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ORIGINAL RESEARCH

Validity of Signal Peptide-CUB-EGF Domain-containing Protein-1 (SCUBE-1) in the Diagnosis of Aortic Dissection

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Abstract

Introduction: Diagnosing aortic dissection (AD) in emergency services still represents a challenging issue as it may manifest not only well-known clinical findings such as sudden onset and severe chest pain but also atypical findings similar to ST elevation myocardial infarction or renal colic. Contrast-enhanced imaging investigations, which are expensive and risky due to possible complications, are necessary for diagnosis. Ultrasonography is not always reliable, which necessitates noninvasive diagnostic tests to support a clinical suspicion. Signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF (epidermal growth factor) domain-containing protein 1 (SCUBE-1) is a cell surface protein produced during embryogenesis. This study was conducted to compare the levels of this novel biomarker between patients with AD and healthy volunteers. **Methods:** This prospective study was conducted on 20 patients diagnosed with AD using contrast-enhanced thoracoabdominal computed tomography angiography. Average age-matched 20 healthy subjects as a control group were included as a reference for biochemical parameters. **Results**: The mean SCUBE-1 levels were significantly higher in patients with AD [24.51 ($\pm$ 3.01) ng/dL] than in the control group [12.11 ($\pm$ 5.31) ng/dL] ($p < 0.001$). Receiver-operating characteristic (ROC) curve was plotted to analyze the specificity and sensitivity of AD diagnosis (with 95% confidence intervals), which revealed 95% sensitivity and 76% specificity when the SCUBE-1 level was $>$ 19.75 ng/dL. A significant correlation was also observed between dissection types and mortality, as well as extravasation state. **Conclusions:** This preliminary study demonstrated that plasma SCUBE-1 level is a better and specific biomarker for AD and may be used for diagnosing AD in emergency services. Wider case series and further clinical studies are required to confirm these findings.

Keywords

SCUBE-1, Aortic dissection, Emergency service

1. Introduction

Aortic dissection (AD) is one of the troublesome diagnoses in emergency services. It requres rapid diagnosis and confirmation. The clinical picture beginning with chest pain has to be confirmed using imaging methods. However, the findings are always not clear, and patients can show only a simple finding imitating mere ischemia. Thus, it is possible that AD could be confused with findings suggesting renal colic, rendering the diagnosis a difficult issue. Ultrosonograpy and D-dimer level are also not always helpful. Contrast-induced and invasive tomography may not be applicable for all clinically suspected patients because of side effects and the cost [1]. Therefore, a helpful and noninvasive biomarker is necessary for clinicians to make a clear diagnosis.

Signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF (epidermal growth factor) domain-containing protein 1 (SCUBE-1) is a surface protein secreted during embryogenesis. In recent years, it was demonstrated that this protein can be used for multiple diagnoses, and there are also ongoing studies investigating SCUBE-1.

In the present study, we investigated whether SCUBE-1 protein could be useful for clinicians as a biomarker in the diagnosis of AD.

2. Materials and methods

2.1 Study setting

This investigation was a prospective study conducted between January 01, 2019, and January 01, 2020. We included 20 patients diagnosed with AD who applied to the emergency department of our university hospital and 20 healthy volunteers. SCUBE-1 levels were assessed for both groups of subjects. Patients and volunteers were aged $\geq$ 18 years. Twenty

healthy subjects as a control group of a similar age as that of the AD group were also included. The control group was formed according to the inclusion and exclusion criteria and the average age of patients. Volunteers with known past medical history and no history of disease/surgery were included in this group.

2.2 Study population

This study was conducted in the emergency service of Izmir Katip Çelebi University and Research Hospital, Izmir, where an average of 700 patients apply daily. It is a 1000-bed hospital and one of the largest in this region. All health conditions requiring critical care, such as AD and cerebrovascular infarcts, are managed and facilities such as coronary angiography units are served at this hospital. It is the only critical care center in this region.

Patients who were considered to have AD or equivalent findings were included in this study. Those aged $\geq$ 18 years, pregnant women, and those with a previous AD diagnosis were excluded. In addition, patients who had primary renal or hematological, severe hepatic, renal, or heart failure, as well as those who had been diagnosed with ischemic conditions such as mesenteric ischemia, were excluded. Patients allergic to contrast agent and those who had or still have a malignant tumor were also excluded. A medical history of a congenital diagnosis of a connective tissue disease such as Marfan syndrome was also considered as an exclusion criterion. Finally, patients who were not aware of their medical history were also excluded.

Patients who were admitted to the hospital with constant shock and vital imbalance were also not included as their statements could not be taken clearly and iatrogenic AD may occur during the procedures.

2.3 Data collection

Patients who were diagnosed with AD and consented to be a participant were primarily taken into the safety circle. Next, their blood samples were collected and analyzed at our hospital's laboratory using model/brand of Spectramax Plus 384 Elisa. The samples were analyzed using an enzyme-linked immunosorbent assay kit (catalog no. E-EL-H5405, Elabscience, Houston, United States) according to the manufacturer's instructions. The acquired data were recorded on forms prepared for patients.

Contrast-enhanced thoracoabdominal computed tomography (CT) angiography was performed for diagnostic purposes in the emergency department due to its high sensitivity and specificity in the diagnosis of AD. Diagnosis was performed using 128 slices of CT scan (Siemens Sonatom Definion AS 128) if the doctor thinks it is necessary. 350/100 mL iodide-equivalent 755 mL of a nonionic omnipac iodinated contrast medium was injected intravenously at the rate of 60 – 70 mL. 0.5 mL/s and 30 – 40 s delay in screening. It is believed that the tomography scans of patients with clear images of all vascular structures are compatible for the study, including the aortic diameter, contour irregularities, intimal flaps, contrast extravasation, and enlargement of aneurysms for acute AD diagnosis.

Blood samples (2 mL) were collected from the patients and the control group in blood collection tubes, blood samples taken were centrifuged at 4000 rpm for 10 minutes, and then stored in a freezer at - 80°C until analysis.

2.4 Statistical analyses

Statistical analyses were conducted using the SPSS 23.0 program for Windows® (IBM Inc., Chicago, IL, USA). Descriptive data were presented as number, percentage, mean, and standard deviation, median, minimum, and maximum values. The Kolmogorov–Smirnov test was used to evaluate the coherence of normal distribution of data. Pearson's chi-square and Fisher's exact tests were used for comparing categorical data. Independent T-test was conducted to compare two independent numerical values, and the Kruskal–Wallis test was used for comparing two different or three different numerical values. Results were considered to be significant at $p < 0.05$ with 95% confidence intervals.

3. Results

Among the study patients, there were 7 males and 13 females. Among the healthy volunteers, 50% (n = 10) were males and 50% (n = 10) were females. The demographic characteristics of both groups were similar with no statistical differences ($p > 0.05$). The average duration between the first arrival of patients to the emergency department and the diagnosis was 61.30 $\pm$ 75.13 (range 7 – 310) min, whereas the average time for the arrival of the cardiovascular surgeon was 33.35 $\pm$ 15.89 (range 10 – 65) min. The average hospitalization duration was 4.60 $\pm$ 3.63 (range 0–10) days. Table 1 shows the information regarding the arrival features of patients, vital signs, and their demographic characteristics, including a summary of their diagnosis.

The outcomes of patients were analyzed according to the types of AD, and no statistical significance was found between AD types and mortality. Type 1 AD cases had a statistically significantly high mortality rate ($p < 0.001$). We also analyzed the correlation between AD types and extravasation state and detected a statistically significantly high relationship in AD type 1 cases ($p < 0.001$). In addition, mortality rate was significantly higher in cases with extravasation ($p < 0.001$) (Table 2).

The overall mean SCUBE-1 level of the study subjects was 16.11 $\pm$ 5.33 (range 7.26 – 27.84) ng/dL, with 24.51 $\pm$ 3.01 ng/dL in the patient group and 12.11 $\pm$ 5.31 ng/dL in the control group. The mean SCUBE-1 level in the patient group was found to be significantly higher than that in the control group ($p < 0.001$). A receiver-operating characteristic (ROC) curve was plotted to determine the specificity and sensitivity of AD diagnosis (with 95% confidence intervals) (Fig. 1), which demonstrated a sensitivity of 95% and a specificity of 76% when the SCUBE-1 level was >19.75 ng/dL. On the other hand, a significant correlation was found between AD types and mortality and the extravasation state. However, there were no significant differences in the mean SCUBE-1 levels based on the AD types, outcomes, and extravasation ($p > 0.05$) (Table 3).

TABLE 1. Details about the cases.

Parameter	n (%) or Average (IQR or ± SS)
Age	
Male	58.35 ± 16.97
Female	65.35 ± 18.62
Gender	
Male	23 (57.5)
Female	17 (42.5)
Admission Complaint	
Chest Pain	14 (70.0)
Back Pain	3 (15.0)
Other	3 (15.0)
Admission Vital Findings	
Pulse (/Min)	93.60 ± 18.72
Systolic Blood Pressure (mmHg)	127.25 ± 29.74
Diastolic Blood Pressure (mmHg)	64.42 ± 16.28
Aortic Dissection Types	
Type I	8 (40.0)
Type II	3 (15,0)
Type III	9 (45.0)
Extravasation Status	
Positive	6 (30.0)
Negative	14 (70.0)
Outcome	
Discharge	10 (50.0)
Exitus	10 (50.0)
HT Status	
Positive	13 (65.0)
Negative	7 (35.0)
CPR* in Emergency Room	
Positive	3 (15.0)
Negative	17 (85.0)
Treatment	
Medical Treatment	2 (10.0)
Preoperative Exitus	5 (25.0)
Postopertive Discharge	8 (40.0)
Postoperative Exitus	5 (25.0)

** CPR: Cardiopulmonary resuscitation.*

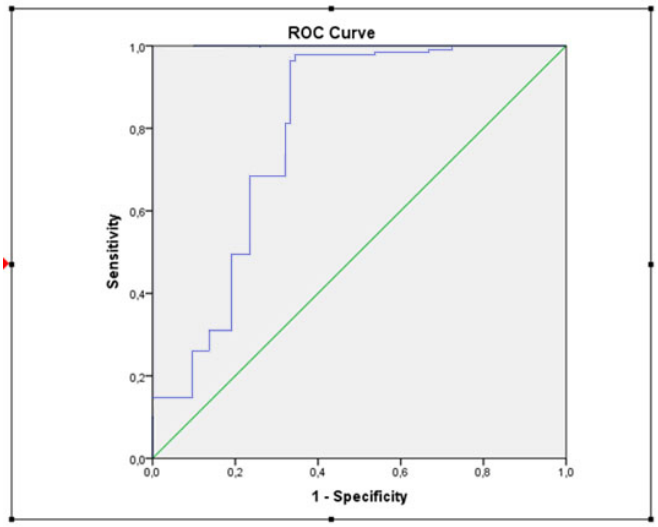

FIGURE 1. ROC analysis of subjects' SCUBE-1 levels. *95% sensitivity and 76% specificity were determined on the cut-off value calculated for AD. AUC = 0.863 (95% CI = 0.70–1). AUC = Area under the curve; AD = Aortic dissection; ROC = Receiver-operating characteristic.*

TABLE 2. Diagnosis/outcome status of cases in the Patient Group.

	Outcome		
Dissection Types	**Exitus**	**Discharge**	**p***
Type I	7 (87.5)	1 (12.5)	
Type II	1 (33.3)	2 (66.6)	< **0.001**
Type III	2 (22.2)	7 (77.8)	
Extravasation			
Positive	6 (42.9)	8 (57.1)	
Negative	4 (66.7)	2 (33.3)	< **0.001**
	Extravasation		
Dissection Types	**Positive**	**Negative**	
Type I	6 (75.0)	2 (25.0)	
Type II	2 (66.6)	1 (33.3)	< **0.001**
Type III	6 (66.7)	3 (33.3)	

**: Fisher's exact test was used.*

4. Discussion

AD comprises the most serious and a life-threatening situation. Although not known clearly, the frequency of AD has been predicted to be 5 – 30 cases in a million population. The diagnosis of AD in emergency services remains a challenging issue. Especially, patients with a history of hypertension are at risk in this context. In the present study, a history of HT was present in 65% of the cases (n = 13). Contrary to the general belief that atypical symptoms are quite common, more than one-third of patients have findings and symptoms secondary to involvement of organ systems. When atypical symptoms are not recognized, potential death is inevitable. These symptoms include 50% pulse deficits, 20% neurological deficits, 5% syncope, and 5% – 10% painlessness [1, 2]. Diagnosis is made using tests such as contrast-enhanced CT angiography, which are expensive and have risks due to radiation and contrast agent. Nonetheless, this test remains the most commonly used diagnostic tool for AD. Hence, diagnostic tests that are inexpensive, noninvasive, and with less risk of complication are necessary. Although D-dimer is a supportive marker for AD, age > 70 years, pregnancy, active malignancy or metastasis, recent surgeries, trauma, deep vein

TABLE 3. Estimated mean SCUBE-1 values.

Parameter	SCUBE-1 Level (ng/dL)	p
Groups		
Patient Group	24.51 ± 3.01	< **0.001***
Control Group	12.11 ± 5.31	
Aortic Dissection Types		
Type I	25.32 ± 3.33	
Type II	26.44 ± 2.04	0.197**
Type III	23.14 ± 2.58	
Extravasation Status		
Positive	25.45 ± 1.67	0.372*
Negative	24.10 ± 3.41	
Outcome		
Discharge	24.39 ± 3.80	0.246*
Exitus	24.62 ± 2.17	

**: Independent T-Test; **: Kruskal–Wallis test was used.*

thrombosis, hepatic failure, rheumatoid arthritis, and presence of infection are potential causes of false-positives. In case of warfarin treatment, the presence of small and isolated thrombi and having symptoms lasting longer than 5 days with false-negative outcomes are detected [2]. Furthermore, D-dimer causes confusion for AD diagnosis, and there are also reports of possible D-dimer negativity in patients with AD [3]. There are also reports of patients with negative D-dimer levels but being operated [4]. Ultrasonography (USG) is also a contributory test for the diagnosis. However, emergency physicians have limited experience in this regard. Reliability of USG is variable because it may provide different results depending on the characteristics of patients and the physician experience. A noninvasive and safe diagnostic method useful for making the diagnosis is unavailable, indicating the crucial need for clinical studies.

SCUBE-1 is a cell surface protein released during embryogenesis. Its family members consist of amino-terminal signal peptide, repeated nine copies of EGF-like N terminal, spacer, CUB-domain signal peptide C terminal sequence, and structural cysteine molecules [5]. This protein is expressed on thrombocytes and vascular endothelial cells. It is involved in thrombosis by taking part in the structure of EGF glycoprotein. It also plays a role in thrombin activation and inflammation [6]. In case of vascular damage, there is an increase in the expression of SCUBE-1.

Acıpayam et al. study reported that SCUBE-1can indicate early stages of thrombosis in patients with beta thalassemia [7]. In recent years, this protein has been investigated because of its role in vascular damage and thrombosis. Dia et al. demonstrated that SCUBE-1 expression can be detected 6 h after cardiac ischemia (SCUBE-1 level in patients with ACS: 205 ng/mL), and it has low sensitivity and is a good marker for thrombosis (In our study, the SCUBE-1 level was measured as 27.84 ng/mL in the majority of AD cases.). In the same study, the authors stated that it is a reliable marker for diagnosing ischemic stroke [8]. Moreover, it has been demonstrated that its expression can be detected within 2 h in cases of mesenteric ischemia [9]. A specific biomarker for pulmonary emboli (PE) is also lacking. In this context, in a study conducted by Türkmen et al., it was reported that SCUBE-1 can be used for PE diagnosis as well [10].

AD is a pathology manifested by vascular endothelial damage and thrombi. SCUBE-1 protein is produced from the vascular endothelium, and its levels in the blood increase in cases of vascular structure damage and thrombi [4]. We observed SCUBE-1 levels can increase in AD as well, in our study. This unexpected increase would support the clinical predictivity. A recommended biomarker for AD is lacking, and although determining D-dimer levels is recommended, its diagnostic value is low. Several studies have stated not to use D-dimer positive and negative values for diagnosis, although there are meta-analyses that have indicated using its negative predictive value [1]. In the present study, we determined the AUC value of SCUBE-1 for AD diagnosis as 0.863, in addition to 95% sensitivity and 76% specificity when the SCUBE-1 cut-off level was 19.75 ng/dL. Based on our study results and in comparison with clinical findings, we believe that SCUBE-1 would be a helpful biomarker for clinicians for diagnosing AD.

5. Conclusion

AD is a diagnosis associated with a high mortality rate in emergency services. Therefore, scientific studies are important to help in the diagnosis. We observed that plasma SCUBE-1 levels have high specificity for AD diagnosis and thus can be valuable. We suggest further studies with larger clinical series to support the resuls of our preliminary study.

LIMITATIONS

The first limitation of our study was the relatively small numbers of patients and controls, although these numbers were predicted by a power analysis. Next, the average age of the subjects was high in our study, as it is known that AD can also present in younger patients. Finally, according to the manufacturer information on the analysis kit, plasma samples cannot be stored in freezers for more than 2 months. This storage period can be a limitation, although the duration was not expired in our study.

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CONFLICT OF INTEREST

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

ETHICAL CONSIDERATION

Approval for conducting the study was obtained from the ethical committee of the university hospital (Izmir Katip Celebi University Clinical Studies Ethics Committee). Ethical committee number: 2019-KAE-0252. Consent forms were obtained from all patients and healthy volunteers or their first-degree relatives. The entire study was conducted according to the criteria of the Declaration of Helsinki.

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