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Evaluation of Serum Cytokeratin 20 and some Trace Elements in Type 2 Diabetic patients with and without COVID19

Samah A. S. Alkubaisi, Shakir F. T. Alaaraji*

Department of Chemistry, College of Education for Pure Sciences, University Of Anbar, Ramadi, Iraq

Abstract

Coronavirus disease 19 (COVID19) is the trend in the healthcare system forthe last two years. The infection with COVID 19 in type 2 diabetes mellitus (T2DM) patients has been signified in the severity of the disease symptoms. Cytokeratin 20 as well as Fe, Cu and Zn elements were investigated in T2DM with and without the incidence of COVID19. The study has included 84 subjects, from Al-Fallujah General Hospital; 28 patients were diagnosed with T2DM, 28 patients were diagnosed with T2DM and infected by the SARS-Cov-2, and 28 healthy people to control the study, then the serum of the fasting individuals was analyzed for glucose, cytokeratin 20, Fe, Cu, and Zn. The results have shown significant ($P<0.05$) elevated levels of glucose, cytokeratin 20, Cu, Fe, and Cu/Zn and Fe/Zn ratios in T2DM patients with and without COVID19, compared to control. The highest obtained significant values were in T2DM patients with COVID19 compared to T2DM patients and control. The level of Zn was reduced significantly ($P<0.05$) in T2DM patients with and without COVID19, with T2DM associated COVID19 having shown the lowest Zn levels. The receiver operating characteristic (ROC) curve has indicated that glucose, cytokeratin 20, and the ratio of Fe/Zn were excellent sensitive markers in the prognosis of T2DM associated COVID19. Furthermore, the ROC indicated that Zn and Cu/Zn ratio are good sensitive markers, while Fe and Cu were fair sensitive markers in the prognosis of T2DM associated COVID19. The results have shown the important role of cytokeratin 20 in the pathophysiology of T2DM and COVID19 which may correlate with abdominal inflammation of COVID19 patients. Moreover, the significant alteration of Fe, Cu, and Zn may follow the oxidative imbalance of T2DM and COVID19 patients.

Keywords: SARS-Cov-2 ;Type2DM iron; zinc; copper; cytokeratin20

Introduction

Today, the pandemic of coronavirus disease 2019 (COVID19) has become versatile and growing in very rapid rates [1]. The disease is developed as the consequence of viral infection of the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) [2]. Since it's first appearance in Dec 2019, SARS-Cov-2 has been adapted and several variants were reported until now, including; i) the original discovered SARS-Cov-2 alpha in 2019, ii) SARS-Cov-2 beta in 2020, iii) SARS-Cov-2 gamma in 2021, iv) SARS-Cov-2 delta in 2021 [3], and v) SARS-Cov-2 omicron in 2021 [4].

The world health organization (WHO) have reported that signs and symptoms accompanied by COVID19 are different in different people, and infectious variant [5]. Yet, the most significant, and abundant signs and symptoms are fatigue, hypoxia, cough, loss of smell and taste senses, pain, and fever [6]. Moreover, the reports have indicated that 75% of COVID19 patients can develop severe chest symptoms [7], and under very severe conditions COVID19 can cause mortality [8, 9]. The association of COVID19 with other health disorders has been documented to increase the severity of COVID19 symptoms. Such reported were perform on type 2 diabetes mellitus (T2DM) [10], obesity [11], hypertension [12], chronic kidney disease (CKD) [13], etc.

Type 2 diabetes mellitus (T2DM) is a metabolic disorder related to insulin defective secretion or action, and causes hyperglycemia [14]. Reports have indicated a progression of inflammation in T2DM patients mediated by the presence of pro-inflammatory cytokines [15, 16]. On this topic, COVID19 has been also characterized by the presence of cytokine mediating inflammation, which is related directly to the severity of the symptoms [17]. But the information

*Corresponding author e-mail: esp.shaker.faris@uoanbar.edu.iq

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of T2DM pathophysiology in COVID19 is still limited [18].

Cytokeratins are important proteins of the extracellular matrix (ECM), secreted by the epithelial cells to preserve the structural integrity of the tissue [19]. There are more than twenty type of cytokeratins that have been recognized, and all are tissue-specific filament, hence, it widely used in the differentiation of malignancy from normal cells [20]. The documents have suggested a key role of cytokeratins in immunomodulation and cell apoptosis [21, 22]. Cytokeratin 20 is a protein of acidic isoelectric point expressed in the gastrointestinal epithelial cells, urothelial cells, and Merkel cells [23]. Therefore, cytokeratin 20 has been used in the differentiation of colorectal cancer [24].

Trace elements are crucial to the health of human body [25]. Metals such as iron (Fe), copper (Cu) and zinc (Zn) are found in the middle of multiple vital functions performing essential roles [26]. Fe is an important component of heme, and the most significant role of Fe is in oxygen transporting of hemoglobin and myoglobin [27]. Heme is also found in other proteins such as the antioxidant enzyme, catalase [28], and most of cytochrome p450 oxidases [29]. Cu and Zn are also important in many proteins such as oxidoreductases [30, 31]. Fe and Cu are pro-oxidant elements, in which they are mediated the production of free radicals and reactive oxygen species (ROS) at elevated levels in Fenton reaction [32, 33]. While Zn is more of an antioxidant, as it presence in the Cu/Zn-super oxide dismutase (Cu/Zn-SOD) [34].

The main goal of the present study was to investigate the role of cytokeratin 20 in T2DM and COVID19 to clarify some of the mysterious progressive conditions in COVID19 associated with T2DM. The role of Fe, Cu, and Zn was also a subject of study.

Materials and Methods

2.1. Subjects

Twenty-eight patients who were diagnosed with T2DM were enrolled in the study their ages ranged from 35 to 65 years old, T2DM patients were collected from Al-Fallujah Hospital. Furthermore, 28 T2DM patients infected by SARS-Cov-2 were enrolled in the Corona Isolation Hall at Al-Fallujah Hospital. COVID19 patients were diagnosed by smear sampling which indicated the presence of SARS-Cov-2 by using real-time reverse transcription- polymerase chain reaction (RT-PCR) according to the clinical diagnostic criteria based on the Novel Coronavirus Pneumonia Diagnosis and Treatment Guideline (6th ed.) [35]. The age range of COVID19 associated T2DM patients was from 35-65 years. The study was controlled with healthy lean people of matching age with patients. The subjects were collected from November 2021 to January 2022.

2.2. Samples collection

Subjects who enrolled in this study volunteered for vein blood donation after 8 hours of fasting. The blood was centrifuged at 1500 xg for 10 minutes to separate the serum. Then the serum was stored in three tubes for each subject at -20 °C until analysis time.

2.3. Methods

The serum of each individual was analyzed for cytokeratin 20 by using a commercial enzyme-linked immunosorbent assay (ELISA) kit purchased from BT LAB Company /China; the measurement was preceded by using an ELISA microplate reader (Human/Germany). Fe, Cu and Zn were analyzed spectrophotometrically in the serum by using Spinreact/Spain, the measurement was proceeded by using enzymatic colorimetric methods. Ferritin and D.dimer were analyzed in COVID19 patients only, and the data were collected from the patient's entry forms. Fasting serum glucose (FSG) was measured for each subject spectrophotometrically.

2.4. Statistical analyses

The outcomes were processed statistically by using GraphPad Prism version 7. The mean comparisons were analyzed by using the analysis of variances (ANOVA), where the p-value of 0.05 or less is considered a significant difference. Highest-significant differences (HSD) post-Hoc test was used as post ANOVA test. The receiver operating characteristics (ROC) was used for testing the possibility of using the parameters in the prognosis of COVID19, by depending on the value of the area under the curve (AUC).

Results and Discussion

3.1. Comparative study

The outcomes of control, T2DM patients, and T2DM with COVID19 patients are expressed by the mean and the standard deviation (SD), as seen in Table 1. According to the criteria of subject enrollment, the age differences were non-significant (P>0.05) among control (50.04±8.988 years), T2DM patients (52±8.628 years), and T2DM with COVID19 (54.31±6.898 year).

Table 1: Comparisons of parameters among the study groups.

Parameter	Healthy controls		T2DM Patients		T2DM with Covid19 Patients		
	Mean	SD	Mean	SD	Mean	SD	p-value
Age years	50.04	8.988	52	8.628	54.31	6.898	0.1505
FSG mg/dL	92.35	9.277	181.9	48.83	216.2	59.94	<0.0001
Cu µg/dL	69.35	18.74	83.64	17.37	97.29	24.95	<0.0001
Zn µg/dL	104.2	16.42	79.46	19.05	73.19	13.01	<0.0001
Fe µg/dL	85.19	17.49	102.8	14.49	126.5	20.57	<0.0001
Cu/Zn	0.6681	0.1976	1.168	0.5317	1.411	0.3957	<0.0001
Fe/Cu	1.328	0.4308	1.266	0.3018	1.317	0.3286	0.7846
Fe/Zn	0.8308	0.189	1.396	0.4917	1.767	0.3637	<0.0001
Cytokeratin20 ng/mL	20.61	8.265	38.85	9.759	51.15	13.08	<0.0001
D-Dimer ng/mL	------	------	------	------	2257	487.3	------
Ferritin ng/mL	------	------	------	------	1279	274.8	------

The level of FSG was significantly higher in T2DM patients, and T2DM associated COVID19 patients, compared to control subjects. Furthermore, T2DM associated COVID19 patients have shown significant higher levels of FSG compared to those without COVID19 patients.

The levels of Cu and Fe were significantly elevated in T2DM patients, and T2DM associated COVID19 patients, compared to control. The highest significant values of Cu and Fe were observed in T2DM associated COVID19 patients. On the contrary, Zn level was reduced significantly in T2DM patients, and T2DM associated COVID19 patients, compared to control.

The ratios of Cu/Zn, Fe/Cu, and Fe/Zn were calculated for each subject and statistically compared among the three groups. The results have indicated significant higher values of Cu/Zn and Fe/Zn ratios in T2DM patients with and without COVID19 compared to control, where the T2DM patients with COVID19 have shown the highest values of Cu/Zn and Zn/Fe ratios. On the other hand, the differences of Fe/Cu ratio were non-significant among the three groups.

The level of cytokeratin 20 was significantly elevated in T2DM patients, and T2DM associated COVID19 patients, compared to control subjects. The highest significant value of cytokeratin 20 was obtained for T2DM associated COVID19 patients.

3.2. Reviver Operating Characteristics

Table 2 contains the values of AUC in the diagnosis of T2DM associated COVID19 with respect to healthy control.

The ROC curve has shown the unusefulness of using age and Fe/Cu ratio in the prognosis of T2DM associated COVID19, in which the AUC of both were less than 0.6 which means that both parameters are considered to fail as sensitive markers, as shown in Figure 1 and 2.

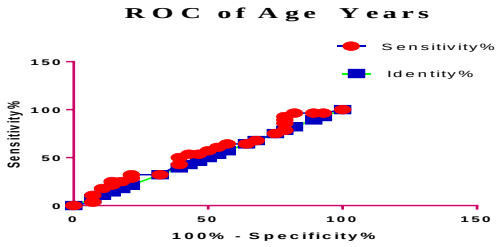

Figure 1: The ROC curve of age in the prognosis of T2DM associated COVID19.

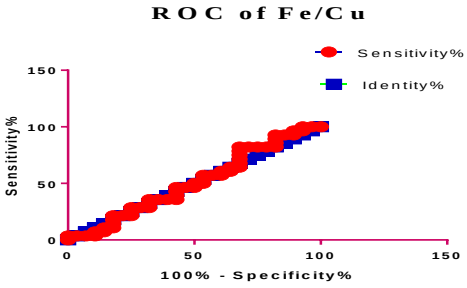

Figure 2: The ROC curve of Fe/Cu ratio in the prognosis of T2DM associated COVID19.

The ROC curve has indicated that FSG (Figure 3), Fe/Zn ratio (Figure 4), and cytokeratin 20 (Figure 5) are excellent sensitive markers in the prognosis of T2DM associated COVID19. The characteristics have

shown that the AUC of FSG was 1.0, Fe/Cu was 0.9069, and cytokeratin 20 was 0.912, all with p-value less than 0.0001.

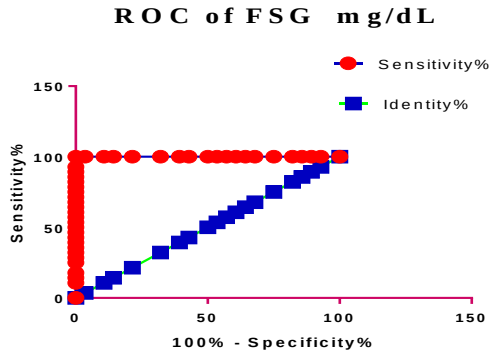

Figure 3: The ROC curve of FSG in the prognosis of T2DM associated COVID19**.**

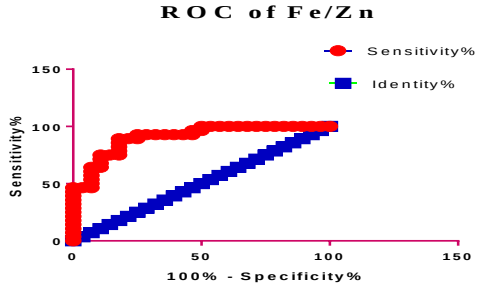

Figure 4: The ROC curve of Fe/Zn ratio in the prognosis of T2DM associated COVID19.

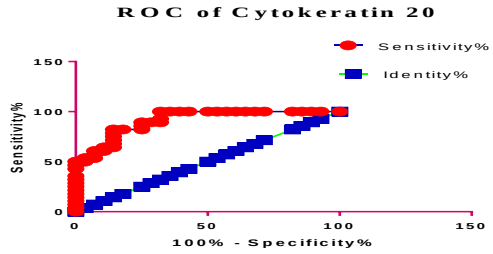

Figure 5: The ROC curve of cytokeratin 20 in the prognosis of T2DM associated COVID19.

Additionally, the ROC curve has indicated that Zn (Figure 6) and Cu/Zn (Figure 7) were good sensitive markers in the prognosis of T2DM associated COVID19, with AUC of Zn 0.8138 and Cu/Zn 0.838. While Fe (Figure 8) and Cu (Figure 9) were fair sensitive markers

The involvement of T2DM in the development of progressive symptoms upon the incidence with SARS-Cov-2 has been reported [36]. In a previous meta-analysis study, the authors concluded that DM was an independent risk factor for increasing the severity and mortality of COVID19 [37].

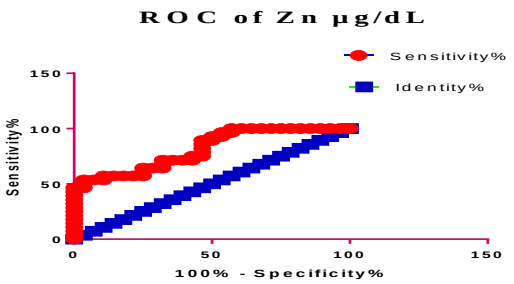

Figure 6: The ROC curve of Zn in the prognosis of T2DM associated COVID19.

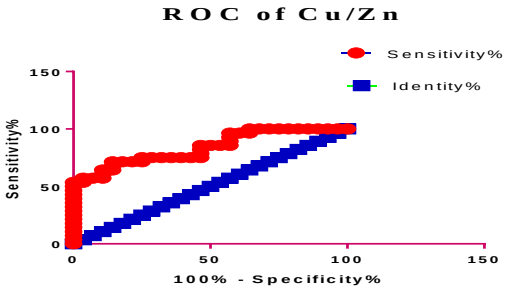

Figure 7: The ROC curve of Cu/Zn ratio in the prognosis of T2DM associated COVID19.

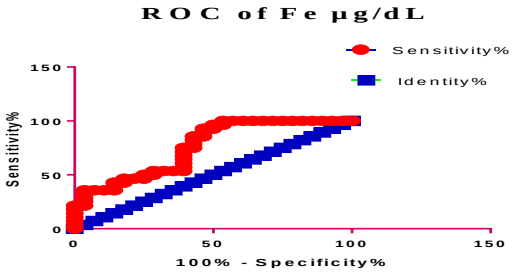

Figure 8: The ROC curve of Fe in the prognosis of T2DM associated COVID19.

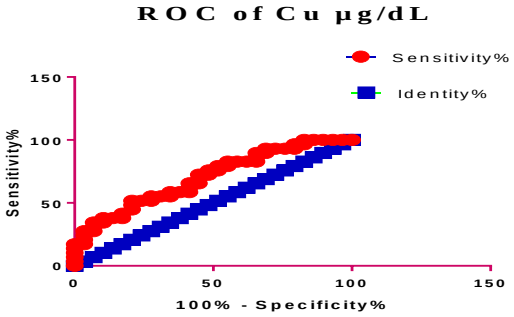

Figure 9: The ROC curve of Cu in the prognosis of T2DM associated COVID19.

We have observed elevated levels of ferritin and D. dimer in T2DM associated COVID19 patients (Table 1). Ferritin is a biomarker of iron storage, it has a key role in mediating immune dysregulation, particularly in very high levels of ferritin, through immediate immunosuppressive and pro-inflammatory impacts, participating in the cytokine storm syndrome [38].

The lethal role of COVID19 has been documented to be associated with the cytokine storm syndrome, that way the researchers have established that the severity of COVID19 is dependent on the degree of the cytokine storm syndrome [39]. Several people with diabetes have high levels of ferritin in their blood [40-42], and it is well recognized that they are more likely to have significant COVID19 problems [43]. According to Poudel et al., the D-dimer value upon hospital entrance is an efficient predictor for identifying morbidity in COVID-19 subjects [44]. Viremia and the cytokine storm syndrome, whereby the increase in pro-inflammatory cytokines is insufficiently regulated by anti-inflammatory agents, overwhelming the coagulation cascade, are the most prevalent reasons reported in the literatures for the increase of D. dimer levels [45].

Hyperglycemia is one of the characteristic signs of T2DM [14], and this was seen in the present study as the ROC curve has indicated a very excellent sensitivity (AUC=1.0) of FSG in the prognosis of T2DM associated COVID19. In COVID19 the majority of patients under treatment, this was attributed to elevated cortisol levels [46]. Several studies have reported elevated blood glucose in COVID19 patients [47-49].

The levels of Cu and Fe were significantly elevated in T2DM patients with and without the incidence of COVID19, encountered by a significant reduction of Zn level. This was in agreement with Hussain et al. who reported significantly elevated Cu levels accompanied by significantly reduced levels of Zn in patients with T2DM [50]. Another agreement was found with Ekin et al. who reported significantly elevated levels of Fe and Cu, as well as the ratios of Cu/Zn and Fe/Zn in T2DM patients compared to control. The authors also reported a significantly low level of Zn in T2DM [51]. Pour et al. have reported that Cu levels were in the normal range in COVID19 patients but Zn level was reduced [52]. Zeng et al. have reported that patients with severe COVID19 symptoms have shown significantly high levels of Fe and Cu with a significant reduction of Zn level [53]. But a disagreement was found with Alipour et al. who reported low levels of serum Fe in COVID 19 patients [54].

The elevated Fe level oxidizes various biomolecules such as nucleic acids, proteins and lipids, which may contribute to T2DM development by decreasing insulin secretion from pancreatic beta cells with a concomitant increase of insulin resistance [55]. While in COVID19, elevated Fe levels may reflect an increase in viral load [56]. Both Fe and Cu are related to increasing the free radicals and ROS (elevated oxidative stress) in the Fenton reaction [32, 33]. Oxidative stress was reported in both T2DM [57] and COVID19 [58], hence it may be attributed, in part, to the elevated levels of Fe and Cu.

In this study, we have evaluated the level of cytokeratin 20 in T2DM and COVID19 for the first time. The novel outcomes have shown elevated levels of cytokeratin 20 in the serum of T2DM patients with and without COVID19. Furthermore, T2DM associated COVID19 was shown the highest significant level of cytokeratin 20. This for sure indicates an important role of cytokeratin 20 in the pathophysiology of T2DM and COVID19, but the mechanism is fully unclear. Cytokeratin 20 was used as a tumor marker for intestinal malignancy, as it is expressed almost exclusively in the epithelium of intestines [59]. We suggest a link between cytokeratin 20 and intestinal pathological alterations that occurs as a consequence of T2DM and COVID19 infection, but it needs more extensive research.

Table 2: ROC outcomes of the study parameters

Parameter	AUC	Std. Error	95% confidence interval	P-value
Age	0.5491	0.0777	0.3968 to 0.7014	0.5281
FSG mg/dL	1	0	1 to 1	<0.0001
Cu µg/dL	0.7021	0.06771	0.5694 to 0.8348	0.0082
Zn µg/dL	0.8138	0.05549	0.705 to 0.9225	<0.0001
Fe µg/dL	0.7589	0.06415	0.6332 to 0.8847	0.0009
Cu/Zn	0.838	0.0524	0.7353 to 0.9407	<0.0001
Fe/Cu	0.5102	0.07829	0.3568 to 0.6636	0.8957
Fe/Zn	0.9069	0.0384	0.8316 to 0.9822	<0.0001
Cytokeratin20 ng/mL	0.912	0.03637	0.8407 to 0.9833	<0.0001

Conclusions

The results have indicated that Fe, Cu and Zn are quite mediators in the pathophysiology of T2DM and COVID19. Patients have shown elevated levels of Fe and Cu, with reduced levels of Zn. In COVID19 patients, Fe may be related to a high viral load of SARS-Cov-2, while both Fe and Cu are linked to increase the oxidative stress. Zn reduction may also a part of increasing oxidative stress in T2DM and COVID19 through decreasing the activity of Cu/Zn-SOD antioxidant enzyme. Moreover, the novel evaluation of cytokeratin 20 in T2DM and COVID19 has indicated significant elevated levels. The ROC was also revealed the usefulness of cytokeratin 20 in the prognosis of T2DM associated COVID19. This suggest an important role of cytokeratin 20 in the pathophysiology of T2DM and COVID19, but further investigations are required to understand the mechanism between T2DM, COVID19 and elevated cytokeratin 20 levels.

Conflicts of interest

In accordance with our policy on Conflict of interest please ensure that a conflicts of interest statement is included in your manuscript here. Please note that this statement is required for all submitted manuscripts. If no conflicts exist, please state that "There are no conflicts to declare".

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