Bacterial co-infection associated with patients suffering from SARS-CoV-2

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Article Information	Abstract
  Corresponding Author E-mail: bdhs56@uomosul.edu.iq Mobile:	Patients with the new coronavirus disease severe acute respiratory syndrome (SARSCoV-2) may be suffering from bacterial infections. The detection of immunoglobulin including IgM and IgG could be provide information on the time of COVID-19 infection. To define and explore the bacterial co-infections in patients with COVID-19 infection, the level of IgM and IgG were determined as well as the level of C-reactive protein. SARS CoV-2 infections were diagnosed as well as bacterial co-infection in Zanko private hospital in Erbil. The samples included both male and female, with an average age of 18-50 years. The antigen-tests were used to detect coronavirus antibodies. Furthermore, the concentration of C-reactive protein was identified by measuring photometric at wavelength 546 nm of antigen-antibody reaction. The reaction was between human CRP antibodies that bound to polystyrene particles and CRP present in the samples. The study showed positive results for both antibodies, but the level of IgM and IgG were low in the first week and after two weeks. The results are showed a high level of C-reactive protein in the first and after two weeks, this could be referring to inflammation in patient's body. The cause of inflammation was investigated by diagnosis the bacterial co-infection. It is found that 25 patients of total group (100) were sever from bacterial co-infection. There were gram positive and negative bacteria. *Staphylococcus aureus* which represents 40%, *Escherichia coli* (28%), *Streptococcus pneumoniae* (12%), both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* which represents (8%). Finally, *Staphylococcus epidermidis* (4%). In conclusion, more tests to find out the cause of inflammation are required because the CRP test does not describe the pathogen that cause the inflammation. In conclusion, the study demonstrated that the CRP test does not explain the microbe that cause or location of the inflammation. Therefore, is required more tests to find out the cause of inflammation

Introduction:

The new coronavirus disease severe acute respiratory syndrome (SARSCoV-2) was discovered in September 2019. The World Health Organization recommendation that the novel coronavirus also known as COVID-19 pandemic, has rapidly spread to lots of countries and has killed lots of people [1]. The coronavirus disease affects the lungs and causes acute respiratory syndrome. It is believed that, the virus is originated from some animals such as

bats. the novel coronavirus was outbreak from the seafood market in the Wuhan city, China [2]. This disease has the ability to transmit from one person to another. The coronavirus characterized by rapidly infection rate, which has led to a global epidemic [3]. The detection of immunoglobulin including IgM and IgG could be provide information the period of getting infection. In SARSCoV-2 infection, IgM antibody levels increased after 6 days and IgG antibody levels increased after 15 days. In some patients with COVID 19, the disease developed lead to multiple organ failure and even death during a short period of time [4,5]. Therefore, the specific antibodies detection, is required for confirmation of SARS-2 infection in patients suspected of being infected [6].

C-reactive protein (CRP) is a protein created by the liver that indicates the presence of infection and inflammation [7]. The normal concentration of CRP in blood of healthy people is less than 5 mg/L [8]. Moreover, CRP increases rapidly within six to eight hours and offers the maximum peak in two days from the bacterial infection onset. CRP binds to polysaccharides (phosphocholine) found out highly on the damaged cells wall. This binding induce immune system and modulates the phagocytosis activity to clear pathogens and damaged cells from the location of the inflammation [9]. CRP concentration start to decreases when the inflammation end and the patient recovers [8]. CRP levels is consider a rapidly and effectively test, can be used in the early stage of diagnosis of infection such as pneumonia and COVID-19 [10]. The patients suffering from pneumonia had high CRP level [11].

Patients with SARS-CoV-2 may be suffering from bacterial infections [12]. Bacterial co-infection are commonly diagnosed in COVID-19 infections and bacterial co-infection is responsible for morbidity and mortality. Early diagnosis of the pathogenic bacteria are important to prescribe antibiotics in patients with coronavirus [13]. it is uncertain, if the patients infected with bacteria early during hospitalisation or appear later during health-care in hospital, more studies are required of bacterial co-infections. In this study I investigated the prevalence of bacterial co-infection in patients with coronavirus and to identify the most common pathogenic bacteria in the patients.

Materials and Methods

COVID-19 was diagnosed in Zanko private hospital in Erbil. The samples (total 100) included both male and female, with an average age of 18-50 years. The antigen-tests was used to detect SARS CoV-2 antibodies, which are IgM and IgG antibodies, using the Kit BioSet Test, Turkish. The principle of test is based on the rapid and accurate detection of antibodies as an immune response to the Corona virus (Fig.2) as well as, Chest CT scans with HRCT was used for confirm diagnosis.
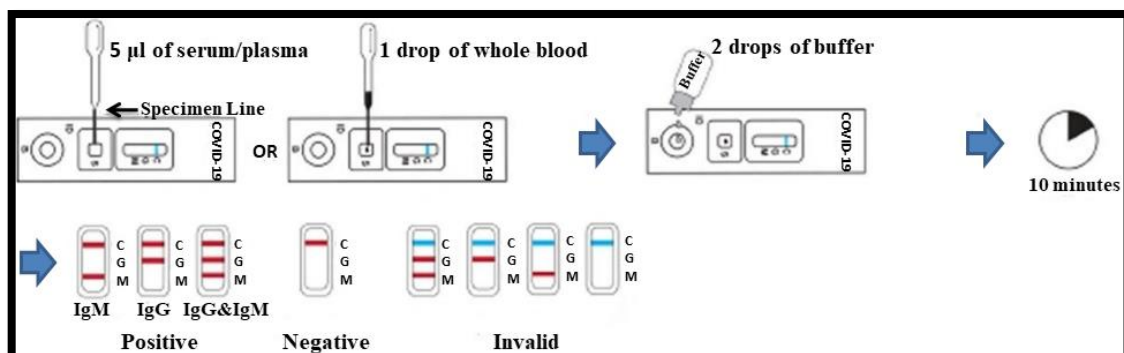
Fig.1: *Showing the procedure of BioSet Test Kit. 5 μl of serum is added in the well, If the result is positive, there are three lines in red, C represent control, G refers to IgG and M is IgM. The negative result, only the control is marked in red. If the kit is invalid, the control is blue.*

CRP levels were also measured using CRP kit. The concentration of CRP was identified by measuring photometric at wavelength 546 nm of antigen and antibody reaction. The reaction was between human CRP antibodies that bound to polystyrene particles and CRP present in the sample.

Bacterial diagnosis

To identify the bacteria, clinical culture were diagnosed using API Analytical profile index kits, Turkish. It is found that 25 patients of total group (100) were severing from bacterial co-infection.

Results and Discussion

The point of this research is to identify the bacterial species that cause co-infection in patients with covid-19 in Erbil Governorate. The private hospital was chosen to obtain this data. Therefore, the level of immunoglobulin and CRP of the hospitalized patients were determined. Result of IgM showed that some data were positive and the other were negative in the first week. The IgM has been increased in the second weeks as well as the level of IGg (Table 1). Previous studies found out that the level of IgM become high during the first week after covid-19 infection and it reached to peak (100 IU/mL) after two weeks, after that decreased to low levels in all patients approximately. IgG antibodies was determined (200 IU/mL) after two weeks and persisted for a long time [14]. Table 1 showed positive results for both antibodies, but the level was low. This could be due to that patients suffer from bacterial co-infections [15].

Table 1: Levels of IgM, IgG and C-reactive protein in patients with coronavirus in first week and after two weeks.

patients	First week			After two weeks		
	IgM (-):≤1 (+):≥1 IU/mL	IgG (-):≤1 (+):≥1 IU/mL	C.R.Protein Adult: ≤5.0 Mg/L	IgM (-):≤1 (+):≥1 IU/mL	IgG (-):≤1 (+):≥1 IU/mL	C.R.Protein Adult: ≤5.0 Mg/L
1	1.01	0.00	12.99	2.00	21	41.38
2	1.00	0.01	16.00	2.70	18	40.25
3	1.07	0.02	21.39	7.05	63.28	170.10
4	1.05	0.05	40.80	13.10	2.1	96.00

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5	1.03	0.01	12.50	17.00	3.90	48.00
6	2.05	0.02	44.11	13.01	4.80	90.00
7	1.01	0.01	10.30	10.00	4.90	85.10
8	1.00	0.00	20.00	3.00	6.70	100.00
9	1.01	0.01	8.70	2.70	9.80	70.10
10	1.05	0.00	42.00	7.11	15.90	96.00
11	1.08	0.05	48.12	8.20	7.10	165.60
12	3.05	0.01	19.00	8.80	9.80	42.20
13	1.02	0.02	30.23	6.70	10.00	95.63
14	1.07	0.01	40.10	9.70	9.6	171.66
15	1.06	0.01	40.32	6.80	2.00	105.00
16	1.00	0.01	27.76	11.91	12.21	87.10
17	1.09	0.02	20.00	6.00	13.80	84.00
18	2.05	0.05	27.27	5.00	25	84.65
19	1.11	0.01	42.01	9.70	18.00	120.00
20	1.00	0.02	50.00	8.05	30.28	166.00
21	1.07	0.00	18.00	17.10	9.10	48.11
22	1.07	0.05	16.78	19.00	20.90	84.00
23	1.03	0.01	48.99	15.01	14.80	125.11
24	2.04	0.02	10.99	18.00	19.90	41.38
25	4.01	0.00	44.65	19.00	25.98	100.21

The level of C-reactive protein(CRP) was measured (Table 1). The results are showing a high level of CRP; this refer to inflammation in patient's body. From Table 1 the level of CRP were > 100 µg/mL for nine samples belong to different patients. Fifteen samples that measured > 40 µg/mL. CRP results could be refer to bacterial co-infection because in bacterial infections, the CRP level increase to reach > 40 µg/mL [16].

The data was statistically analysed using One-way ANOVA test, GraphPad Prism5 software (GraphPad Software, Inc., San Diego, California, USA). The titer of antibodies was measured in the first and second week Fig. 2. There is no significant differences in titer of both antibodies in the first week compared to control (-): ≤1(+): ≥1 IU/mL (IgG 0.0168 IU/mL, IgM 1.3612 IU/mL, n=25). Whereas, there are significant differences in the titer of both antibodies in the second week compared to control (IgG 15.154 IU/mL, IgM 9.8656 IU/mL, n=25). (***p<0.001).
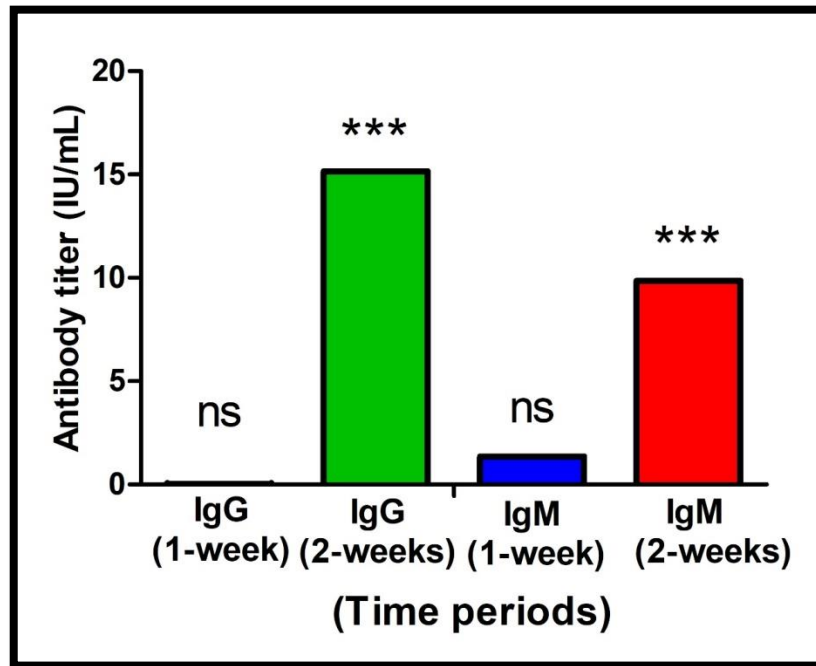
Fig. 2: *The titer of immunoglobulin IgG and IgM during two weeks. There are significant differences in level of both antibodies in second week compared to control (+):≥1 using One-way ANOVA, (***p<0.001). There is no significant differences in the first week compared to control.*

The levels of CRP were measured also in the first and second week Fig. 3. There are significant differences in the level of CRP in the first and second week (28.5204 Mg/L, 94.3032 9.8656 Mg/L respectively, n=25) compared to control ≤5.0Mg/L, (**p<0.01, ***p<0.001).
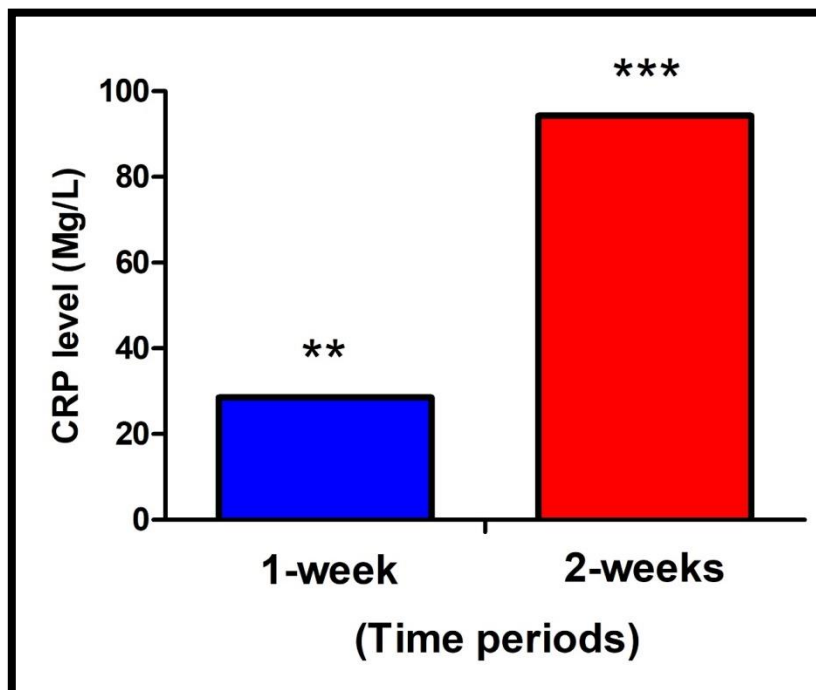
Fig. 3: *The levels of CRP during two weeks. There are significant differences in the level of CRP in the first and second week compared to control ≤5.0Mg/L using One-way ANOVA, (**p<0.01, ***p<0.001).*

The high levels of CRP in the blood samples are a marker of inflammation. When the results are not normal, this require more tests to find out the cause of inflammation [17]. In this study, with regard to the subject of diagnosis of these Inflammation, more than one different bacterial species were isolated and diagnoses. There were gram positive and negative bacteria in general Fig.4. In figure 2, ten patients have inflammation caused by *Staphylococcus aureus* which represents 40% and the other seven patients have infection with *Escherichia coli* (28%). Three patients infected with *Streptococcus pneumoniae* (12%). Two patients infected with *Klebsiella pneumoniae* and other two suffering from *Pseudomonas aeruginosa* which represents (8%). Finally, one patients infected with *Staphylococcus epidermidis* (4%).
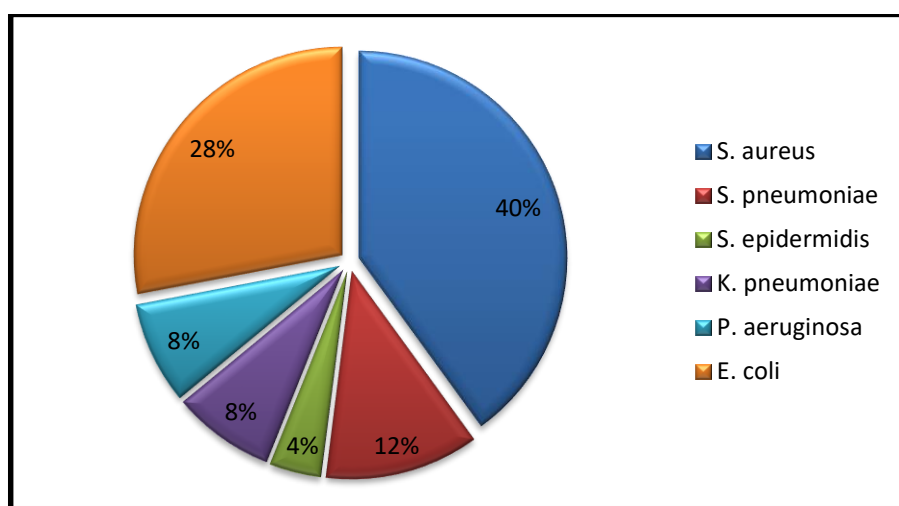
Fig.4: *Percentage of bacterial co-infection associated with patients have infection with SARS-CoV-2.*

Conclusion:

In conclusion, the study demonstrated that the higher levels of CRP are an indicator of inflammation. Therefore, is required more tests to find out the microbe that cause of inflammation. The more common bacterial co-infection are *S. aureus*, *E. coli*, *S. pneumoniae*, *K. pneumoniae*, *P.s aeruginosa*, *S. epidermidis*.

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البكتريا المصاحبة للمصابين بفايروس كورونا

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معلومات المؤلف

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الخلاصة:

ان المرضى المصابين بفايروس كورونا المتلازمة التنفسية الحادة الوخيمة قد يصاب بعدوى ثانويه مثل الاصابة البكتيرية. ان الكشف عن هذه الاجسام المضادة IgM و IgG قد يعطي مؤشر لبداية العدوى بالفايروس. لتحديد العدوى البكتيرية المشتركة في المرضى المصابين بعدوى COVID-19، تم قياس مستوى IgM و IgG فضلا عن قياس مستوى بروتين سي التفاعلي. تم تشخيص عدوى SARS CoV-2 في مستشفى زانكو الاهلي في أربيل. وشملت العينات كلا من الذكور والإناث بمتوسط أعمار 18-50 سنة. تم استخدام الاختبارات المصلية للكشف عن الأجسام المضادة لفيروس كورونا. علاوة على ذلك، تم تحديد تركيز بروتين سي التفاعلي عن طريق تفاعل الاجسام المضادة بالمستضد والقياس عند طول موجي عند 546 نانومتر. اظهرت الدراسة انخفاض في مستويات الاجسام المضادة IgM وIgG في الاسبوع الاول والثاني. من جهة اخرى اظهرت النتائج ارتفاع في مستويات بروتين سي التفاعلي في الاسبوع الاول والثاني وهذا دليل على وجود التهابات لدى المريض. تم التحقق عن مسببات الالتهاب، في هذه الدراسة اتضح ان السبب يعود الى البكنريا الموجبة والسالبة لصبغة كرام. *Staphylococcus aureus* شكلت نسبتها 40% بينما *Escherichia coli* شكلت 28% في حين *Streptococcus pneumonia* شكلت 12% واخير كل من *Klebsiella pneumoniae* و *Pseudomonas aeruginosa* نسبتها 8% واخير *Staphylococcus epidermidis* شكلت 4%. تم الاستنتاج من هذه الدراسة ان هنالك ضرورة لمزيد من الاختبارات لمعرفة سبب الالتهابات لأن اختبار بروتين سي التفاعلي لا يحدد العامل الممرض الذي يسبب الالتهاب.