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# TUMOR BIOMARKERS CEA, CA19.9, CA15.3 AND AFP LEVELS IN THE SERUM OF PATIENTS WITH COVID-19

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*Early diagnosis is very important to reduce morbidity and mortality in COVID-19 infected patients. The aim of this study was to detect of tumor antigens CEA, CA19.9, CA15.3, and AFP and to compare their levels in the serum of 69 COVID-19 patients and 69 healthy individuals who did not have COVID-19. The levels of CEA, CA19.9, CA15.3, and AFP in the serum were measured using ELISA. The levels of the tumor biomarkers in the serum of COVID-19 patients were found to be 7.74 ± 4.65 ng/ml for CEA, 29.33 ± 16.35 U/ml for CA19.9, 23.24 ± 13.48 U/ml for CA15.3 and 7.46 ± 5.57 ng/ml for AFP, while in the serum of healthy control patients 9.73 ± 43.58 ng/ml for CEA, 20.66 ± 11.1 for CA19.9, 19.64 ± 10.99 U/ml for CA15.3, and 3.83 ± 9.20 ng/ml for AFP, indicating no differences in the levels of the studied tumor biomarkers in the two experimental groups. It is concluded that tumor biomarkers CEA, CA19.9, CA15.3, and AFP cannot be used as effective screening tools for patients with COVID-19.*

*K e y w o r d s: tumor biomarker, CEA, CA19.9, CA15.3, AFP, COVID-19.*

In late December 2019, cases of severe acute respiratory syndrome coronavirus (SARS-CoV-2)-infected pneumonia were discovered in Wuhan City, Hubei Province, China [1]. The 2019 Coronavirus disease (Covid-19) outbreak has spread rapidly throughout the country and around the world [2, 3], with the World Health Organization (WHO) declaring the outbreak a pandemic on March 11th [4-6]. Identifying asymptomatic cases that result in the virus spreading to close contacts is one of the many challenges in containing the spread of COVID-19. A study of passengers on a Diamond Princess Cruise ship quarantined due to an early COVID-19 outbreak estimated the asymptomatic proportion (among all infected cases) at 17.9% (95%CrI: 15.5-20.02%). As a result, the actual number of SARS-CoV-2-infected people may be significantly higher than what is currently being accounted for based on positive test results. Accurate, convenient, and rapid testing for widespread use can aid in the elimination of COVID-19 silent transmission by asymptomatic viral carriers [7]. SAR-SCoV-2 is a single-stranded RNA virus in the Betacoronavirus genus. It is based on the coronavirus genome's most conserved sequence, the open reading frame 1a/1b (ORF1a/1b), which is responsible for replicase encoding [8]. The RNA genome is 29 891 nucleotides long and shares 79%t sequence identity with SARSCOV and 50% with Middle East respiratory syndrome coronavirus [8, 9].COVID-19 is a multisystem disease caused by a diffuse systemic process involving a complex interplay of the immune, inflammatory, and coagulative cascades. Understanding what the virus does to the body and how the body responds to it has led to the discovery of plethora of potential biomarkers [10].According to growing evidence, this novel disease can affect multiple organs, including the heart, liver, and gastrointestinal tract, and result in abnormalities in several biomarkers [11, 12]. Multiple organ damage can occur quickly in COVID-19 infected patients. Previous research has shown that many laboratory biomarkers in covid-19 patients were not within normal ranges [13]. Several biomarkers with prognostic and therapeutic implications in SARS-CoV-2 infections have been described [14]. Recent research has looked into the carcinoembryonic antigen (CEA) as a prognostic biomarker in SARS-CoV-2 patients [15, 16]. Additionally, some tumor markers, such as CEA and Carbohydrate antigen125 (CA-125), have been found to be elevated and positively correlated with the pathological progression of COVID-19 infection,

potentially increasing the cancer detection rate [17]. Many cancer biomarkers, such CEA and carbohydrate antigens (CA), have been found to be elevated in inflammatory pulmonary conditions in the lungs [18-20]. CEA is a 200 kDa oncofetal acid glycoprotein [21], first described by P. Gold and S. O. Freedman in 1965 [22].

CEA is found on the periphery of tumor cell membranes and is released into body fluids. It is thought to play a role in cell adhesion and apoptosis inhibition in the physiologic state, which is why it is expressed in normal mucosal cells and over-expressed in adenocarcinoma (colorectal, gastric, pancreatic, breast, lung, and others) [23, 24-27]. CEA is a well-known tumor marker for many common cancers and has been found to be highly expressed in the serum of patients with severe pneumonia [28]. CA15.3 is a 300-450 kDa glycoprotein produced by a variety of cells, particularly breast cancer cells [29, 30]. It has been found to be elevated in cancers such as breast, ovarian, pancreatic, lung, and colorectal [31].

Carbohydrate antigen19.9 (CA19.9) is an intracellular adhesion molecule. The antigen is a 210 kDa tumor-associated glycoprotein antigen found as a carbohydrate determinant on glycolipids and glycoproteins [32]. CA19.9 antibody detection, has been identified as the most useful tumor marker for pancreatic adenocarcinoma and gall bladder carcinoma since its first mention in 1979 [23, 26-33]. Alpha-fetoprotein is the most commonly used biomarker for HCC surveillance (AFP). It is a 70 kDa glycoprotein produced by the fetal liver and yolk sac during the first trimester of pregnancy and rapidly diminishes after birth [34]. Serum AFP levels are undetectable in healthy people but can be elevated in a number of conditions, including hepatocellular carcinoma (HCC), seminoma and nonseminomatous germ cell tumors, and gastric, biliary, and pancreatic cancers [35]. As a result, early detection and treatment are critical in reducing morbidity and mortality in COVID-19-infected patients. Until now, no comprehensive study has evaluated the value of CEA, CA19.9, CA15.3, and AFP in the diagnosis of COVID-19 in the Libyan population. The aim of this study was to evaluate the ability of several tumor biomarkers parameters, including CEA, CA19.9, CA15.3, and AFP to detect COVID-19 and to compare the levels of these in COVID-19 patients with the levels of these in healthy individuals in Libya.

## Materials and Methods

*Patients and specimens.* This is a retrospective study that was conducted at the Department of Biotechnology, Faculty of Science, Sebha University, and Respiratory Clinic, Sebha Branch from December 24$^{th}$, 2020 to April 18$^{th}$, 2021, with a total of 138 patients, 69 patients (62.14 ± 16.43), with the age range 12-87 years, with covid-19 as COVID-19 group, and 69 healthy individuals (40.90 ± 16.80, with the age range 16-88 years, without COVID-19 as control group. The study was approved by the scientific Committee of Biotechnology department, Sabha University approved number 31/2020 (date 09/06/2020). All participants signed an informed consent form.

*Collection of serum samples.* Following disease diagnosis, 5 ml whole blood samples were collected from patients and healthy individuals and allowed to clot at lab temperature. The blood was put in a plain tube for separation by centrifugation at 4500 rpm for 5 min to separate serum from whole blood, and it was stored at –20°C until used. An immunoassay analyzer was used to measure the levels of CEA, CA15.3, CA19.9, and APF serum biomarkers (AIA 360 Diagnostics, IN, USA). CEA levels were reported as ng/ml, CA15.3 levels as U/ml, CA19.9 levels as U/ml, and AFP levels as ng/ml. CEA, CA15.3, CA19.9, and AFP serum levels were recommended cut-off values to be 5 ng/ml, 37 U/ml, 35 U/ml, and 7 ng/ml, respectively.

*Statistical data analysis.* The current study's results were statistically analyzed using the PSPP version 1.2.0-g0fb4db software (PSPP, Inc., 51 Franklin Street, USA). To compare the values of patients with COVID-19 and healthy individuals, the results were expressed in terms of mean ± SD. At 5% probability, the results were statistically significant ($P < 0.05$). Using Receiver Operating Characteristic (ROC) analysis of COVID-19 biomarker proteins, the cutoff values, Areas under the Curve (AUCs) with 95% CIs, and Standard Errors (SEs) of each marker's ability to detect were determined.

## Results and Discussion

COVID-19, an illness caused by a novel SARS-CoV-2 that has spread globally, has become one of the leading causes of death in some areas [36, 37]. Biomarkers are quantitative measurements used in clinical settings for a wide range of conditions that

reflect pathological development; they can help clinicians initiate treatment and closely monitor patients [38]. Though biomarkers may help improve prognosis and outcomes, the study's findings may be influenced by their significant variability between patients [39].

The current study included 138 patients divided into two groups, including the first group 69 patients with COVID-19 who were diagnosed with the disease using a PCR test. As a control group, 69 patients with non-COVID-19 were included in the second group. The current study aims to discover new tumor markers that are sensitive and specialized in detecting COVID-19 regardless of age. The mean ± SD of age among all patients with COVID-19 was (62.14 ± 16.43) years, while the mean ± SD of age among all healthy individuals was (40.90 ± 16.80) years. Our findings were supported by others, such as R. H. Du et al., who discovered that people over the age of 60 had a 3.7-fold increased risk of COVID-19 infection [40]. Another study discovered that 6% were 85 years old, 25% were 65 to 84 years old, 18% were 55 to 64 years old, 45 to 54 years old, and 29% were 20 to 44 years old [41]. A similar study by A. Lingaiah et al. discovered that 44.3% of COVID-19 infected patients and inflammatory markers were elderly [42]. In this study, we discovered 43 (62.32%) males and 26 (37.68%) females in the COVID-19 group, while 37 (53.62%) males and 26 32 (46.38%) females in the control group, which is similar to A. Lingaiah et al. discovery of 83 (72.3%) of men [42].

The study reported that the mean ± SD of serum level CEA for COVID-19 group and healthy control group were 7.74 ± 4.65 ng/ml and 9.73 ± 43.58 U/ml, respectively, and the mean ± SD of serum level CA19.9 for COVID-19 group and healthy control group were 29.33 ± 16.35 and 20.66 ± 11.10 U/ml, respectively, and the mean ± SD of serum level CA15.3 for COVID-19 group and healthy control group were 23.24 ± 13.48 and 19.64 ± 10.99 U/ml, respectively, and the mean ± SD of serum AFP for COVID-19 group and healthy control group were 7.46 ± 5.57 and 3.83 ± 9.20 U/ml, respectively as shown in Table 1.

In fact, as shown in Table 1, there is no difference between all tumor markers in the group of patients with COVID-19 and without COVID-19. Our findings contradict other authors' studies, like as J. Yu et al. They discovered that CEA was highly expressed in the serum of COVID-19 patients who did not have cancer, and serum CEA levels were found to be increased in patients with severe or critically severe SARSCoV-2 infection, implying that CEA may serve as a novel prognostic marker of COVID-19 [16]. As a result, their findings suggest that CEA could be used as a novel prognostic marker for COVID-19 [16], whereas X. Wei et al. examined serum biomarker levels in COVID-19 patients (mild: 131; severe: 98; critical: 23) [17]. B. He et al. discovered that all five tumor biomarkers were significantly higher in COVID-19 patients' plasma than in healthy controls in another study [43]. Our findings agree with those of others, such as J. Yu et al., who found no difference in AFP levels [16].

The level of specialization of the four tumor markers was evaluated to determine their efficacy in distinguishing between the COVID-19 group and the healthy group. According to the ROC curve analysis, the area under the curve (AUC) of COVID-19 for estimating CEA was 0.44 (95% CI, 0.32–0.56; $P$ = 0.407). For estimating CA19.9, COVID-19 had an AUC of 0.54 (95% CI, 0.42–0.67; $P$ = 0.565). For estimating CA15.3, COVID-19 had an AUC of 0.53 (95% CI, 0.42–0.65; $P$ = 0.629). As shown in Table 2

*Table 1. Levels of CEA, CA19.9, CA15.3 and AFP (mean ± SD) in sera of COVID-19 patients and healthy controls subjects*

| Parameters | COVID-19 patients, mean ± SD or N (%) | Healthy controls, mean ± SD or N (%) | *P* - Value |
|---|---|---|---|
| Age | 62.14 ± 16.43 | 40.90 ± 16.80 | 0.645 |
| Male | 43 (62.32%) | 37 (53.62%) | 0.647 |
| Female | 26 (37.68%) | 32 (46.38%) | 0.647 |
| CEA ng/ml | 7.74 ± 4.65 | 9.73 ± 43.58 | 0.698 |
| CA19.9 U/ml | 29.33 ± 16.35 | 20.66 ± 11.1 | 0.356 |
| CA15.3 U/ml | 23.24 ± 13.48 | 19.64 ± 10.99 | 0.752 |
| AFP ng/ml | 7.46 ± 5.57 | 3.83 ± 9.20 | 0.433 |

*Table 2. AUC for COVID-19 estimating serum biomarker CEA, CA19.9, CA15.3 and AFP*

| Serum Biomarkers | Cutoff | St Error | *P*-Value | 95% Confidence interval |
|---|---|---|---|---|
| CEA | 0.44 | 0.07 | 0.407 | 0.32–0.56 |
| CA19.9 | 0.54 | 0.08 | 0.565 | 0.42–0.67 |
| CA15.3 | 0.53 | 0.07 | 0.629 | 0.42–0.65 |
| AFP | 0.53 | 0.08 | 0.696 | 0.40–0.65 |

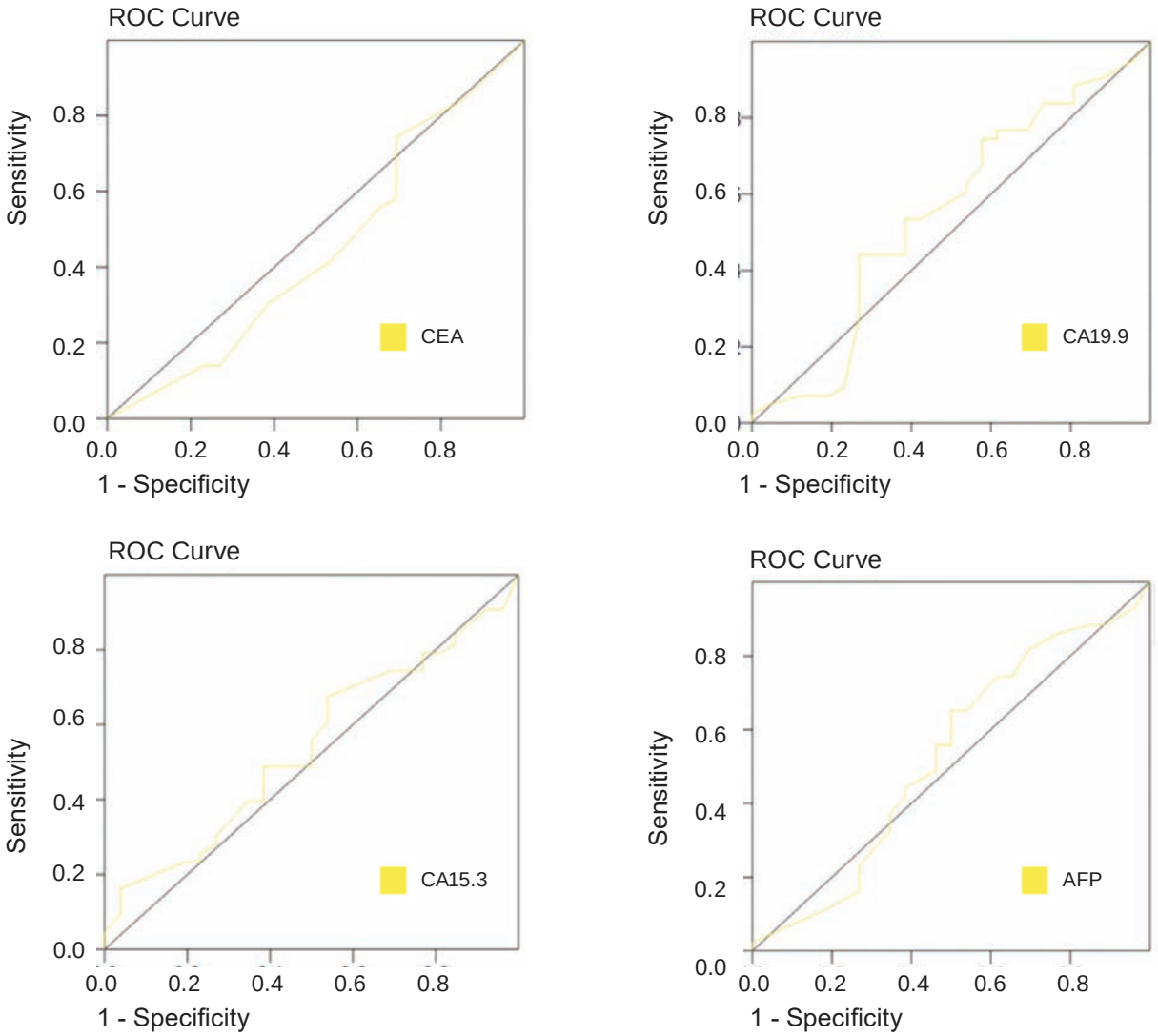


*Fig. The ROC curve analysis of COVID-19 estimating serum biomarker CEA, CA19.9, CA15.3 and AFP*

and Figure, COVID-19's AUC for estimating AFP was 0.53 (95% CI, 0.40–0.65; $P = 0.696$), indicating the highest specificity and relative sufficient sensitivity that could be identified as an independent predictive factor for COVID-19. COVID-19 had no effect on the tumor markers CEA, CA19.9, CA15.3, and AFP, according to the study. Y. E. Purut et al. and A. H. Ali [13, 44] both reported similar findings.

*Conclusion.* Several tumor marker parameters, including CEA, CA19.9, CA15.3, and AFP, cannot be used as screening tools for patients with COVID-19. Additional research is needed to identify novel biomarkers for early COVID-19 detection.

*Limitations.* To the best of our knowledge, this is the first study of its kind in the Libyan population; the number of patients in our study can be increased

in the future to confirm these findings, and we can add other tumor biomarkers to make our study more comprehensive.

*Conflict of interest.* Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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## РІВНІ ПУХЛИННИХ БІОМАРКЕРІВ CEA, CA19.9, CA15.3 ТА AFP У СИРОВАТЦІ КРОВІ ПАЦІЄНТІВ ІЗ COVID-19

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Рання діагностика дуже важлива для зниження рівня захворюваності і смертності у пацієнтів, інфікованих COVID-19. Метою даного дослідження було виявити пухлинні антигени CEA, CA19.9, CA15.3 та AFP та порівняти рівні цих антигенів у сироватці крові пацієнтів хворих на COVID-19 та здорових людей, які не хворіли на COVID-19. У дослідження було залучено 69 пацієнтів з COVID-19 і 69 здорових людей. Рівень CEA, CA19.9, CA15.3 та AFP у сироватці крові визначали за допомогою ELISA. Встановлено, що рівень пухлинних біомаркерів у сироватці крові пацієнтів із COVID-19 складав 7,74 ± 4,65 нг/мл для CEA; 29,33 ± 16,35 од/мл для CA19.9; 23,24 ± 13,48 од/мл для CA15,3 та 7,46 ± 5,57 нг/мл для AFP, у той час, як рівень біомаркерів у сироватці крові осіб, які не хворіли на COVID-19 був 9,73 ± 43,58 нг/мл для CEA; 20,66 ± 11,10 од/мл для CA19.9; 19,64 ± 10,99 од/мл для CA15.3 та 3,83 ± 9,20 нг/мл для AFP. Таким чином, показано відсутність відмінностей у рівнях пухлинних біомаркерів двох досліджуваних груп. Зроблено висновок, що пухлинні біомаркери CEA, CA19.9, CA15.3 та AFP не можуть бути використані як ефективні інструменти скринінгу пацієнтів на COVID-19.

Ключові слова: пухлинні біомаркери, CEA, CA19.9, CA15.3, AFP, COVID-19.

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