Relation between severity of re-infection and vaccination with first and second dose Covied-19 virus

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Abstract :

Avidity is consider one of the important and significant manifestations of the humeral immune response to virus covid-19), representing the binding strength antigen with antibody, in many viral infections low avidity consider risk factor for reinfection. While many of the infected people have mild symptoms or no symptoms, there is a group of patients have strong symptoms and need intensive care. Imbalance in cytokine production is the main mechanism for disease progression, an increase of selected pro-infammatory and anti-infammatory cytokines correlate with severity of disease, Therefore, assessing the level of certain cytokines can give an indication of disease progression and thus provide an accurate protocol of treatment. The purpose of this paper was to study the relation between severity of reinfection and vaccination by compression the values of antibody level and IgG avidity, also measure certain cytokines (IL-10, IL-6, IL-8, IL-7, IL-2, IFN-γ, TNF-α, IL-1β, TGF-β) in vaccinated reinfection patients (with first and second dose) and non-vaccinated reinfection covid-19 patients. Blood samples were pulled in (first, third and sixth) months after infection from 60 reinfection patients with covid-19: 30 patients were vaccinated with first and second dose, they were asymptomatic or show mild symptoms and 30 patients were non-vaccinated they have severity symptoms. The serum were obtained from all patients and evaluated for the Antibody levels, IgG-avidity, also inflammatory and proinflammatory cytokines were analyzed. There are clearly differences in all evaluated parameters were observed when comparing severe (group B non-vaccinated) and mild (group A vaccinated). The vast majority of samples contained **s** and **n**-antibodies (82%, 80% respectively in group A n=30 and 80%, 81 respectively in group B n = 30), level of total n-antibodies in group A clearly rises within one to three months (median value: 20.8, 31.2 s/co $p<0.001$), and clearly decreases significantly through three to six months (median value: 16,1 $p<0.001$), also in group B the levels increase then decrease (18.3, 22.4,14.8 respectively, $P<0.001$). there is clearly decrease in the level of (IgG) anti-spike (30.2, 21.4 and 16.2 RU/ml, $p<0.001$) were in group A also in group B (23.6, 18.4, 15.8 $P< 0.001$), there were a significant increase in the IgG-avidity index (median values 48.6, 56 and 61%, $p<0.001$) in group A were observed from (1 to 3 to 6) months also in group B (32.4, 41.2, 49.3 $P<0.001$), at the same time a significant differences in IgG-avidity index were shown between A and B groups. There was significant differences at ($P<0.001$)for all tested cytokines between the group A (vaccinated mild

reinfection patients) and group B (non-vaccinated sever reinfection patients), group B had significantly higher serum IL-10, IFN-γ, IL-6, IL-2, IL-1β, IL-8, TGF-β concentrations than group A. But the level of IL-7, TNF-α significantly decrease in group A as compared to group B as shown in (table 6). our results indicates that the vaccination that precedes the infection prepares the body to be a strong immune environment to resist the virus, represented by strength of binding or affinity between antibody and antigen, An inhibitors for some selected cytokines may be a good idea to alter induced hyperinflammation (cytokine storm) then prevent disease progression and dysfunction of many organ that contribute to an increasing mortality of covied-19 patients.

Key words: vaccination, re-infection, Avidity

الخلاصة

يعتبر انخفاض قوة الارتباط بين الجسم المضاد والجسم الغريب عامل خطر لتكرار العدوى. ان عدم التوازن في إنتاج السيتوكين هو الآلية الرئيسية لتطور المرض حيث يرتبط زيادة بعض السيتوكينات المؤيدة للالتهابات والمضادة للالتهابات بشدة المرض ، لذلك فإن تقييم مستوى بعض السيتوكينات يمكن أن يعطي مؤشرًا على تطور المرض وبالتالي يوفر بروتوكولًا دقيقًا الغرض من هذه الدراسة هو معرفة العلاقة بين شدة الإصابة الثانية والتلقيح عن طريق تقيم مستوى الاجسام المضادة .للعلاج (IL-1β , IFN-γ , TNF-α , IL-2 ، IL-7 ، IL-8 ، IL-6 ، IL-10 ، وكذلك قياس بعض السيتوكينات (IgG ونوعية اصابة ثانية ومستكملين للجرعة الأولى والثانية من اللقاح والمرضى المصابين بفيروس كوفيد -19 في المرضى المصابين تم تم سحب عينات الدم في الأشهر (الأول والثالث والسادس) بعد الإصابة من 60 مريضًا مصابًا بفيروس كرونا.غير الملقحين تطعيم 30 مريضًا بالجرعة الأولى والثانية ، ولم تظهر عليهم أعراض أو ظهرت عليهم أعراض خفيفة وكان 30 مريضًا غير مصابين بالعدوى. تم الحصول على المصل من جميع المرضى وتم تقييم مستويات الأجسام المضادة ، وتم تحليل IgG-avidity ، وكذلك بعض السيتوكينات . لوحظ وجود اختلافات واضحة في جميع المتغيرات المقيمة عند المقارنة الشديدة (لجميع P <0.001(المجموعة ب غير الملقحة) والمعتدلة (المجموعة أ الملقحة). كانت هناك فروق ذات دلالة إحصائية عند (مرضى عودة العدوى الحادةB (مرضى عودة العدوى الخفيفة الملقحة) والمجموعة **A**السيتوكينات المختبرة بين المجموعة

introduction

Covid-19 declared to be pandemic in march 2020 by WHO. From that time, there are 179 people infected with Corona in the world, including 8 million have died [1]. The treatment is represented with supportive care , such as oxygen in high dose, supplement tocilizumab and corticosteroids have some effect [2]. Recently, vaccination have contribute to limited spread of the virus. All immunological manifestations, whether after infection with the virus or after vaccination, are important indicators for studying the disease and limiting its epidemiology. within three weeks after infection with SARS-CoV-2 the humoral immunity stimulates to make antibodies to attack spike-glycoprotein (S) also nucleocapsid protein (N) [3] .The humoral response evaluated by the avidity of the antibodies also by the quantity, and the neutralizing capacity of the antibodies, avidity is the determination of the intensity of binding between

antigen and antibody [4]. During the initial response to a viral infection the avidity is low, then increases with time [5]. The decline avidity of IgG is involved to rise risk of re-infection with virus [6]. Eidge et al. have illustrate that the declining percentage of antibodies against corona virus is correlated with a re-infection after one year in high probability [7]. So that the avidity and Antibody levels can be used as values to determine the risk of re-infection, either after vaccination or after the first infection.

COVID-19 infections including two parts of immune response; start with incubation (non-severe stages), to control over virus proliferation specific adaptive immune response is necessary to eliminate the virus, so that to prevent the progression of disease. Then, in some patients with specific genetic background, comorbidities and older age the cytokine causes syndrome (cytokine storm) leading to dysfunction and severe status [8]. since the host immune system is on one side necessary to resistance and limited of COVID-19 infection, but on the other play important role in the pathogenesis and disease prognosis [9], the immune system activated by COVID-19 by different receptors; (TLR-3, 7, and 3) Toll-like receptors the binding between TLRs and COVID-19 leading to produce IL-1β and IL-6 whose are (the central proinflammatory molecules) leading to clinical symptoms and lung inflammation [10]. The elevation of other chemokines and cytokines (IL2R, IL-8, IL-10, TNF-α and IL-6) been observed in severe patient as compare to mild cases [11,12], also higher concentrations of inflammatory markers observed in severe COVID-19 patients as compared to moderate patients [11], the incrase level of IL-6 was most important predictors in non-survivors cases [13]. from the above information it can be suggested that, in addition to antiviral treatment, control in immunosuppression for example by using the inhibitors for selective cytokines could be an effective way to repel the disease and prevent its complications then prevent severe and dysfunction of organ that involved in high mortality of COVID-19 patients [10,14].

The vaccine improves the immune response to various types of virus which the infected person this illustrated when Sera from the persons after vaccination showing a height neutralization response to the Beta variant, so that the vaccination can provide improved protection for previously infected persons. [15]

Material and method

Study design: This study including 60 re-infection Covied-19 patients, (they were PCR-confirmed covid-19), 30 vaccinated both first and second dose (11 females 19 males), and 30 non-vaccinated (14 females, 16 males), age range was (21–53 y) in both groups, all vaccinated re-infected patients were asymptomatic or mild symptoms whereas all non-vaccinated re-infected patients have severe symptoms, patients were divided into two groups: group A (covied-19 mild) contain patients whose oxygen saturations level was (95%) or higher, throughout the illness, they did not need to enter intensive care, while group B (covied-19 severe) contain patients whose oxygen saturations level was less than (92%), also the ratio of (PaO2)/ (FiO2) was ≤ 300 mm Hg and need to enter intensive care [16]. Blood samples drawn at 1, 3 and 6 months from 60 patients

with covid-19 patients obtain from the private specialized laboratories in Karbala Governorate, their phone numbers were selected, and they were contacted to agree on the dates of blood withdrawal. Then centrifuge all serum separator tubes and stored at − 80 °C. The serum was tested for the following parameters:

-Antibody detection : detection antibodies against N-protein were evaluated by (ElecsysAnti-SARS-CoV-2) assay uses a recombinant protein representing the nucleocapsid (N) antigen for the limitation of antibodies against SARS-CoV-2. Results are mentioned as cut-off index (signal sample/cut-off, s/co) with values >1 considered as positive, Serum samples from 2014 (n=20) conceder as as negative controls. SARS-CoV-2 antibodies against S-protein (IgG) were evaluated using Anti-SARS-CoV-2QuantiVac ELISA (Euroimmun) manually. The results are mentioned in RU/ml and according to manufacturer ≥11 RU/ml is considered as positive, ≥8 - 8 RU/ml as cut-off for detectable antibodies, Serum samples from 2014 (n=20) conceder as negative controls.

-Avidity ELISA: Samples with detectable spike-antibodies were measured using AntiSARS-CoV-2QuantiVac,ELISA(IgG) (Euroimmun) according to the manufacturer`s instructions but integrating a soaking step in urea containing wash buffer. Samples were added in duplicate to the wells coated with antigen. After the first incubation step, (300 µl 4 M) urea containing wash buffer was added to one well then wash buffer without urea to the other well. 10 min after that, wash steps. The avidity index was described as (OD urea/OD reference) and multiplied by 100 to be reported as percentage.

-Cytokines assay: Serum were tested for the concentration of Interleukin (IL)-1β, IL-2, IL-6, IL-7, IL-8, IL-10, Interferon (IFN)-γ, (TNF)-α, using the Bio-Plex multiplex Human Cytokine and Growth factor kits (Bio-Rad) according to the manufacturer's protocol.

Statistics: we used SPSS statistic vergion 27 to analyze all data. T-test were used to analyze differences between two groups, cytokine data is presented as the median and range, Avidity index of spike antibodies is presented as (%),The level of total s and n-antibodies were presented as median value, P value of < 0.001 was considered statistically significance.

Results: there are clearly differences in all evaluated parameters were observed when comparing severe (group B non-vaccinated) and mild (group A vaccinated). The vast majority of samples contained **s** and **n**-antibodies (82%, 80% respectively in group A n=30 and 80%, 81 respectively in group B n = 30), level of total n-antibodies in group A clearly rises within one to three months (median value: 20.8, 31.2 s/co p<0.001), and clearly decreases significantly through three to six months (median value: 16,1 p<0.001), also in group B the levels increase then decrease (18.3, 22.4,14.8 respectively P<0.001). A significant decrease in the IgG anti-spike levels (median value 30.2, 21.4 and 16.2 RU/ml, p<0.001) were in group A also in group B (23.6, 18.4, 15.8 P< 0.001), there were a significant increase in the IgG-avidity index (median values 48.6, 56 and 61%, p<0.001) in group A were seen from 1 to 3 to 6 months also in group B

(32.4, 41.2, 49.3 P<0.001), at the same time a significant differences in IgG-avidity index were shown between A and B groups. There was significant differences at (P<0.001)for all tested cytokines between the group A (vaccinated mild reinfection patients) and group B (non-vaccinated sever reinfection patients), group B had significantly higher serum IL-10, IL-6, IL-8, IL-2, IFN-γ, IL-1β, TGF-β concentrations than group A. But the level of IL-7, TNF-α significantly decrease in group A as compared to group B as shown in (table 6).

Table (1): Characteristics of study patients

Adjective	Group A (n= 30)	Group B (n= 30)
disease	2 Diabetes, 3 Hypertension	4 Hypertension
Symptoms	asymptomatic or mild	Severe
Age range (years)	21-51	23-53
male	19	17
Fe male	11	13

Table (2): Levels of n-antibodies (s/co), s-antibodies (RU/ml), Avidity index (%) for IgG spike-antibodies.

Parameters	group	1 month	3 month	6 month	P Value
Nucleocapsid antibodies (s/co)	A	20.8	31.2	16.1	P< 0.001
	B	18.3	22.4	14.8	P< 0.001
Spike antibodies (RU/ml)	A	30.2	21.4	16.2	P< 0.001
	B	23.6	18.4	15.8	P< 0.001
Avidity index of spike antibodies (%)	A	48.6	56	61	P< 0.001
	B	32.4	41.2	49.3	P< 0.001

Table (3): serum concentration of n-antibodies, s-antibodies, Avidity index in group A and B after one month of reinfection.

Parameters	Group A (n= 30)	Group B (n= 30)	P value

Nucleocapsid antibodies (s/co)	20.8	17.3	P< 0.001
Spike antibodies (RU/ml)	30.2	23.6	P< 0.001
Avidity index of spike antibodies (%)	48.6	32.4	P< 0.001

Groupe A: vaccinated reinfection patients (mild symptoms)
Groupe B: non-vaccinated reinfection patients (sever symptoms)

Table (4): serum concentration of n-antibodies, s-antibodies, Avidity index in group A and B after three month of reinfection

Parameters	**Group A (n= 30)**	**Group B (n= 30)**	**P value**
Nucleocapsid antibodies (s/co)	31.2	22.4	P< 0.005
Spike antibodies (RU/ml)	21.4	19.4	P< 0.005
Avidity index of spike antibodies (%)	56	41.2	P< 0.005

Table (5): serum concentration of n-antibodies, s-antibodies, Avidity index in group A and B after sex month of reinfection

Parameters	**Group A (n= 30)**	**Group B (n= 30)**	**P value**
Nucleocapsid antibodies (s/co)	16.1	14.8	P< 0.001
Spike antibodies (RU/ml)	16.2	15.8	P< 0.001
Avidity index of spike antibodies (%)	61	49.3	P< 0.001

Table (6): median and range of serum concentration of cytokines (pg/ml)

Cytokine(pg/ml)	Group A (n= 30)	Group B (n= 30)	P value
IL-10	13.2 (9.07-14.16)	17. 09 (13.18- 21.09)	P< 0.001
IL-6	12.14 (8.09-29.08)	94.09 (35.05-151.5)	P< 0.001
IL-8	60.81 (31.93-168.07)	173.08 (61.82- 1119)	P< 0.001
IL-7	35.45 (30.43- 41.59)	47.21 (24.34-56.17)	P< 0.001
IL-2	12.42 (13.83-15.66)	13.75 (12.98-17.75)	P< 0.001
IFN-γ	18.53 (17.1- 25.21)	27.21 (21.41- 30.08)	P< 0.001
TNF-α	103.8 (80.9-110.3)	122.9 (63.9-140.9)	P< 0.001
IL-1β	5.04 (3.96-4.91)	5.72 (4.66-8.02)	P< 0.001
TGF-β	102.8 (80.3-121.4)	122.9 (75.04-149.9)	P< 0.001

Discussion:

infection with covied-19 causing the humoral immunity to make antibodies against spike-glycoprotein (S) and nucleocapsid protein (N), always in 3 weeks of infection [3].The avidity of the antibodies can be using as measurement for humoral response, during the initial response the avidity to a viral infection was low, then increases gradually over time over time [5].

It been noticed antibody concentrations decreased with decreased avidity In seasonal corona-virus infection, and it expected this impermanent immune response be an expected cause for reinfection within first year after infectio [7, 4]. So the determining of dynamics and magnitude of avidity for antibodies against covied-19 are very important to recognize how the body's immunity works.

Our result shown that the avidity ripeness is progresses over time with increase level of avidity above 3 months after infection, at the same time the antibodies level are decrease, there were increase in the index of IgG-avidity (median values 48.6, 56 and 61%, p<0.001) in group A were observed within 1 to 3 to 6 months also in group B (32.4, 41.2, 49.3 P<0.001), at the same time a significant differences in IgG-avidity index were shown between A and B groups. Some studies recorded increase in avidity level after infection but it was slight to a short follow-up time [17,18,19]. Benner et al observed rising in avidity level association with higher titers of neutralizing antibodies, this indicates that the level of avidity, might be dependent as an indicator

to predict the level of immunity and to diagnose patients who are candidates for recurrence of infection. We showed a considerable variation in the avidity levels between reinfection patients groups, like others studies [20, 16, 21]. our result proved the presence of anti-spike and anti-nucleocapsid in most of the infected people, and these antibodies remain detectable for 6 months after infection, our result elucidate a decrease in level of antibody with time, the decrease was significant from 1 to 3 to 6 months for spike-antibodies, while the level of nucleocapsid-antibodies was increase from 1 to 3 months (with high levels at 3 months), then a significant decrease, this result agree with line of other studies [22, 23] .

virus-specific IgM, IgG, and IgA antibody produce by B cell which response rapidly in infection with covied-19, then occur neutralizing capacity against the S and N protein receptor-binding domain (RBD). The neutralizing antibody are remains active for a period of (three to six months), then decreases gradually. The power of humoral protection after infection with covied-19 is dependent on the stability of (antigen-specific memory B cells) and longevity of plasma cells [24].

Our study shown significantly increase in avidity index in group A (vaccinated reinfection patients) as compare with group B, this perhaps it highlights the importance of the vaccine before infection, since the Prior exposure to specific parts of the virus, makes memory B cells produce high avidity IgG antibodies.

during infection with covied-19 the cytokines are play an important role in immunopathology and immunity, in our paper we reported a significant differences in level of cytokines between group A and B, this result agree with some study [25] while disagree with other study B [26]. So it is possible that the measured cytokines consider an indication for disease progression and give a more accurate method of treatment.

A large number of cytokines produce by irregular T cells and infammatory monocytes whose activated rapidly after infection, so initiated a “cytokine storm”. Many studies on the first wave of the virus covied-19 indicated an increase in the production of pro-infammatory and anti-infammatory cytokines, whose levels have correlation with severity of disease in different ages [27,28, 29].

the basis for cytokine storm and disease development risk is increase the producing of pro-inflammatory markers in severe infection covied-19 [30]. some studies provide that the severity of symptoms in covied-19 are associated with increase levels of proinflammatory cytokines and chemokines (IL-1b, IL-2, sIL-2RA, IL-6, IL-7, IL-17, IL-18, TNF-α and other) [31,32]. Infected pulmonary epithelia cell produce IL-1b, IL-6 and IFN-I/III, whose involving in activation of macrophage and the mobilization of (monocytes, granulocytes and lymphocytes) in the blood. Appropriate (IL-6 and TNF-α) release by effective monocytes leading to hyperinflammation cascades then cytokine syndrome [33]. No equilibrium between the generation of pro-inflammatory and anti-inflammatory cytokines causes malfunction in the complex immune

response mechanism . Cytokine storm that is followed by a decrease in T-cells leads to aggravation and continue of the inflammatory state then causes the failure of some organs and death.

patients with persistent antibodies (six months after infection) have anti covied-19 spike and T-cellular responses specific for internal protien. It is reported by some study that IL-2 and IFN-γ, levels of specific IgG and neutralizing antibodies, were implicated in severity of disease [34].

Covied-19 infection stimulate activation of innate immunity, NK cells the first step for cellular response by generation of cytokine (mostly IL-6) Then it starts attacking the infected cell , after this step, CD8+ T cells demolition all infected cells, at the same time CD4+ T cells stimulate antibodies generation by B cells. Continuing infection leads to depletion of many T-cells with lymphopenia also high ratio of (CD8+/CD4+) is consider a distinctive sign of the progression of the disease to become more severe [24].

Conclusion: We observed in this study a marked increase in the level of avidity over time after Covid-19 reinfection in group A more than in group B, This result indicates that the vaccination that precedes the infection prepares the body to be an immune environment to resist the virus, represented by strength of binding or affinity between antibody and antigen, whilst the levels of antibodies were declining, suggesting a possible aspect of long-term immunity. The level of most inflammatory and proinflammatory cytokines parameters elevated in sever reinfection non-vaccinated patients (group B), this is the cause of severity of reinfection. An inhibitors for some selected cytokines may be a good idea to alter induced hyperinflammation (cytokine storm) then prevent disease progression and dysfunction of many organ that contribute to an increasing mortality of covied-19 patients.

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