Statistical correlations between demographical and clinical risk factor with immunological determination of Toll-like receptor markers (TLR2, TLR4 and TLR9) in patient associated with covid_19

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Abstract

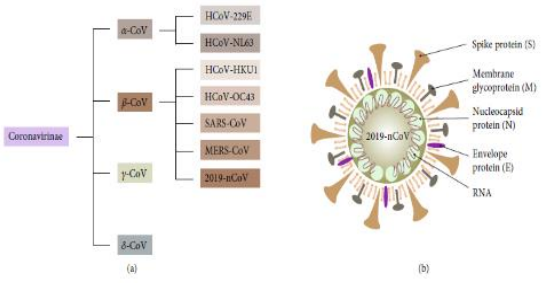
Coronavirus disease–2019 (COVID-19) was detected in Wuhan, China, in December 2019 and has resulted in a 2% fatality rate. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the virus that causes this illness, and it is a newly discovered zoonotic strain. Toll-like receptors (TLRs), which recognize microbial components derived from invading pathogens, are involved in both the initiation of innate responses against SARS-CoV-2 infection and the hyperinflammatory phenotype of COVID-19. Aim: Determine the molecular structure of Toll-like receptor genes and clarify the immunopathogenesis of TLRs associated with covid 19 to provide insight into the role and early detection of covid 19. Results: are the highest age group of patients with covid_19 was the decades (45-54) years were 26 (29.5%) from total study patients. In immunological study there are only TLR2 and TLR9 statistically significantly between patient and control groups. The study found no significant positive effect of smoking and non-smokers. Conclusion: The most common clinical symptoms of covid_19 were Fever, Headache, Muscle/ body ache and Loss of taste and/ or smell. There are positive correlation between toll like receptor 9 (TLR9) and smoking patients.

Keywords: covid_19, TLRs, CRP, D dimer.

1. Introduction

Coronavirus disease 19 commonly called as COVID-19 is caused by a recently identified beta coronavirus classified as severe acute respiratory syndrome coronavirus 2. (SARS-CoV-2).This virus only contains a single strand of RNA.After the first incidence was detected in December 2019 in Wuhan, China, COVID-19 has swiftly spread over the globe and become pandemic, with over 563 million individuals infected and over 5 million fatalities in over 200 nations. Since the beginning of the century, the COVID-19 outbreak is the third CoV outbreak. [1]. The first pandemic, which started in China in November 2002 and concluded in July 2003, was caused by SARS-CoV, resulting in 8,422 cases reported and 772 mortality in 26 countries. [2]. The second one appeared in Saudi Arabia in June 2012 and was caused by a novel subtype of the Middle East respiratory syndrome coronavirus (MERSCoV) [3].The MERS-CoV epidemic continues, with 2,499 cases and 858 deaths reported in 27 countries as of December 2019 [1].

The molecular structure of SARS-CoV-2 has four key proteins: the spike (S), envelope (E), membrane (M), and nucleocapsid (N). SARS-CoV-2 shares approximately 80% of its genetic sequence with SARS-CoV the virus causative for the pandemic of SARS in 2002. [4]. Despite the similarities the Severe acute respiratory S protein which enables the virus to connect to the ACE2 receptor is many amino acids lengthier than the SARS-CoV S protein. It is possible that this is the reason why COVID-19 has expanded so rapidly over the globe, in contrast to SARS, which was immediately restricted to its original location.[5]. Human angiotensin-converting enzyme 2 (ACE2) is a type I membrane protein that binds viral S proteins. SARS-CoV-2 binds to ACE2 10 to 20-fold more strongly than SARSCoV, which may explaining reason human-to-human transfer is simpler.



Figure(1) (a) A classification of the genera of coronaviruses (b) A schematic representation of the CoV-2019 [6]

The clinical presentation of SARS-CoV-2 infection might vary greatly from patient to patient. [7], Approximately 80% of patients had moderate illness 14% had significant signs or symptoms, such as shortness, hypoxia, or lungs infiltrates encompassing more than fifty percent of the parenchyma of the affected lung(s), and 5% had indications suggestive of severe illness, including shocks, respiratory failure, or multiple organ failure. Notably, the

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total mortality rate for the group was 2.3%.[8]. Fever, a dryness cough, and exhaustion are often the symptoms that are experienced by the majority of people in the early stages of a COVID-19 infections. [9]. Less common symptoms include nausea or vomiting, muscular or joint pain, dry mouth, lack of odour, rhinitis, conjunctivitis, headaches, different skin rashes, diarrhoea, chills, and disorientation. [10]. In the advanced stages of the illness, the patients will have severe difficulty breathing, decreased blood oxygen (hypoxia), damage of the lungs, and various organ failure. [11]. Other consequences of COVID-19 sickness include more extreme and uncommon neurological symptoms such as stroke, encephalitis, insanity, and nerve damage. [12].

Transmission can occur by coughing and sneezing and inhaling droplets as well as direct contact with the mouth, nose, and eyes. [4]. from Human-to-human occurs through common ways such as contact transmission, direct transmission, and airborne transmission [13]

Viral life cycle and replication begin with viral integration onto ACE2 receptors in mucosal, pulmonary, cardiac, and renal epithelia. Age-related ACE2 expression increases adult ACE2 receptors [14]. Antigen-presenting cells (APCs), which include dendritic cells and macrophages, are able to take in the virus and then pass it along to T cells for processing. APCs are responsible for the induction and differentiation of T cells, as well as the following massive release of cytokines once these processes have taken place [15]. The innate immune system of the host Utilizes pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) to identify viral components. The production of inflammatory factors that can mediate lung inflammation and fibrosis is stimulated by TLR binding [16].

The ten members of the TLRs family (TLR1–TLR10) are expressed on macrophages, epithelial cells, and fibroblast cells. Multiple pathogen-associated molecular patterns (PAMPs) from bacterial, virus, and other foreign pathogens may activate TLR. [17]. TLRs initiate innate immune activity by producing inflammatory cytokines, IFN, and other mediators. TLR 1, 2, 4,5, 6, 10 are cell surface receptors; TLR 3, 7, 8, and 9 are endosome receptors.[18]. Dendritic cells (DCs), macrophages, natural killer cells, adaptive T cells, and B cells express TLRs. TLR3 recognises double-stranded RNA, TLR4 recognises lipopolysaccharide, TLR7/8 recognises single-stranded RNA, and TLR9 recognises unmethylated CpG DNA [19]. TLR activation through MyD88- and TRIF-dependent pathways causes nuclear translocation of NF-B, IRF-3, and IRF-7, resulting to the production of pro-inflammatory cytokines (IL-1, IL-6, TNF-) and type I IFN-/, which are necessary for anti-viral responses [20].

2. Materials and Methods

2.1 Sampling and sources

Cases for the case-control research came from the Al Hussein Teaching hospital in Dhi Qar in southern Iraq. All patients who participated had a positive nasopharyngeal swab result from RT-PCR as well as a positive CT scan and/or X-ray result.

2.2 Hematological assays methods:

In the haematology laboratory at Al Rifai General Hospital five par, haematological parameters were run on EDTA blood utilising (SPINREACT / china) to estimate the quantities and percentages of white blood cells. An EDTA tube was used to collect the whole blood sample. Total white blood cells, monocytes, lymphocytes, neutrophils, eosinophils, basophils, and platelets were measured with the use of an automated haematology analyzer (SPINREACT / spin).

2.3 The estimation C reactive protein test assay

The UPT Analyzer will be warmed and stabilized for 20 minutes. Press setting button on screen of device and choose read parameter. Enter, scan the barcode, insert the test card to get the result. The result that was obtained after five minutes

2.4 The estimation D dimer rapid quantitative test

Finecare's FIA system requires that you inserted your ID chip the pipette was used for sampling. 15 pL of whole blood or 10 pL plasma was collected, and then added to the Detection Buffer tube. Results presented on the main screen and may be printed using the "Print" button.

2.5 ELISA Method for Serological Detection of Toll-Like Receptors

Using 96-well microplates, a Sandwich ELISA test was carried out in order to determine the titer of Toll Like Receptors (2,4, and 9) in the serum samples taken from patients as well as controls. MybioSurce: Reagents, serum, standard, controls and the pre-coated plate were carried before reaching room temperature. The plate was sealed and incubated for 90 minutes at a temperature of 37 ° C. A 100µl Biotin-labeled antibody solution for all plate wells at the bottom, without touching the sidewall, was added and covered for 60 minutes, at 37°C. A TMB Substrate was equilibrated for 30 min at 37 °C before adding into wells to plot a standard curve for eachToll Like Receptor detecting test.

3. The Results

3.1 Demographical study

A case control study was carried on an overall instances of covid 19 patients were (88) that obtained from Al hussein Teaching hospital in Dhi Qar province through period of 14th october 2021 to 1st June 2022, thier age were arounded (29 - 73) years. Taked (88) individuals regarded as control group were checked and confirmed to be free from any respiratory diseases or any other health problems.

distribution of covid_19 patients and controls within age groups:

Table (3-1) The highest age of patients with covid 19 was (45-54) years with 26 (29.5%) from total study patients 88 (100%), followed by (55-64) years with 21 (23.9%), (35-44) years with 20 (22.7%), (65) years with 12 (13.6%), and (30) years with 9 (10.2%). (100.0 percent). This difference was non-significant (p=0.128*).

Table (1): Distribution of studied group according to age group (Years)					
Categorial age group (Yrs.)		Studied Groups		Total	P-value
		Patient	Control		
ss	Count	9	11	20	0.128*
	%	10.2%	12.5	11.3%	
31-40	Count	20	28	48	
	%	22.7%	31.8%	27.2%	
41-50	Count	26	25	51	
	%	29.5%	28.4%	28.9%	
51-60	Count	21	17	38	
	%	23.9%	19.3%	43.2%	
> 60	Count	12	7	19	
	%	13.6%	7.9%	10.8%	
Total	Count	88	88	176	
	%	100%	100%	100%	
covid_19 patients and controls distribution within Gender					

In Table (2) from 88 study patients, 47 (53.4%) males and 41 (45.8%) females had covid 19 (100.0 percent). 38 (43.2%) male research controls against 50 (56.3%) female. This difference (sig = 0.451) was not statistically significant.

Table (2): Distribution of studied group according to gender group					
Gender		Studies groups		Total	sig
		Patient	Control		
male	Count	47	38	85	0.451**
	%	53.4%	43.2%	48.3%	
Female	Count	41	50	91	
	%	45.8%	56.3%	51.7%	
Total	Count	88	88	176	
	%	100%	100%	100%	
covid_19 patients and controls distribution within Smoking. covid_19 patients and controls distribution within Smoking.					

there was no any significant statistical difference in smoking and nonsmoking among Patient and control groups.

Table (3): Smoking of the study population				
Characteristic	Patient	Control	Total	Sig.
Smoking				
Yes	31	22	53	0.454**
	35.2%	25.0%	30.1%	
No	57	66	123	
	64.7%	75.0%	69.9%	
* Mann Whitney U Test ** Chi2 Test				

correlation of (TLRs) with demographical study.

there was no any significant statistical difference in Toll like receptors levels between male and female groups, in patients group as show in Table (4). Also, there were no significant statistical differences in the levels of Toll like receptors between smokers and non-smokers except in patients' TLR9 show in Table (5)

Table (4): Differences in Toll like receptors according to sex in patients group				
Patients Sex:		TLR2	TLR4	TLR9
Male	N	47	47	47
	Mean	1.12310	1.00946	30.76764
	Std. Deviation	0.898710	1.538786	6.138672
Female	N	41	41	41
	Mean	1.16945	0.96579	29.06015
	Std. Deviation	1.127377	1.463145	6.209694
Sig.*		0.756	0.852	0.229
* Mann Whitney-U Test				

Table (5): Differences in Toll like receptors according to smoking in patients group.				
Patients Smoking		TLR2	TLR4	TLR9
Yes	N	31	31	31
	Mean	1.09172	1.43640	32.61416
	Std. Deviation	1.018429	2.034140	6.211205
No	N	57	57	57
	Mean	1.17234	0.75170	28.58657
	Std. Deviation	1.004368	1.058733	5.762297
Sig.*		0.723	0.312	0.012
* Mann Whitney-U Test				

3.2 Clinical study

Clinical symptoms

Table (6) presents the distribution of clinical features among patients. The most frequent symptom was fever (82.9%) and the least was diarrhea (21.6%).

Table (6): Clinical features of patients		
Feature	Number*	Percent*
Fever	73	82.9
Headache	62	70.4
Muscle/ body ache	59	67
Loss of taste and/ or smell	45	51.1
Cough	44	50.0
Shortness/ difficulty of breathing	32	36.3
Congestion or runny nose	33	37.5
Chest pain	28	31.8
Nausea or vomiting	24	27.2
Diarrhea	19	21.6
* A patient may have more than one symptom. Toll like receptors levels associated with the certain symptoms.		

By testing the differences in the Toll like receptors levels associated with the presence of certain symptoms: fever, cough,shortness/ difficulty of breathing,muscle/ body aches,loss of taste and or smell, headache, nausea with/without vomiting, congested/ runny nose, diarrhea, and chest pain, the only significances documented were the presence of headache and the presence of congested/ runny nose.

Table (7) Toll like receptors levels associated with the certain symptoms							
Clinical symptoms	%	Mean±SD			p. value		
		TLR2	TLR4	TLR9	TLR2	TLR4	TLR9
Fever	82.9	1.18±0.98	1.03±1.58	30.27±5.88	0.44	0.59	0.48
Headache	70.4	1.18±0.94	0.92±1.456	31.15±5.435	0.278	0.507	0.022
Muscle/ body ache	67	1.16±0.98	0.91±1.44	30.25±6.01	0.566	0.291	0.731
Loss of taste and/ or smell	51.1	1.10±1.00	0.89±1.49	30.23±5.96	0.616	0.235	0.978
Cough	50.0	1.17±0.93	1.35±1.94	31.15±6.11	.521	.813	.063

Shortness/ difficulty of breathing	36.3	1.45±1.21	0.88±1.27	28.93±6.20	0.137	.277	.207
Congestion or runny nose	37.5	0.75±0.59	1.06±1.25	29.77±6.05	0.044	0.349	0.740
Chest pain	31.8	1.36±1.25	1.05±1.39	28.93±6.31	0.526	0.121	.234
Nausea or vomiting	27.2	1.25±1.11	1.26±1.89	28.48±5.17	0.753	0.606	0.175
Diarrhea	21.6	0.79±0.77	0.82±1.11	29.30±5.76	0.113	0.850	0.551

3.3 Laborotary study

Comparison of leukocyte among studied groups

Table (8) clarifies that the only significant statistical differences were in eosinophil and basophil levels when compared between patients and controls.

Table (8): Comparison of leukocyte indices between patient and control groups

Category		WBCs (109/L)	Neu (109/L)	Lym (109/L)	Mon (109/L)	Eos (109/L)	Bas (109/L)
Patient	N	88	88	88	88	88	88
	Mean±SD	8.90±3.81	5.86±3.01	2.33±1.24	0.72±0.42	0.04±0.15	0.0006±0.002
Control	N	88	88	88	88	88	88
	Mean±SD	7.70±1.72	4.18±1.03	2.23±0.61	0.86±0.36	0.36±0.23	0.07±0.09
Sig.*		0.463	0.061	0.705	0.164	0.0001	0.0001
* Mann Whitney-U Test							

Comparison of D dimer and CRP among studied groups

In Table (9), it is shown that D-dimer and CRP levels differed statistically significantly between patients and controls.

Table (9): Comparison of D-dimer and CRP indice between patient and control groups

Category		D-dimer (ng/ml)	CRP(mg/L)
Patient	N	88	88
	Mean±SD	1489.70±1947.52	53.16±39.01
Control	N	88	88
	Mean±SD	233.19±113.40	5.97±7.60
Sig.*		0.0001	0.0001
* Mann Whitney-U Test			

3.4 Immunological study

Comparison of TLR markers between patient and control groups

From Table (10), it can be clarified that TLR2 and TLR9 statistically significantly between patient and control groups.

Table (10): Comparison of TLR markers between patient and control groups

Category		TLR2 ng/ml	TLR4 ng/ml	TLR9 ng/ml
Patient	N	88	88	88
	Mean±SD	1.14±1.00	0.99±1.49	29.98±6.19
Control	N	88	88	88
	Mean±SD	2.33±0.80	0.32±0.32	24.22±4.27
Sig.*		0.0001	0.056	0.0001
* Mann Whitney-U Test				

4. The Discussion

Since its identification at the end of 2019, the 2019 Coronavirus Disease (COVID-19) has become a global public health concern. The predominant site of infection for coronavirus 2 in severe acute respiratory syndrome(SARS-CoV-2) is the lower airway [21]. The current outbreak of coronavirus disease 2019 (COVID-19) is a global emergency because of its quick spread and high fatality rate, both of which have caused significant disruptions. [22].

Demographical factors (Risk factors)

A case control study was carried on an overall case of covid_19 patients were (88), thier age were arounded (29 - 73) years. In addition to (88) persons observed as control group, in this investigation the highest age group of patients with covid_19 was the group (45-54) years were 26 (29.5 %) from patients total 88 (100.0%), followed by the group (55-64) years were 21 (23.9 %) from patients total, at the group (35-44) years they were 20 (22.7%), the group (≥ 65) years covid_19 patients were 12 (13.6%), while less cases of covid_19 patients appeared at (25-34)were 9 (10.2%) from total study cases 88 (100.0%).The results agree with Dhuaif [23] that observed the Patients were separated into three age groups (15–45 years old, 46–65 years old, and 66–85 years old), with 169 (30%), 235 (43%) and 156 (27%) patients in each age group, respectively. [23]. Other study of Huang et al. [24] saying that In general, individual are susceptible to COVID-19; however, those who are older or in the middle years of their life are more likely to be affected by COVID-19 (median age of onset is about 55 years) [25].

In this investigation, the most incidences of covid 19 were documented among male groups 47 (53.4 percent), compared to the female group's 41 (45.8 percent), out of a total of 88 patients who participated in the study (100.0 percent). The greater mobility of men in comparison to women, particularly when they are required to go out to work, may be to reason for the larger incidence of COVID-19 instances found in men. In addition to the possibility of movement within the workplace, biologically speaking, men are more prone to contracting infectious diseases. according to results that supported with study of Hikmawati et al. [26] Males made up the vast majority of COVID-19 cases (56.5 %) [26].the study of Rabaan et al. [4] agree with this study by showed males (58%) have higher risk factors for covid_19 than females (42%) [4].

In this study the most cases of covid_19 recorded non_smoking 57 (64.7%versus smokers were 31 (35.2%)from total study cases which reflects no significant positive effect of smoking among studied patients groups(p value =0.454). this results supported by [27] observed that the number of patients who have never smoked is greater than the number of smokers. Ten studies conducted in China found that the prevalence of smoking among hospitalised covid 19 patients ranged from 3.8 percent to 14.6 percent, but the prevalence of smoking among the general Chinese population is 27.7 percent. A low smoking prevalence was noted among hospitalised patients in both Korea and the United States. [28].

5. Clinical factors

In the current study that the most clinical symptoms was fever (82.9%) and the least was diarrhea (21.6%).,Headache(70.4%),Muscle/ body ache(67%),Loss of taste and/ or smell(51.1%),Cough(50%),Congestion or runny nose(37.5%),Shortness/ difficulty of breathing (36.3%),Chest pain(31.8%),Nausea or vomiting (27.2%). In line with the findings of some other studies, which came to the conclusion that clinical symptoms of COVID-19 comprised fever, cough, lethargy, pneumonia, headache, diarrhoea, hemoptysis, and dyspnea. (Hikmawati & Setiyabudi, 2020).other study of Rabaan (2020) reported that The majority of the clinical manifestations were comparable to those seen in SARS-CoV infection, dry cough (67.7%), fever (87.9%), myalgia (34.8%) and fatigue (69.6%). A few infected patients also presented rhinorrhoea,, diarrhea and pharyngalgia (Rabaan et al., 2020).Some showed dyspnea and hypoxemia, which eventually could lead to acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndromes (MODS) in one week [29]. In case series involving COVID-19, fever, dyspnea, shortness of breath and coughing, were identified as the symptoms that were reported the most frequently. A confusion, sore throat,, anosmia, dysgeusia, myalgia, diarrhoea, nausea, odynophagia, vomiting, headache, and are some of the other symptoms that may be present. [30].

laboratory study

Viruses rarely raise white blood cells. Neutropenia is seen in individuals with Herpes viruses (EBV, CMV, HHV6, and HSV), respiratory viruses (, influenza A and B, RSV, and parainfluenza), and hepatitis viruses, as well as measles,chickenpox, and German measles (Rubella). Neutropenia occurs early in viral illness and lasts 3–8 days. [31]

The findings of this study demonstrated that COVID-19 patients with an elevated leukocyte count had a greater number of neutrophils and lymphocytes in their peripheral blood. the only significant statistical differences were in eosinophil and basophil levels when compared between patients and controls. Other study [32] reported that Monocyte and eosinophil counts linked inversely with sickness severity. Basophil numbers didn't affect COVID-19 severity. NLR and NMR were positively linked with COVID-19 illness severity. In severe cases of COVID-19 pneumonia, neutrophilia due to the cytokine storm and/or lymphocytopenia were associated with a poor prognosis. Neutrophil to lymphocyte ratio (NLR) predicts early SARS CoV-2 illness severity. In tiny trials, severe patients had fewer granulocytes than non-severe patients. [33].

Our study shown that D-dimer and CRP levels differed statistically significantly between patients and controls. [34] reported that CRP and D dimer are a significant marker of COVID-19 inflammation. Higher levels of CRP and D dimer are associated with higher mortality in people with severe COVID-19 disease. And supported by [25] showed that Elevated serum concentrations of CRP, PCT, D-dimer, and ferritin were related with an increased risk of mortality and severe COVID-19. Other research indicated that the blood CRP and D-Dimer concentrations (for diabetic patients) were favourably connected with the age of COVID-19 patients; however, no significant correlation was observed when comparing the prevalence of COVID-19 diseases by geographical region. [35].the study of Milenkovic et al. [34] was found that substantial abnormalities of laboratory parameters were found in patients with COVID-19 who were being treated in the intensive care unit. There was an increase in the levels of IL-6, CRP, PCT, and D-dimer. [34]

TLRs (2,4,9) as a diagnostic marker for covid_19

The most recent findings suggest that the detection of PAMPs from SARS-CoV-2 requires the participation of a number of different TLRs. It has been hypothesised that TLR2, TLR3, TLR4, TLR7/8, and TLR9 all play a role in the antiviral responses that the body mounts against SARS-CoV-2 infection. [36]. In the current investigation, three different TLR receptors (TLR2, TLR4, and TLR9) were chosen so that their associations with covid 19 could be investigated.

Toll-like receptors 2

Toll-like receptor (TLR)2 acts as a sensor of SARS-CoV-2 and other -coronaviruses, which stimulates the production of inflammatory cytokines and may contribute to the downregulation immune response seen in individuals with severe COVID-19. [37]. In this study we shown that the mean concentration of TLR2 (1.14±1.00) among patient and its mean concentration (2.33±0.80) among control. According to this results there are statistically significantly between patient and control groups.in study of [38] observed that The first genetic evidence that TLR2 can sense -coronavirus infection by recognition of the E protein to activate release of inflammatory cytokines including TNF- and IFN, and the activation of TLR2 signalling was independent of viral entry and replication. The same study [38] observed that Similarly to what was seen with MYD88 expression, the pattern of TLR2 expression improved with severity of COVID-19. This phenomenon was also detected with MYD88 expression, which exhibited a positive link with the pathogenesis of COVID-19..other study [39] found that Due to the fact that the SARS-CoV-2 S protein is a strong viral PAMP that is implicated in the generation of inflammatory cytokines and chemokines via TLR2-dependent activation of the NF-B pathway, it is a vital addition to the enormous scientific effort that is being put forth to combat COVID-19.

Toll-like receptors 4

TLR4 is a remarkable pattern recognition receptor (PRR) that belongs to the innate immune system. It identifies various pathogen-associated molecular patterns(PAMPs) from bacteria, viruses, and other diseases [40]. During the course of these investigations, it was discovered that the mean concentrations of TLR4 (0.99±1.49 ng/ml) among patients of covid_19 and (0.32±0.32 ng/ml) among control. there is no statistically significantly between patient and control groups. Aboudounya et al. [40] explained that SARS COV-2 most likely utilises TLR4 to enter cells and/or to enhance ACE2 expression. According to the biocomputational analysis of the S spike to SARS-COV-2 glycoprotein, the virus may be using TLR4 signalling

to induce the expression of ACE2 (or another receptor) through which it enters the same or surrounding cells. [40]. Choudhury et al. [41] demonstrated that TLR4 exhibits the strongest protein-protein interaction with the SARS-spike CoV-2's glycoprotein when compared to the other TLRs. [41]

Toll-like receptors 9

Toll-like receptors (TLRs) are a family of 13 conserved transmembrane receptors that play a prominent role in regulating innate and adaptive immune responses against invading bacteria, fungi, viruses, and parasites [42]. During the course of these investigations, it was discovered that the mean concentrations of TLR9 (29.98±6.19 ng/ml) among patients of covid_19 and (24.22±4.27ng/ml) among control. there is statistically significantly between patient and control groups.the study of Zhong et al. [2] was foud that TLR9 expression was considerably increased in patients with severe COVID-19 infection. In contrast, TLR3 expression did not correlate with COVID-19 disease progression, while TLR7 expression was only elevated in individuals with mild COVID-19 disease [38].

6. Conclusion

TLRs are important constituents of the immune system and recognize a wide variety of PAMPs that are conserved molecular signatures of bacteria, bacteria, and viruses. Of the TLRs that have been identified, six represent a subclass that recognizes viral ligands. These immune responses often contribute to viral clearance and disease resolution but sometimes can be harmful to the host. The most common clinical symptoms of covid_19 were Fever, Headache, Muscle/ body ache and Loss of taste and/ or smell. There are positive correlation between toll like receptor 9 (TLR9) and smoking patients.

7. Recommendation

Carrying out other studies that explain the reason for multiorgan dysfunction in patients covid_19, conducting a comparative study between vaccinated and unvaccinated of covid_19 patients using other biomarkers and conducting a study showing how dangerous infection with covid_19 is for pregnant women and their fetus.

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