Seromucoid and Protein-Bound Hexose as Inflammatory Markers in Sera of COVID-19 Patients

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Abstract:

Most common inflammatory markers like C-reactive protein, which is a protein produced by the liver in response to inflammation and infection in the body, is used to quantify circulating molecules that are released as a cause of an inflammatory response in clinical studies.

The aim of this study is to estimate the levels of serum seromucoid and protein bound-hexose (PBH) as inflammatory markers in sera of COVID-19 patients in comparison with normal subjects. For this purpose, Thirty COVID-19 patients were selected as control (male and female) in addition to 30 healthy subjects as the control group. The findings indicated that seromucoid and PBH levels were highly significant increase in COVID-19 patients (P< 0.0001) comparing with normal subjects This study provides evidence that, despite some potential physiological differences, the levels of seromucoid and PBH showed no significant differences for both male and female patients. The results from both parameters showed that COVID-19 severity is associated with inflammatory markers.

Key words: COVID-19, Glycoproteins, Inflammatory markers, Seromucoid.

المخاط المصلي والسكر السداسي المرتبط بالبروتين كعلامات التهابية في مصول مرضى كوفيد -١٩

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الخلاصة:

يستخدم على نطاق واسع ويتم فحصه كنظام لتوصيل الأدوية المعوية لإطالة وقت Superporouse Hydrogel تُستخدم العلامات الالتهابية الأكثر شيوعًا مثل البروتين التفاعلي C، لتحديد الجزيئات المنتشرة التي يتم إطلاقها كسبب للاستجابة الالتهابية في الدراسات السريرية، والغرض من هذه الدراسة هو تقدير مستويات المخاط المصلي والبروتين المرتبط بالسكر السداسي(PBH) في مصول ٣٠ مريضًا من كوفيد -١٩ (ذكورا وإناثا) ومقارنتها مع تلك الموجودة في مصول ٣٠ اشخاصا من الاصحاء. تشير النتائج إلى أن مستويات المخاط المصلي PBH اظهرت فروقًا ذات دلالة إحصائية معنويه في مرضى كوفيد -١٩ ($p<0.0001$) مقارنة مع الأشخاص الاصحاء. أظهرت الدراسة عدم وجود اي فروقات ذات دلالة إحصائية معنويه بين مجاميع المرضى من الإناث والذكور فيما يتعلق بمستويات المخاط المصلي PBH وتوضح النتائج أن كلاهما ذو اهميه عالية على الارتباط بين علامات الالتهاب وشدة كوفيد-١٩. وهذا يتطلب المزيد من الدراسة على المعلمات كل من المخاط المصليPBH للبحث في هذه العلامات الالتهابية لمراقبة وتطور عدوى كوفيد-١٩، وتقييم فعالية الاساليب العلاجية المختلفة.

الكلمات المفتاحية: كوفيد -١٩، بروتينات سكرية، علامات الالتهابية، المخاط المصلي

Introduction

The global pandemic of coronavirus disease in 2019 (COVID-19) has had a significant impact on virtually all aspects of life around the world and poses a previously unheard-of public health, sociological, and monetary threat to society as a whole [1,2]. There is growing evidence that inflammation contributes significantly to the development of COVID-19 [3,4]. Clinical and epidemiological research assess circulating molecules that are produced as a cause or outcome of an inflammatory process using the most prevalent biomarker for inflammation. The most common biomarker for inflammation, C-reactive protein, is used to quantify circulating molecules that are released as a cause or result of an inflammatory response in clinical and epidemiological studies [5, 6].

Seromucoid is insoluble in phosphotungstic acid but dissolves in 0.6 mol/L perchloric acid. The majority of these are acute-phase compounds with a glycoprotein component rich in carbohydrates. These acute inflammatory reactants, which are highly sensitive to stressors, resulted in an increase in the number of liver hepatocytes could enter the bloodstream during an illness; trauma and inflammatory, degenerative, and malignant conditions [7]. The response pattern of this heterogeneous group's increased biosynthesis in different clinical circumstances and stresses may vary significantly. Thus, the seromucoid may appear in blood to varying degrees in various types of tissue injuries. A component of membrane proteins, the seromucoid portion is generated from the connective tissue's intercellular matrix, which is regulated by a variety of physiological and pathological processes, including severe damage, morphogenesis, tissue repair, and malignancy, among others. [8,9]. The PBHs are proteins that have a carbohydrate part that is chemically attached to their peptide part like enzymes, hormones, blood proteins, and clotting factors are all examples of glycoproteins, which are only a few of their many roles. organic compounds, and extracellular membranes. The constituents of these organic compounds are including proteins and monosaccharides, mostly the sugar hexose and hexosamine, Serum glycoproteins concentrations of the different kinds are kept within a narrow range in a healthy person [10], but are abnormally high in many conditions, such as autoimmune disease, Tuberculosis, and other pathological severity [11].

The current study aimed to identify seromucoid and PBH levels as potential markers for the diagnosis and prognosis of COVID-19, as their changes can indicate the severity of infection or the efficacy of treatment.

Materials and Methods

Materials:

All chemicals and reagents used in this study were of analytical quality and obtained from Fluka (UK), Hopkins, and Sigma.

Sample collection

The study included 30 hospitalized patients, who conducted (8 females and 22 males) aged between (35-65) years, collected at central laboratory in Erbil City, Kurdistan region –Iraq. The control groups included 30 subjects (males and females) healthy volunteers. The sample collection was completed during three months (October to December 2021). A 5-ml blood sample was obtained from patients and control. The blood samples were drawn into plane tubes and centrifuged for 15 minutes at 3000 rpm. The study was carried out at Salahaddin University's Chemistry Department in the College of Science.

Methods:

1- **Serumucoid determination.**

Weimer and Moshin have identified assay for determination of serumucoid; proteins other than serumucoid were precipitated by

perchloric acid; serumucoid was precipitated from the filtrate by phosphotungestic acid; and finally, serumucoid was dissolved in alkali after being precipitated from the filtrate by phosphotungestic acid. The oligosaccharide is separated from the polypeptide through the process of hydrolysis, and the hexose is turned into furfural by the use of prolonged heating. The color that is produced through condensation with orcinol is measured at 520 nm (12).

2- **Glycoprotein (protein-bound hexose) determination**

The hexose moiety of protein-carbohydrate conjugate (previously precipitated with 99% ethanol at 25 °C) was determined in alkaline medium by the orcinol reaction. The method depends on the principle that after the polypeptide has been hydrolyzed, the oligosaccharide is released, and the hexose is transformed to furfural through prolonged heating (45 min). The color resulting from condensation with orcinol can be read at 520 nm [13].

Statistical Analysis: The data was presented as mean± SE. The obtained data was evaluated in order to draw relevant conclusions. The assay results were analyzed using GraphPad Prism 8.0 software. Un-paired t-test for significance was performed to compare between the normal control subjects and patients. A receiver operating characteristic (ROC) curves and areas under the curve (AUC) values were calculated to evaluate the diagnostic potential of the biomarkers, and to determine which ones had a higher ability to distinguish between patients with COVID-19 and healthy subjects

Results:

Table (1) shows the mean ± SE of serume seromucoid and PBH levels of the patients in Covid -19 group and normal healthy subjects, expressed as mg/dL, as well as of their sex, statistical analysis, and t-test. The Serum seromucoid level in Covid-19 patients are significantly higher (80± 5.6) than in the normal healthy subjects (15± 0.52 mg/dL) ($P< 0.0001$). Fig (1). There is no significant differences in the level of seromucoid between males and females Fig (3).

Table (1) and Figure (2) reveal that serum PBH levels are higher in Covid -19 patients (375± 30) than in normal subjects (100± 2.5) ($P< 0.000$). Fig (2). On the other hand, statistical analysis showed no difference at ($P < 0.05$) in the level of PBH between males and females Fig (4).

Table.1 Mean ± SE of serum serumucoid and PBH levels (mg/dL) in covid-19 patients and control subjects.

Statistical Analysis	Control	Patients		
		Total	Female	Male
Serumucoid (mg/dL)				
No.	30	30	8	22
Mean	15^{a}	80^{b}	78^{NS}	85^{NS}
SE	0.52	5.6	8.9	4
PBH (mg/dL)				
No.	30	30	8	22
Mean	100^{a}	375^{b}	391.6^{NS}	350.3^{NS}
SE	2.5	30	44.09	38

Note: significant differences between the compared groups in the same raw were indicated using different letters (at $P<0.0001$), while (NS) indicates non-significant differences.

Discussion:

Elevated levels of serum seromucoid and BPH were found in COVID-19 patients in the present study, compared to those of normal subjects to establish which of these markers would be most relevant for diagnosis and following the progression of Covid-19.

Recent studies have shown that there is a significant interest in biochemical variances in glycoprotein concentration as a possible inflammatory marker for many diseases [14,15]. According to earlier studies, elevated serum glycoprotein levels were associated with a higher risk of inflammatory disease [16,17], like acute myocardial infraction, and other conditions [18].

Regarding table 1, shows the mean ± SE of seromucoid level in serum of Covid -19 patients and normal control subjects, serum seromucoid level in Covid-19 patients are significantly higher than in the normal control subjects as shown in Figure (1), Several investigators have suggested a number of explanations, yet the precise cause of this rise remains uncertain. The reason may involve release of glycoproteins and seromucoid into the blood due to tissue overgrowth, severe cell damage, or inflammatory processes [19,20]. Other fractions of glycoprotein are also biomarkers for inflammation and can contribute to the increase as well. Seromucoid molecules are somewhat more carbohydrate-rich while making up a very minor portion of serum glycoproteins. These are a diverse group of inflammatory markers that also include ceruloplasmin, plasminogen, and α- acid glycosaminoglycans. Since the interstitial matrices of the cells and the liver's production are both sources of the acute phase reactants that originate in serum. [8,9]. Many inflammatory biomarkers can

be used to trace and diagnose infection severity and mortality with some degree of accuracy [21]. The elevated risk of developing severe covid-19 has been observed to be highly correlated with inflammatory markers such as serum C-reactive protein and interleukin-6 [22,23,24]. Protein - bound hexose levels in the COVID-19 patients were higher, as shown

in Table 1 than in normal subjects were significantly higher compared to normal subjects. Fig (2). COVID-19 patients have higher levels of protein-bound hexose compared to healthy subjects.

The reason for this increase in protein-bound hexose levels is believed to be due to the glycosylation process, where sugar molecules attach to proteins. It has been suggested that the virus may alter the host's glycosylation process, leading to changes in the immune response and increased inflammation [25]. Neither the seromucoid nor PBH levels of female patients differed significantly from those of male patients, based on the results of this study Fig (3 and 4).

The exact reasons for this lack of difference in protein-bound hexose and seromucoid levels between male and female patients is not clear. It is possible that the response of the immune system to the virus may be similar between males and females, leading to comparable changes in protein-bound hexose and seromucoid levels. It is also possible that other factors, such as age or underlying health conditions, may play a more significant role in determining the levels of these markers in COVID-19 patients [25]. The area under the ROC curve (AUC) results were regarded as satisfactory for AUC values ranging from 0.9 to 1, indicating that receiver operator characteristic (ROC) curves for both markers. Both Seromucoid and PBH showed the best overall ability to differentiate between Covid-19 patients a healthy subject with an area under then curve (AUC) of 1, and 0.9 respectively. Both seromucoid and PBH are excellent inflammatory markers, this requires further global research to better comprehend the observed changes in this study.

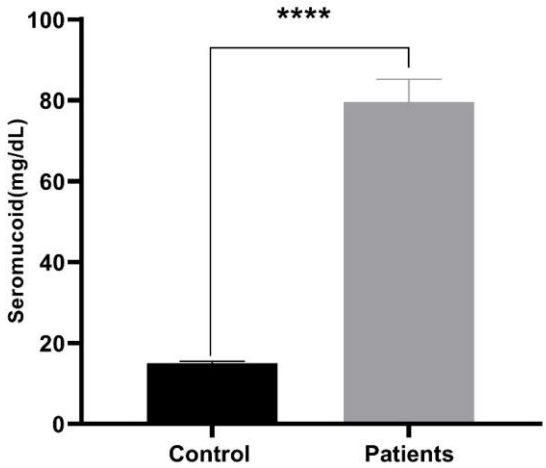

Figure. 1 Serum seromucoid in sera covid-19 patients and control groups (** denotes *P*<0.0001)**

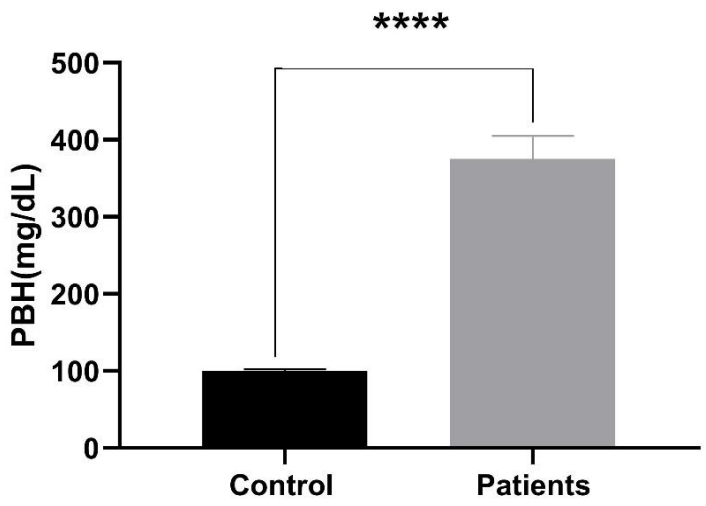

Figure.2 Serum protein- bound hexose in sera covid-19 patients and control groups (** denotes *P*<0.0001)**

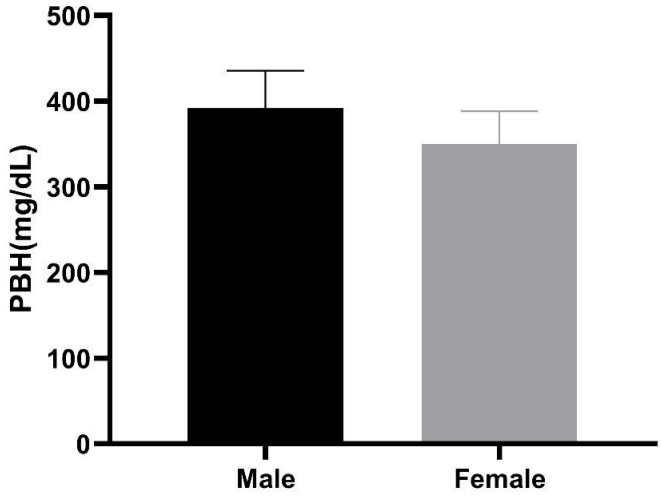

Figure. 3 Serum seromucoid in sera covid-19 patients (male and female) groups

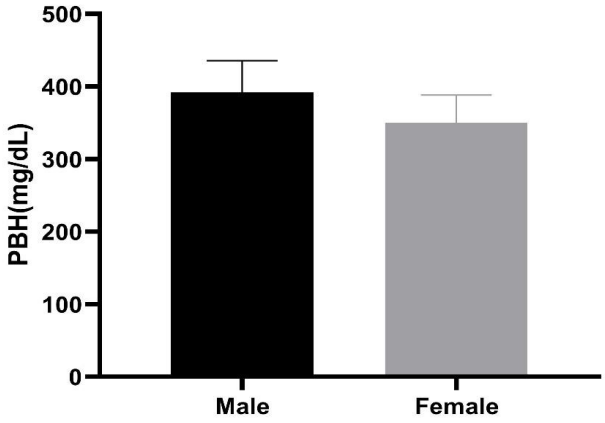

Figure.4 Serum protein-bound hexose in sera covid-19 patients (male and female) groups

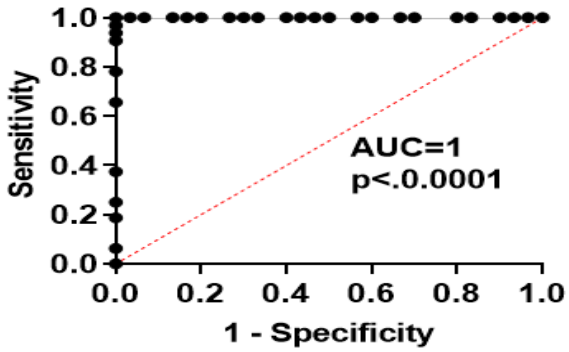

Figure. 5 ROC curve assessing overall diagnostic abilities of seromicoid marker in sera covid-19 patients

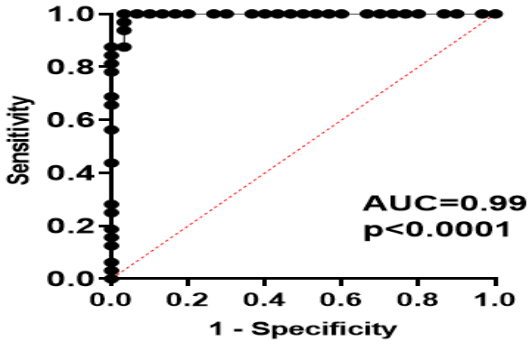

Figure. 6 ROC curve assessing overall diagnostic abilities of serum protein-bound hexose marker in sera covid-19 patients

Conclusions:

High significant differences in serum seromucoid and PBH levels were found in COVID-19 patients. Inflammatory indicators have been linked to the severity of COVID-19, as shown by both seromucoid and PBH. Monitoring and assessing the severity and prognosis of COVID-19 may be helped by the measurement of inflammatory markers, according to clinicians. By measuring these markers, clinicians can gain a more detailed understanding of the effects of COVID-19 on an individual's immune system, and make more informed decisions about their treatment

Conflict of interest

The author declared no conflict of interest.

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