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Foxp3 serum level in patients with severe nasal and respiratory symptoms due to COVID-19 infection

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Abstract

In this study, (76) cases of confirmed COVID-19 patients were included, of them, (38) severe cases and (38) non severe cases. There were many demographic data included in the present study includes; age, sex, geographic distribution, ABO system, vaccine. All of 76 cases of COVID-19 subjected to detection of ELISA FOXP3 concentration. Out of 38 cases of severe COVID-19, there were 23(30.3%) male and 15(19.8%) female. Out of 38 cases of non-severe COVID-19, there were 24(31.6%) male and 14(18.4%) female. The cases of COVID-19 categorized according to age(years) into 4 categories from 18 to 96 years, and the results show 35(46%) of the non-severe case in 18-40 years, while most severe cases in 41-96 years. The cases of COVID-19 categorized according to geographic distribution into 2 categories Village areas and Urban areas, the results show 29(38.8) of the non-severe case in Urban areas, while most severe cases in Village areas. The cases of COVID-19 categorized according to vaccine into 2 categories vaccinated and non-vaccinated, the results show 23(30.2%) of the non-severe case in Vaccinated, while most severe cases in Non-Vaccinated.

Keywords: COVID-19; FOXP3; Regulatory T-cell; Immune system

1. Introduction

In late 2019, SARSCoV-2, which is new member emerge from the family Corona viridae, infects many persons in Wuhan, China. The virus causes severe pandemic with more than 203 million COVID 19 cases and high mortality rate through out the world on August 11, 2021. 5–10% of patients has severe and critical symptoms which may need hospital admission, mechanical ventilation and have fatal complications although the majority end with good outcome [1, 2]

The immune system has defined and well specified response to various pathogens. Inflammatory pathways usually activated after exposure to these pathogens which is controlled.Although uncontrolled severe inflammatory reaction sometimes result in exaggerated response [3-5] .

Pro-inflammatory cytokines, like interleukin 1 beta (IL-1β), tumor necrosis factor (TNF), interleukin 6 (IL-6),and gamma interferon (IFN-γ), are necessary for the signalling of innate antiviral immune response.Sometimes,the immune respose is exaggerated and can results in severe immune response. IL-1β stimulates the production of cycloxygenase-2 (COX-2) with increament in production of prostanoid which cause inflammation.IL-6 primarly affects pro-inflammatory signaling, regulates massive cellular reactions, and involved in many inflammatory and cancer cases [6,7]. The regulatory T cells (Tregs) controls the immune responsiveness by suppression and down regulation in specific way for the host immune system. The fork head box P3 (FOXP3) protein is aspecific gene found on the Tregs outer surface [8, 9]. This protien plays an essential role in the down regulation of many allergic, inflammatory, and autoimmune diseases, like pulmonary inflammation [10, 11].

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A cytokine storm which is a serious sequel occurs acutely due to sudden icreament of pro-inflammatory cytokines that results in chemotaxis of neutrophils, macrophages, and lymphocytes to the area of infection. Measurement of these cytokines may help in the differentiation between mild, moderate and severe cases [3]. The immune response to viral stimulation in moderate COVID-19 infection, behaves normally in a robust way. While in severe COVID-19 infection, exaggerated and dysregulated immune response lead to complications and bad outcome [12] .

Corona virus as a cause of common cold is firstly recognized in 1960.later on many studies tries to focus on this virus.One of them done 1n 2001 analyzes more than 500 patients presented with flu-like symptoms. Virological study by polymerase chain reaction(PCR) showed that 3.6% of these cases were positive for the HCoV-NL63 strain (PCR)[13] .Corona virus was regarded as a relatively simple, nonfatal virus;until an outbreak in 2002–2003 in Guangdong province in China, and that outbreak spread to different countries,including nearby countries like Taiwan, Thailand,singapore and then worldwide causing severe acute respiratory syndrome with increasing mortality in large numbers of patients. Specialist in infectious diseases studied this form of infection who found that a new form of corona virus is responsible for this outbreak.

In 2012 an outbreak in Saudi Arabia leads to high mortality and affects nearby countries and then spread worldwide, make scientist more interesting to explore this new strains of corona virus[14].

SARS-CoV-2 can spread to human directly (respiratory droplet from infected person) and by indirect contact with contaminated surfaces and airborne particles. Personal protective equipment may participate as a source of airborne transmissions[15].

Patient coughs, sneezes, or even sings or talks can transmit infected droplets to normal person. Droplets cannot cross more than six feet and stay in the air for a short time. However, SARS-CoV-2 remains intact and contagious in droplets. The virus could suspend in the air for three hours[16].

Certain measures like airborne isolation, good ventilation, and use of disinfectant might limit virus spread. Touching SARS-CoV-2 contaminated area, could transmit infection if the hand come in contact with the mouth , nose or eye [17,18] .

In a total of 32 583 confirmed COVID-19 patients , females confirmed to have higher rate of confirmed cases than males, although males shows a tendency todevelop critical and severe illness.Another study carried out in China suggesting a higher crude fatality rate among men in comparism to women (2.8% vs 1.7%)[19]. Among critically ill patients one study shows that more men were affected (67%) in comparism to women (33%). Different factors may implicate for such difference between males and females. One explanation is men were more likely to be current smokers. Also comorbid conditions may play a role for such a difference [20, 21].

Regarding the age, younger people have a lower rate of infection[19, 22], with the rate continued to rise in children and adolescents over the time. Among children and adolescents, infants show higher number of reported infections. Special concerns must be given to highly susceptible people including elderly and young children[23].

During SARS-CoV infection many cytokines are released in high levels like IL-1β, IL-6, IL-8, IL-12, IFN-γ and MCP-1 while others may be found in low level like Th2 cytokine IL-4. MERS-CoV infection increase the level of IFN-γ, TNF-α, IL-15,and IL-17 [24,25].Certain cytokines secreted in higher levels in people who had severe infection like MCP-1, TNF-α, IL-2, IL-6, IL-7, IL-10, IP-10, and granulocyte-CSF in comparism to those with less severe infection. Some of these cytokines shows little or no fluctuations (e.g, TNF-α, IL-6, and IL-10) [26, 27]

The number of lymphocyte shows significant decrease especially $CD8^+$ T cells, while the neutrophil count shows increament especially in patient wirh severe infection. Decrease in number of T cell increased inflammatory reaction due to loss of T cell control on this immune response; while T cell upregulation can decrease inflammation during active infection.The neutrophil-to-lymphocyte ratio (NLR) can be a predictive of outcome in patient with COVID-19 infection. A study done by (*Ong ,et al*,2020) found that most cytokines, with the exception of IL-1, markedly increases after respiratory function nadir, and this indicates that impaired respiratory function may not caused only by cytokine expression in COVID-19 patients.Severity of this infection is related to T cell lymphopenia and active cytokine storm.Identification of patients susceptible to develop severe infection may be based on these finding[28].

Severe clinical symptoms with high mortality rate may result from this cytokine storm especially in critically ill patient. The ability to control this cytokine storm by immune modulator drugs especially in early cases can result in much favourable outcome[29].

The role of regulatory T cells (Tregs) that express the transcription factor FoxP3 is crucial in maintaining self tolerance, immune reaction homeostasis, and to prevent uncontrolled immune responses.These cells use several mechanisms to regulate and control the activation of several lines of adaptive and innate immune systems. These cells also involved in other functions as regulating tissue inflammation, and repair of tissue.The effect of this cell may extend to involve suppression of stimulated cytotoxic immune response in tumor cells by adopting a distinctive phenotype .thus these cells participate in balance of disease progression to mild or severe infection by a definit regulatory role to immune system [30-34]. This research aims to detection of severe and non-severe cases according to different demographic data and correlate this with FOXP3 serum level.

2. Materials and method

2.1. Study design and population

This study was designed as a cross-sectional study. A total of 76 cases of confirmed COVID-19 enrolled in this study from Al-Hillah /Babylon province, during the period from November to December 2021.the patients separated into 2 groups (severe and non severe cases).Severe group has severe nasal and respiratory tract infection symptoms, respiratory frequency ≥ 30/minutes,blood oxygen saturation ≤ 93%,with CT scan chest finding of >50% infiltrate (*Xi zhou, et al ,2020*).The blood samples collected from Marjan medical city and a private laboratory (Al-Mustaqbal Laboratory) and under the supervision of specialist physician.

2.2. Study samples

Three ml of freshly venous blood were collected from the patients. The blood is kept in the gel tube without anticoagulant for the purpose of serum.

2.3. Detection of FOX P3

This ELISA kit involves the technique of the Sandwich-ELISA principle. The micro ELISA plate is prepared by pre-coating by an antibody that is unique for Human FOXP3. Samples (or Standards) are included within the micro ELISA plate wells allowing them to be included with the specific antibody.After that adding of a biotinylated detection antibody specific for Human FOXP3 and Avidin-Horseradish Peroxidase conjugate to the micro plate wells with incubation.Washing a way of any free component. Then each well is provided with substrate solution.Only wells which contain Human FOXP3, biotinylated detection antibody and Avidin-HRP conjugate will show blue color. This reaction is ended through adding stop solution which turns the color to yellow.By spectrophotometer, measuring of the optical density (OD) at a wavelength of 450 nm ± 2 nm. This OD value is proportionate to Human FOXP3 concentration. Lastly, calculation of Human FOXP3 concentration through comparism between the OD of the samples to that of the standard curve.

2.4. Inclusion and exclusion criteria

All samples of COVID-19 cases that included in our study were confirmed by clinical and laboratory criteria and the samples with co infection by any other disease such hepatitis, anemia, renal failure, diabetes mellitus type1, 2 had been excluded.

2.5. Ethical approval

Verbal informed consent was taken from legally authorized representatives before the study, and verbal approval was taken for each case.

2.6. Statistical analysis

Managing of data is done through SPSS version 27 software. The significance tested at probability < 0.05

3. Results and Discussion

3.1. Age and severity of COVID-19

The age of most severe cases were over forty years while most non severe cases were under thirty years. The present study results appear highly significant differences between severe and non-severe cases where p value was less than 0.0001, as shown in table (1).

Table 1 Age distribution with severity of cases

Cases-of COVID-19	Mean ± SD	95% CI	t-statistic	P Value
Severe n=38	42.15 ± 19.69	23.1917 to -9.8041	-4.911	P < 0.0001
Non severe n=38	25.66 ± 6.39			

Several studies tries to assess age related severity of clinical manifestations, a meta-analysis study carried out by Barek et al.,in (2020) that assess the severity in different age groups concluded that elderly or older patients (age ≥50 years) have increasing risk to develope severe manifestations, with direct influense on prognosis and outcome of COVID-19 infection[35].

The virus also affect pediatric age group with over 3.54 million infection cases have been reported although severe infection is less common in comparism to the adult ,even hospital admission, enterance to intensive care unit and death are reported in children and adolescents[36].

3.2. Detection of distribution of age range and number of COVID-19

Different ages are involved in this study, 37 patients (48.7%) were between ages 18-25. The severe cases were 13 (17.1%). and non-severe cases 24 (31.6%). In the age group 26-40 years, the total cases were 19 (25%). The severe cases were 8 (10.6%), while the non-severe cases were 11 (14.4%).in the age group 41-60 years the number of cases were 11 (14.5%). The severe cases were 8 (10.6%), while the non-severe cases were 3 (3.9%).While the ages from 61-96 make a number of 9 (11.9%) Of them the severe cases, 9 (11.9%) and the non-severe cases were 0 (0%). As shown in the figure (4.1).

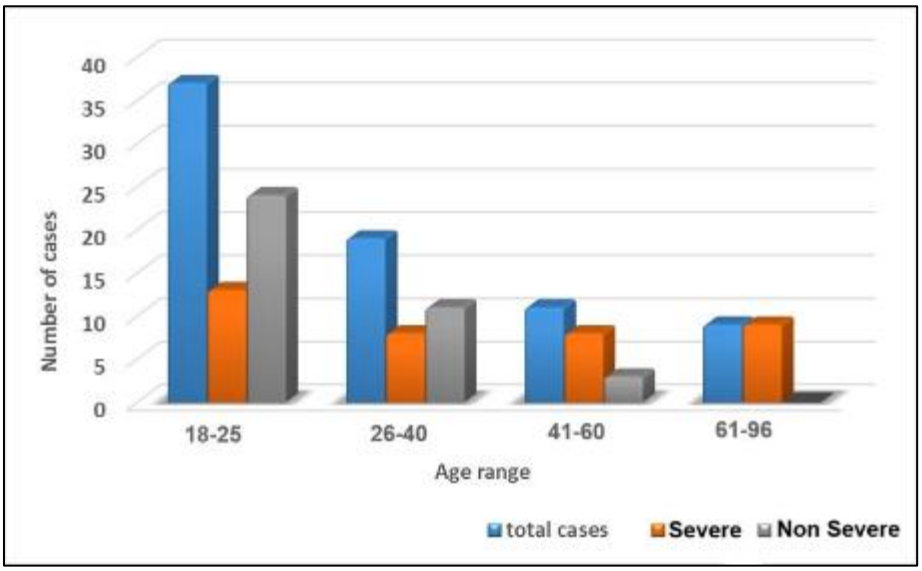

Figure 1 Distribution of age range and number of COVID-19 cases

Some age shifts occur in COVID-19 cases that affect larger proportion of young age in period from June to August as compaired to period from January to May 2020 [37].

In the united states the shift toward younger ages appeared by increase number of confirmed COVID-19 cases, number of positive COVID-19 PCR tests,and rate of disease related emergency department visits. In Europe, a similar shift is noticed through decreasing the median age of COVID-19 cases from 54 years in the period from January to May to 39 years during the period from June to July, and the patients aged 20–29 years made the largest percent of the cases (19.5%) [38].

Studies shows that older patients (≥65 years old) tend to develop more serious disease especially men with higher rate of mortality among this age group [39].

3.3. Detection of Geographic distribution of COVID-19:

The Geographic distribution of most severe cases was in rural area while most non severe cases were in urban. The present study results appear highly significant differences between severe and non-severe cases where p value was less than 0.0046, as shown in table (2).

In a study conducted in South Carolina, it's found that total number of confirmed cases is higher in urban areas although it form lower case rates. Close residency in this area may facilitate virus transmission, resulting in increase the number of confirmed cases of COVID-19 infection.Standardization the cases per 100,000 to the size of population shows that the case rate of confirmed infections from early March to early September was higher in rural areas than in urban areas .This is possibly due to lower accuracy in detecting all COVID-19 cases in rural area with high dependency on private clinics and hospitals. There may be a difficulty to have adequate sites for testing, testing, and contact tracing for halting the transmission of the virus, noticeably in rural areas. From the first seen, the fatality is lower in rural area but with the normalized mortality rates the rural areas were indeed is higher in comparism to urban areas[40,41].

Table 2 Geographic distribution of COVID-19 cases**.**

COVID-19 Cases	Rural areas	Urban areas	Chi square	P value
Total n=76 (100%)	35 (46%)	41 (54%)	0.477	0.4898
Severen=38(100%)	26 (68.4%)	12 (31.6%)	4.412	0.0357*
non severen=38(100%)	9 (23.7%)	29 (76.3%)	8.016	0.0046**

3.4. Detection of Sex distribution in severity of COVID-19:

In our study, the male sex constitutes the most variant both in severe and non severe cases. The present study results appear highly significant differences where p value was less than 0.0001, as shown in Figure (2).

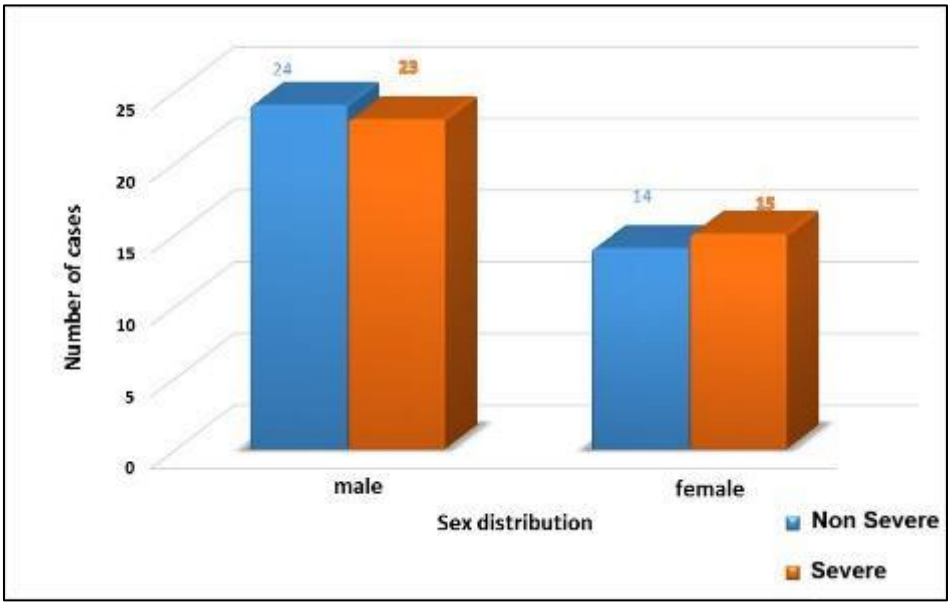

Figure 2 Sex distributions in severity of COVID-19 cases.

One research demonstrates the high mortality among male patient that reach 2.4 times the women mortality.although the male COVID 19 infection forms 56% of cases.In a study held by *Zhang, et al*.,2020 on 140 patient, males forms 50.7 % of the cases,with older patients have high mortality [42].

3.5. Detection of relationship between vaccination and severity of COVID-19:

In our study, the percent of most severe cases were in Non- vaccinated patients while most non severe cases were in vaccinated one. The present study results appear highly significant differences where p value was less than 0.0045, as shown in table (3).

Table 3 Vaccination and severity of COVID-19 cases relationship

COVID-19 cases	Vaccinated	Non vaccinated	Chi square	P value
Total cases n=76 (100%)	35 (46%)	41 (54%)	0.477	0.4898
Severe n=38 (100%)	9 (23.6%)	29 (76.4%)	8.082	0.0045**
Non severe n=38 (100%)	26 (68.4%)	12 (31.6%)	4.412	0.0357*

This results support people to be less hesitate to take vaccine and encourage their trust in efficacy of vaccination. Kreps et al. implies that the efficacy of received vaccine was a very good predictor of vaccine uptake in the United States. Other studies concentrate on attitude of patients to take vaccine and improved health quality following vaccination [43-46].

3.6. Detection of Evaluation of FOXP3 concentration and severity of COVID-19:

The high concentration of FOXP3 of most severe cases was in male. The non severe cases show no great difference between males and females. The present study results appear NO significant differences between severe and non-severe cases regarding FOXP3 concentration, as shown in table (4).

Table 4 Evaluation of FOXP3 concentration and severity of COVID-19 cases

Cases of COVID-19	Male FOXP3 concentration Mean ± SDn=22	Female FOXP3 concentration Mean ± SDn=16	P value	Total FOXP3 concentration Mean ± SDn=38
Severe n=38	2.988 ± 3.334	1.846 ± 4.458	0.3716	2.507±2.742
Cases of COVID-19	Male FOXP3 concentration Mean ± SDn=23	Female FOXP3 concentration Mean ± SDn=15	P value	Total FOXP3 concentration Mean ± SDn=38
Non severe n=38	1.874 ± 2.054	1.756 ± 0.985	0.8373	1.827±1.697
p value	0.1821	0.9397		0.1977

Studies show that altered immune system in critically ill patient is attributed in part to decrease in number of regulatory T cells. The true need for mechanical ventilation is associated with high level interleukin-6 (IL-6). The use of tocilizumab to inhibit IL-6 appears to be safe and of benefit especially in patient develops severe COVID-19 infection that has significant elevation of IL-6 level. An interesting study carried out by Sadeghi to assess the response of Treg and Th17 cells in COVID-19 intensive unit patients and comparing it with healthy controls. A significant decrease in Treg cell number, decrease in FoxP3 mRNA expression level is clarified in those patients. Other cytokines levels like IL-10 and TGF-β are also decreased in those patients in comparison to healthy controls [47, 48].

4. Conclusions

The study concluded low serum level of FOXP3 can be associated with increasing mortality of COVID-19 infection. The study concluded increasing in severe cases of Non-vaccinated patients, while there is a decreasing in the severe cases of the vaccinated patients. The study concluded increasing in severe cases of male compared to female. The study

concluded increasing in severe cases of rural areas compared to urban areas. The study concluded the age of most severe cases were over forty years while most non severe cases were under thirty years.

Compliance with ethical standards

Disclosure of conflict of interest

No Conflict of interest to be disclosed.

Statement of informed consent

Informed consent from all participants was obtained.

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