lung disfunction through prompting the aggregation of immune cells inside the lungs [8,9]. The T helper-17 in lung produce IL-17 in response to viral infection [10]. There is emerging evidence supporting the role of interleukin-17 in SARS-CoV-2 pathogenesis, including a report on the first anatomopathologic lung analysis with a high number of T helper-17 lymphocytes in the alveolar space [11]. Furthermore, IL-17 promotes viral survival by inhibiting apoptosis in concert with IL-6. This study aims to evaluate the level of pro-inflammatory cytokines such as interleukin-6 and interleukin-17 among patients with COVID-19.

MATERIALS AND METHODS

Study Design

A case- control study, 78 confirmed cases with COVID-19 (58 males and 30 females; their age ranged between 15-83 years old) and compared with 10 apparently healthy individuals (8 males and 2 females) involved in this study, were admitted to Epidemiological monitoring unit, Emergency Department in Baqubah Teaching Hospital during period from 1st of October to the 1st of December 2020. The diagnosis of each case was established using clinical diagnosis and laboratory diagnosis using real time polymerase chain reaction, as well as case history was taken from all study population.

Sample Collection

Nasopharyngeal swabs were collected from all patients included in this study and directly placed in tube contain 2ml of viral transport medium (V.T.M) then stored at -20 ºC until the time of RNA extraction (Cat. No.H 200201 Zybio Inc- Germany) and real time polymerase chain reaction Go Script® (Cat. No. A5003 promega, USA) processing. Blood samples (5 ml) were obtained from patients and placed in serum separator tube (SST) with specific gel to easy sorting the serum. Then labeled and was left for 20 minutes at room temperature. After coagulation, sera were separated by centrifuge 4000xg for 15 minutes and directly stored at -20 ºC to be analyzed later to quantitative detection of IL-6 using (Cat No: abx152020, Abbexa Ltd, Cambridge, UK) and IL-17 (Cat No: abx152020, Abbexa Ltd, Cambridge, UK).

Statistical Analysis

Data analyzed using Statistical Analysis System- SAS (2012) program [10].

RESULTS

Based on results of ELISA assay, the mean serum level of IL-6 in COVID-19 patient and control were 136.25±12.55µg and 72.93±15.02µg respectively and statistically significant (P= 0.0398), while the level of IL-17 was 37.87±4.83µg and 13.48±2.23µg respectively and statistical analysis showed a significant difference (Table 1).

TABLE 1. Serum Level of IL-6 and IL-17 in Study Population.

Study Population	No. (%)	Serum Level of IL-6 and IL-17(µg)	
		IL-6	IL-17
Patients	78(100%)	136.25 ±12.55	37.87 ±4.83
Control group	10(100%)	72.93 ±15.02	13.48 ±2.23
T-test		60.792	17.015
P value		0.0398	0.046

The most noteworthy of patients was inside the age group 15-35 year (43.6%) followed by those within the age group 36-50 year (29.50 %) and the least rate was within the age group71-90 year (6.40%) while in control group: half of them (50%) was from the age group 71-90 year, the statistical analysis revealed highly significant differences (p=0.0001). About 64.10% of patients were males and 35.90% were females, while 70% of control group were males and 30% were females and statistically highly significant (0.0001) as shown in Table 2.

TABLE 2. Age and Sex Distribution in Study Population.

Variables factor		Patients No. (%)	Control group No. (%)	T-test (P value)
Age (year)	15-35	34(43.60 %)	2(20%)	0.0001 **
	36-50	23(29.50 %)	2(20%)	
	51-70	16(20.50%)	1(10%)	
	71-90	5 (6.40%)	5(50%)	
Sex	Males	50(64.10%)	7(70%)	0.0001 **
	females	28(35.90%)	3(30%)	0.0085 **
Total		78 (100%)	10(100%)	----------

DISCUSSION

The current study demonstrated that the level of IL-6 was higher in patients than that in control group (136.25 ±12.55 and 72.93 ±15.02 respectively), the difference was statistically significant (p= 0.0398) also the level of IL-17 was higher in patients than that in control group (37.87 ±4.83 and 13.48 ±2.23respectively) the difference was statistically significant(p=0.046). This result in agreement with several report found increase in level of IL-6 among SARS-COV-2 patient in Wuhan- China [12,13]. So, the level of IL-6 elevates in severe cases but not all patient in different clinical pictures [14-18]. Another finding from Hirano and Murakami (2020) reported that cytokine storm in SARS-COV-2 patients mainly characterize by increased level of IL-6 and TNF-α [19]. Interlukin-6 was significantly high in 11 studies involved measuring the level of IL-6 in serum of patents with SARS-COV-2 [20]. Increase level of IL-6 in large number of cases was noticed during SARS-CoV-1 [21-24]. Also, the same finding was observed among Middle East Respiratory Syndrome- corovirus (MERS-CoV) patients in Korea, the level of IL-6 increased in the serum of patients in the second week [25].

On other hand IL-6 is the key mediator in toxicity of cytokine release syndrome CRS and increase its level had significantly correlated with severity outcome of the disease [26]. Two studies report there is a strong correlation between the high level of IL-6 and occurrence of respiratory failure in SARS-COV-2 patients [27,28]. Interleukin-6, interferon and lymphopenia is associated with early immune response [29,30]. This also may due to IL-6 is a key cytokine release of innate immunity as its release by the cells of innate immunity as defenses mechanism against viral infection.

The level of IL-17 was significantly higher in patients than that in control group, this result agreed with several studies. [31], reported high level of IL-17 among 140 SARS-COV-2 patients in Wuhan-China, [32], demonstrated that peripheral blood of a SARS-COV-2 patient had a significantly high number of CCR6þ Th17 cells further suggesting a Th17 type cytokine storm in this disease. Interleukin-17 levels were higher in intensive-care COVID-19 patients compared to mild SARS-COV-2 and control group [16]. It has been hypothesized that blocking IL-17 could increase SARS-COV-2's aberrant immune response and ARDS-related mortality [33].Interleukin-17 functions are critical in a variety of viral infection environments, with targeting it as an effective alternative treatment. In some cases, viral infections are suppressed while tissue pathology is minimized diseases that affect humans [34]. Many studies reported there is positive correlation between high level IL-17 and pro-inflammatory cytokines [35-38]. During cytokine storm several types of cytokines are release including TNFα, IL-1β, IL-2, IL-6, IFN α, IFN β, IFN γ, IL-7 and MCP-1. These cytokines cause immune cells to produce a large number of free radicals, which are the primary cause of ARDS and multiple organ failure [39].Increase level of cytokine may be a predictor factor for development of severe form due to harmful effect of these cytokine on body cell and excessive production lead to dead large number of cells in multiple organs and poor outcome of cases.

According to result of this study, the rate of infection was higher in males than that in females (64.1% and 35.9 respectively) with significant differences. The result of this study is comparable with result of several studies from them two studies in Saudi Arabia, first one found that among 1519 confirmed SARS COV-2 the rate of infection in male was 54.4% [40]. The second report confirmed that 80% of cases were males [41]. The third one found that 66% of patients with SARS-CoV-2 infection were males [42]. On other hand, several studies in different period of epidemic and various countries reported that females had significantly high rate of infection with SARS COV-2 than

males, other study done in KSA found among total confirmed cases the infection rate in female was51.1% [43], while other was found that female (57.6%) have high rate of infection than males study in UK [44], and this result is disagreed with the result of current study, There is a number of study demonstrate that there no difference or very small difference in range of getting SARS COV-2 infection between males and females from these Iranian researchers recorded that among confirmed cases there is no significant difference in SARS COV-2 infection between male and female [44,45].

Data obtained from this study demonstrated that the highest rate was from young age group 15-35 years old as their percentage 43.60% and the lowest rate of patients was those with age above >70 years and the result was statistically significant. This result comparable with the result of study reported that the age of most patients was between 29-50 years old, from them those aged 30-39 year being the most infected range in Iraq [46,47]. Younger adults, particularly those younger than 35 years are often have high frequency of infection [48]. In contrast, these results unlike with result of study showed that older individuals were more exposed to the thrilling types of this disease [49]. Large study consist of 4880 confirmed cases SARS COV-2 infection using real time PCR in China, the authors were divided the patients into six age group and the result was most infection within age group as following: 482 (18-29 year), 1097 (30-39 year), 841 (40-49 year), 1011 (50-59 years), 886 (60-69 years), 563(> 70 years) [50].

CONCLUSION

The level of pro-inflammatory cytokine (IL-6 and IL-17) was higher in the patients than that in the control group. The rate of infection was higher among males than that in females and within the age group 15-35 year.

REFERENCES

1. C. Gebhard,V. Regitz-Zagrosek , H.K. Neuhauser , R. Morgan and S.L. Klein, "Impact of sex and gender on COVID-19 outcomes in Europe," Biol. Sex. Differ., Vol. 11, pp. 1-13 ,2020.
2. P Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li , C. L. Huang, H. D. Chen, J. Chen, Y. Luo, H. Guo, R.D. Jiang, M.Q. Liu, Y. Chen, X.R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F.X. Zhan, Y.Y. Wang, G.F. Xiao and Z.L. Shi. "A pneumonia outbreak associated with a new coronavirus of probable bat origin," Nature, Vol. 579, no.7798, pp. 270-273,2020.
3. CDC COVID-19 Response Team, S. Bialek, E. Boundy, V. Bowen, N. Chow, A. Cohn, N. Dowling, S. Ellington and R. Gierke. "Severe outcomes among patients with coronavirus disease 2019 (COVID-19) United States, February 12–March 16," MMWR., Vol. 69, no. 12, pp. 343-346, 2020.
4. Center for Systems Science and Engineering. "COVID-19 Dashboard by at Johns Hopkins University. ArcGIS," Johns Hopkins University. https://systems.jhu.edu/ (2021).
5. D. Wang, B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B.Wang, H. Xiang, Z. Cheng, Y. Xiong and Zhao, Y. "Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan-China," Jama. Vol. 323, no. 11, pp. 1061-1069, 2020.
6. E. Antalis, A. Spathis, C. Kottaridi, A. Kossyvakis, K. Pastellas, K. Tsakalos, A. Mentis, C. Kroupis and S. Tsiodras, "Th17 serum cytokines in relation to laboratory-confirmed respiratory viral infection: A pilot study," J. Med. Virol., Vol. 91, no. 6, pp. 963-971, 2019.
7. E. Goldstein, M. Lipsitch and M. Cevik, "on the effect of age on the transmission of SARS-CoV-2 in households-schools, and the community," J. Infect. Dis., Vol. 223, no. 3, pp. 362-369, 2021.
8. Z. Wu and J.M. "McGoogan, characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China -summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention," JAMA, Vol. 323, no. 13, pp. 1239-42, 2020.
9. Z. Xu, L. Shi, Y. Wang, J. Zhang, L. Huang, C. Zhang, S. Liu, P. Zhao, H. Liu, L. Zhu and Y. Tai, "Pathological findings of COVID-19 associated with acute respiratory distress syndrome," Lancet Respir Med. Vol. 8, pp. 420–422, 2020.
10. F. Alamri, Y. Alsofayan, Y. AlRuthia, A. Alahmari, Y. Almuzaini, F.A. Gazalah, F. Alradini, T. Alaama and A. Khan, "predictors of hospitalization among older adults with COVID-19 in saudi

arabia: a cross-sectional study of a nationally representative sample," Risk Management and Healthcare Policy, Vol. 14, pp. 875, 2021.
11. F. Najafi, N. Izadi, S.S. Hashemi-Nazari, F. Khosravi-Shadmani, R. Nikbakht and E. Shakiba, "serial interval and time-varying reproduction number estimation for COVID-19 in western Iran," New Microbes New Infect., Vol.36,pp. 100715, 2020.
12. N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y., Wei and T. Yu, "Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China a descriptive study," Lancet, Vol. 395, no. 10223, pp. 507-513, 2020.
13. D. Sun, H. Li, X.X. Lu, H. Xiao, J. Ren, F.R. Zhang, and Z.S Liu, "Clinical features of severe pediatric patients with coronavirus disease 2019 in Wuhan: a single center's observational study," World J Pediatr, Vol. 16, no. 3, pp. 251-259, 2020.
14. Y. Gao, T. Li, M.Han, X. Li, D.Wu, Y. Xu, Y. Zhu, Y. Liu, X. Wang and L. Wang, "Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19," J.med.virol., Vol. 92, no. 7, pp. 791-796, 2020.
15. J. Gong, H. Dong, S.Q. Xia, Y.Z. Huang, D. Wang, Y. Zhao, W. Liu, S. Tu, M. Zhang, Q. Wang, and F. Lu, "Correlation Analysis Between Disease Severity and Inflammation-related Parameters in Patients with COVID-19 Pneumonia," medRxiv, (2020).
16. C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G.Fan, J. Xu, X. Gu and Z. Cheng, "Clinical features of patients infected with 2019 novel coronavirus in Wuhan-China,"Lancet, Vol. 395, no.10223, pp. 497-506, 2020 .
17. Z. Wang, B. Yang, Q. Li, L. Wen and R. Zhang, "Clinical features of 69 cases with coronavirus disease 2019 in Wuhan," China. Clin. Infect. Dis., Vol. 71, no. 15, pp. 769-777, 2020.
18. F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, B. Song X. Gu and L. Guan, "Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study," Lancet, Vol. 395, no. 10229, pp. 1054-1062, 2020.
19. T. Hirano and M. Murakami, "COVID-19: a new virus, but a familiar receptor and cytokine release syndrome," Immunity, Vol. 52, no. 5, pp. 731-733, 2020.
20. H. Mojtabavi, A. Saghazadeh and N. Rezaei, "Interleukin-6 and severe COVID-19: a systematic review and meta-analysis," Eur.Cytokine. Netw., Vol. 31, no. 2, pp. 44-49, 2020.
21. Y. Zhang, J. Li, Y. Zhan, L. Wu, X. Yu, W. Zhang, L. Ye, S. Xu, R. Sun, Y. Wang and J. Lou, "Analysis of serum cytokines in patients with severe acute respiratory syndrome," Infect. Immun., Vol. 72, no. 8, pp. 4410-4415, 2004.
22. K.J. Huang, I.J. Su, M. Theron, Y.C. Wu, S.K. Lai, C.C. Liu and H.Y. Lei, "An interferon-γ-related cytokine storm in SARS patients," J. Med. Virol., Vol. 75, no. 2, pp. 185-194, 2005.
23. Y. Jiang, J. Xu, C. Zhou, Z.Wu, S. Zhong, J. Liu, W. Luo, T. Chen, Q. Qin and P. Deng "characterization of cytokine/chemokine profiles of severe acute respiratory syndrome," Am J Respir Crit Care Med., Vol. 171, no. 8, pp. 850-857, 2005.
24. J.Y. Chien, P.R. Hsueh, W.C. Cheng, C. Yu and P.C. Yang, "Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome," Respirology, Vol. 11, no. 6, pp. 715-722, 2006.
25. E.S. Kim, P.G. Choe, W.B. Park, H.S. Oh, E.J. Kim, E.Y. Nam, S.H. Na, M. Kim, K.H. Song, J.H. Bang and S.W. Park, "Clinical progression and cytokine profiles of Middle East respiratory syndrome coronavirus infection," J. Korean Med. Sci., Vol. 31, no. 11, pp. 1717, 2016.
26. M. Norelli, B. Camisa, G. Barbiera, L. Falcone, A. Purevdorj, M. Genua, F. Sanvito, M. Ponzoni, C. Doglioni, P. Cristofori and C. Traversari, "Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells," Nat. med., Vol. 24, no. 6, pp. 739-748, 2018.
27. X. Chen, B. Zhao, Y. Qu, Y. Chen, J. Xiong, Y. Feng, D. Men, Q. Huang, Y. Liu, B. Yang and J. Ding, "Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients," Clin. Infect. Dis., Vol. 71, no. 8, pp. 1937-1942, 2020.
28. E.A. Coomes and H. Haghbayan, "Interleukin-6 in COVID-19: a systematic review and meta-analysis," Rev. Med. Virol, Vol. 30, no. 6, pp. 1-9, 2020.
29. M. Merad and J.C. Martin, "Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages," Nat. Rev. Immunol., Vol. 20, pp. 355-362, 2020.

30. M. Zheng, G. Yong, W. Gang, S. Guobin, L. Siyu, S. Dandan, X. Yuanhong and T. Zhigang, "Functional exhaustion of antiviral lymphocytes in COVID-19 patients," Cell. Mol. Immunol. Vol. 17, pp. 533-535, 2020.
31. C. Zhang, Z. Wu, J. W. Li, H. Zhao and G.Q. Wang, "Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality," Int. J. Antimicrob. Agents., Vol. 55, no. 5, pp. 105954, 2020.
32. Z. Xu, L. Shi, Y. Wang, J. Zhang, L. Huang, C. Zhang, S. Liu, P. Zhao, H. Liu, L. Zhu and Y. Tai, "Pathological findings of COVID-19 associated with acute respiratory distress syndrome," Lancet Respi. Med., Vol. 8, no. 4, pp. 420-422, 2020.
33. E. Antalis, A. Spathis, C. Kottaridi, A. Kossyvakis, K. Pastellas, K., Tsakalos, A. Mentis, C. Kroupis and S. Tsiodras, "Th17 serum cytokines in relation to laboratory-confirmed respiratory viral infection: A pilot study," J. Med. Virol., Vol. 91, no. 6, pp. 963-971, 2019.
34. W.T. Ma, X.T. Yao, Q. Peng and D.K. Chen, "The protective and pathogenic roles of IL-17 in viral infections: friend or foe? ," Open Biol., Vol. 9, no. 7, pp. 190109, 2019.
35. C.R. Crowe, K. Chen, D.A. Pociask, J.F. Alcorn, C. Krivich, R.I. Enelow, T.M. Ross, J.L. Witztum and J.K. Kolls, "Critical role of IL-17RA in immunopathology of influenza infection," The Journal of Immunology, Vol. 183, no. 8, pp. 5301-5310, 2009.
36. A. Kudva, E.V. Scheller, K.M. Robinson, C.R. Crowe, S.M. Choi, S.R. Slight, S.A. Khader, P.J. Dubin, R.I. Enelow, J.K. Kolls and J.F. Alcorn, "Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice," J. Immuno, Vol. 186, no. 3, pp. 1666-1674, 2011.
37. W.H. Mahallawi, O.F. Khabour, Q. Zhang, H.M. Makhdoum and B.A. Suliman, "MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile," Cytokine, Vol. 104, pp. 8-13, 2018.
38. P. Mehta, D.F. McAuley, M. Brown, E. Sanchez, R.S. Tattersall and J.J. Manson, "COVID-19: consider cytokine storm syndromes and immunosuppression," Lancet, Vol. 395, no. 10229, pp. 1033-1034, 2020.
39. J.R. Tisoncik, M.J. Korth, C.P. Simmons, J. Farrar, T.R. Martin and M.G. Katze, "into the eye of the cytokine storm," Microbiol Mol. Biol. Rev., Vol. 76, pp. 16-32, 2012.
40. Y.M. Alsofayan, S.M. Althunayyan, A.A. Khan, A.M. Hakawi and A.M. Assiri, "Clinical characteristics of COVID-19 in Saudi Arabia: A national retrospective study," Journal of Infection and Public Health, Vol. 13, no. 7, pp. 920-925, 2020.
41. A. Al-Omari, W.N. Alhuqbani, A.R.Z. Zaidi, M.F. Al-Subaie, A.M. AlHindi, A.K. Abogosh, A.K., Alrasheed, A.A. Alsharafi, M.N. Alhuqbani, S. Salih and M.A. Alhedaithy, "Clinical characteristics of non-intensive care unit COVID-19 patients in Saudi Arabia: a descriptive cross-sectional study," J. Infect., Vol. 13, no. 11, pp. 1639-1644, 2020.
42. M. Barry, A. AlMohaya, A. AlHijji, L. Akkielah, A. AlRajhi, F. Almajid, A. Alsharidi, F.S. Al-Shahrani, N.H. Alotaibi,A. Alanazi and L. Ghonem, "Clinical characteristics and outcome of hospitalized COVID-19 patients in a MERS-CoV endemic area," J. Epidemiol. Glob. Health, Vol. 10, no. 3, pp. 214, 2020.
43. F. Alamri, Y. Alsofayan, Y. AlRuthia, A. Alahmari, Y. Almuzaini, F.A. Gazalah, F. Alradini, T. Alaama and A. Khan, "Predictors of Hospitalization Among Older Adults with COVID-19 in Saudi Arabia: A Cross-Sectional Study of a Nationally Representative Sample. Risk Manag, Vol. 14, pp. 875 ,2021.
44. S. De Lusignan, J. Dorward, A. Correa, N. Jones, O. Akinyemi, G. Amirthalingam, N. Andrews, R. Byford, G. Dabrera, A. Elliot and J. Ellis, "Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study," Lancet Infect. Dis., Vol. 20, no. 9, pp. 1034-1042, 2020.
45. S.F. Jalali, , M. Ghassemzadeh, S. Mouodi, M. Javanian, M. A. Kani, R. Ghadimi and A. Bijani, "Epidemiologic comparison of the first and second waves of coronavirus disease in Babol, North of Iran," Caspian J Intern Med, Vol. 11, pp. 544, 2020.
46. A.A. Khan, Y. AlRuthia, B. Balkhi, S.M. Alghadeer, M.H. Temsah, S.M. Althunayyan, S.M. and Y.M. Alsofayan, "Survival and Estimation of Direct Medical Costs of Hospitalized COVID-19 Patients in the Kingdom of Saudi Arabia (Short Title: COVID-19 Survival and Cost in Saudi Arabia) ," Int. J. Environ. Res. Public Health., Vol. 17, no. 20, pp. 7458, 2020.

47. A.R. Sarhan, M.H. Flaih, T.A. Hussein and K.R. Hussein, "Novel coronavirus (COVID-19) Outbreak in Iraq: The First Wave and Future Scenario," Lancet, Vol. 69, no. 12, pp. 343-346, 2020.
48. E. Goldstein, M. Lipsitch and M. Cevik, "On the Effect of Age on the Transmission of SARS-CoV-2 in Households-Schools, and the Community," J. Infect. Dis., Vol. 223, no. 3, pp. 362-369, 2021.
49. Q.L. Xue, "Frailty as an integrative marker of physiological vulnerability in the era of COVID-19," BMC Med., Vol. 18, no. 1, pp. 333, 2020.
50. R. Liu, H. Han, F. Liu, Z. Lv, K. Wu, Y. Liu, "Feng and C. Zhu, Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan-China," Clinica. Chimica. Acta., Vol. 505, pp. 172-175, 2020.