Research Article

health science

# RELATIONSHIP BETWEEN CORONARY COLLATERAL DEVELOPMENT AND SCUBE (SIGNAL PEPTIDE-CUB-EGF DOMAIN-CONTAINING PROTEIN) LEVELS IN PATIENTS WITH CORONARY ARTERY DISEASE

**Burak Ogulcan YILDIRIM[1*], Cemil ZENCIR[2]**

***Keywords***

*Coronary artery disease, Coronary collateral development, SCUBE1, Signal Peptide CUB-EGF like Domain Containing Protein*

## ABSTRACT

*Our study focused on exploring the connection between serum SCUBE1 (Signal Peptide CUB-EGF like Domain Containing Protein) levels and the formation of CCC (Coronary Collateral Circulation) in patients diagnosed with CAD (coronary artery disease). In the study, we included 80 patients who underwent coronary angiography in the cardiology clinic between April 25, 2019 and April 25, 2020. Their mean age was 63.75 ± 10.7 years. Of them, 30 with normal coronary arteries were assigned to Group 1, 19 with CAD to Group 2, and 31 with CAD who developed CCC to Group 3. The mean SCUBE1 level was 30.61±2.6ng/ml in all the patients. There was no significant difference between the groups (P=0.272). As a result, we compared SCUBE1 levels according to the development of CCC in patients with CAD for the first time in the literature. The findings demonstrated that the measurement of SCUBE1 levels alone might be insufficient to predict the development of CCC in patients with CAD. To confirm these results, multicenter studies with larger samples should be conducted.*

## INTRODUCTION

Even with the progress made in science and technology, CAD continues to be a major contributor to mortality rates in developed nations. CAD most commonly develops in patients with atherosclerosis. Explaining the risk factors and underlying biological and anatomical connections of atherosclerosis complications can be challenging[1]. Collateral vessels, which help to protect the myocardium in patients with CAD, limit the infarct area and can prevent the development of left ventricular aneurysm and heart failure, which significantly reduces mortality and morbidity rates[2].

Therefore, it is important to investigate the biological factors likely to affect the development of collateral vessels[3]. Mechanisms of angiogenesis and arteriogenesis play a role in the development of CCC[4]. In studies conducted within this context, SCUBE (Signal Peptide CUB-EGF like Domain Containing Protein) molecule has been indicated to play a role in platelet aggregation, angiogenesis, arteriogenesis, carcinogenesis and the development of the nervous system[5,6]. Signal Peptide CUB-EGF Domain-Containing Proteins belong to the EGF superfamily, characterized by diverse domain structures such as epidermal growth factor (EGF)-like repeats, cysteine-rich repeat motifs, and the CUB (complement protein C1r/C1s, Uegf and Bmp1) domain.

Volume: 1
Issue: 2
Page: 61-68

Received:
30.06.2023

Accepted:
03.08.2023

Available Online:
15.10.2023



DOI:10.5281/zenodo.8414505

[1*] *Mengucek Gazi Education and Research Hospital, Medical Faculty, Erzincan Binali Yildirim University, Department of Cardiology, Erzincan, Turkey, b.ogulcan@hotmail.com, ORCID: 0000-0003-3448-6187*
[2] *Aydin Adnan Menderes University, Cardiology Department, Aydin, Turkey, ORCID: 0000-0002-8734-8987*

The SCUBE molecule has been detected predominantly in the gonads, developing tissues, central nervous system, dermomyotomes, finger mesenchyme, limb buds, endothelium, and alpha granules of inactive platelets during early embryogenesis[7]. There are three different isoforms of the protein in mammals, namely SCUBE1, SCUBE2 and SCUBE3. It may exist in the cell as a peripheral membrane protein or as a protein secreted to the extracellular region[8]. SCUBE is highly expressed in most of the vascularized tissues. Endothelium[7,9] and platelets[10] are very rich in SCUBE1.

The protein SCUBE1 is expressed in the vascular endothelium and plays a significant role in both the inflammatory response and thrombosis[9]. SCUBE1 exists in the α-granules of platelets in high levels. It has been shown that SCUBE1 has been indicated to strengthen ristocetin-induced platelet agglutination and adhesion[10]. SCUBE1 proteins have also been detected in various cardiovascular diseases[11]. SCUBE protein levels reach their peak value at the earliest 6 hours after the onset of symptoms in acute coronary syndrome (ACS) and stroke, but continue to decrease until the 96th hour after the 36th-60th hours[5]. In their study, Yang et al. determined a direct proportional relationship between angiogenesis, which has an important place in the pathogenesis of rheumatoid arthritis, and SCUBE1 level[12].

The results of these studies suggest that SCUBE1 might be effective in one of the mechanisms of collateral vessel formation in CAD. According to our review of studies conducted so far in the literature, there is no data on the relationship between CCC development and SCUBE1 levels in patients with coronary artery disease.

In our study, we aimed to investigate the relationship between serum SCUBE1 (Signal Peptide CUB-EGF like Domain Containing Protein) levels in patients with CAD (coronary artery disease) who developed CCC. Thus, we contributed to the literature by presenting information to reveal the possible role of SCUBE1 levels in the development of CCC in patients with CAD.

## MATERIALS AND METHODS

### *Determination of patient (inclusion) criteria and groups*

We planned this study as a prospective single center study to investigate the relationship between coronary collateral development and SCUBE level. The article was produced from the thesis.

We included 80 patients who underwent coronary angiography performed by us in the Cardiology Clinic of the Cardiology Department of Adnan Menderes University Hospital between April 25, 2019 and April 25, 2020 in the study. We obtained the approval to conduct the study from the Non-Interventional Clinical Research Ethics Committee (Approval number: 66, Approval Date: April 25, 2019). We assigned the 80 patients into three groups: group 1: patients with normal coronary arteries, group 2: patients with CAD and group 3: patients with CAD who developed CCC. We compared them in terms of their SCUBE levels.

The inclusion criteria for the patients with normal coronary arteries were as follows:

1. Being in the age group of 18-90 years and having been diagnosed to have normal coronary arteries during coronary angiography

2. Having had hemogram and biochemical analysis of their blood samples

The inclusion criteria for the patients with CAD were as follows:

1. Being in the age group of 18-90 years, having been diagnosed to have 50% or more stenosis in at least one of his or her coronary arteries during coronary angiography and having no collateral circulation

2. Having had hemogram and biochemical analysis of their blood samples

The inclusion criteria for the patients with CAD who developed CCC were as follows:

1. Being in the age group of 18-90 years, and having been diagnosed to have coronary collateral circulation during coronary angiography

2. Having had hemogram and biochemical analysis of their blood samples

The exclusion criteria were as follows:

1. Having a history of acute coronary syndrome, acute cerebrovascular accident, acute pulmonary embolism, acute mesenteric ischemia and acute arterial occlusion in the past month

2. Having malignancy or rheumatic disease

Immediately after the coronary angiography procedure, we obtained informed consent from the patients who volunteered to participate in the study. Then, we took approximately 5 ml of arterial blood samples into biochemistry tubes (red caps). After keeping the samples in the room temperature for 5 minutes, we centrifuged them at 3,500 rpm for 10 minutes using centrifuge device. Then we portioned the serums obtained into Eppendorf tubes to include at least two samples and stored them at -80°C. After reaching the desired number of cases, we removed all the samples stored at -0°C and raised their temperature to room temperature.

We analyzed the blood samples obtained from the volunteers using the human SCUBE1 ELISA (Sunred Biological Technology Co. Cat No: 201-12-5378, Shangai, China) kit and Epoch 2, BioTek brand device in accordance with the kit protocol of the manufacturer company. The sensitivity for the Human SCUBE1 ELISA kit is 0.852 ng/mL, and the measurement range is 1-300 ng/mL.

### *Statistical analysis*

We analyzed the results of the obtained data using the Statistical Package for the Social Sciences (SPSS, version 23) statistically. We used Kolmogorov-Smirnov test to find out whether the data were normally distributed, Mann-Whitney test to compare pairwise continuous variables in the analysis of the non-normally distributed data, Kruskal Wallis test to compare triple groups, and the chi-square test to compare categorical variables. We performed the correlation analysis of the data with the Spearman Correlation test.

We performed the ROC (Receiver operating characteristic curve) analysis to calculate the sensitivity and specificity of serum SCUBE1 level in predicting the presence of CAD and CCC.

## RESULTS

### *Demographic and clinical data of the participants*

The mean age of 80 volunteers included in the study was 63.75±10.7 years. Of them, 46 (57.5%) were men. Their demographic and clinical characteristics were presented in Table 1.

According to the results of coronary angiography, of the participants, 30 had normal coronary artery (Group 1), 19 had CAD (Group 2), and 31 had CAD and developed CCC (Group 3).

Of the participants, 33 (41.25%) had diabetes, 40 (50%) had hypertension, 31 (38.75%) had heart failure, 6 (7.5%) had chronic renal failure, 46 (57%) had hyperlipidemia. and 32 (40%) were smokers.

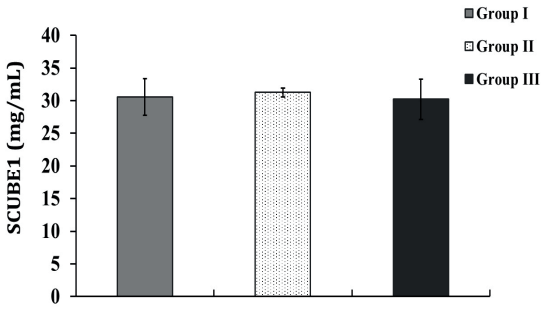
According to the Rentrop classification, used to determine and classify the coronary collateral circulation angiographically, of the patients in Group 3, 4 (12.9%) were Grade 1, 11 (35.5%) were Grade 2 and the majority (51.6%) were Grade 3 (Table 1).

**Table 1.** *Demographic and clinical characteristics of the participants.*

| Characteristics | Total (N = 80) | Group 1 (N = 30) | Group 2 (N = 19) | Group 3 (N = 31) | P value |
|---|---|---|---|---|---|
| **Age, X ± SD**<br>**Median [Min-Max]** | 63.75 ± 10.7<br>65 [35–83] | 59.23 ± 12.4<br>62 [35–77] | 68.84 ± 9.2*<br>69 [48–82] | 65.0 ± 8.2<br>64 [48–83] | 0.027 |
| **Sex N (%)**<br>**Men**<br>**Women** | <br>46 (57.5)<br>34 (42.5) | <br>13 (43.3)<br>17 (56.7) | <br>9 (47.4)<br>10 (52.6) | <br>24 (77.4)<br>7 (22.6) | 0.016 |
| **Diabetes N (%)** | 33 (41.25) | 6 (20.0) | 11 (57.9) | 16 (51.6) | 0.010 |
| **Hypertension N (%)** | 40 (50) | 12 (40.0) | 15 (78.9) | 13 (41.9) | 0.015 |
| **Heart failure N (%)** | 31 (38.75) | 9 (30.0) | 4 (21.1) | 18 (58.1) | 0.015 |
| **Renal failure N (%)** | 6 (7.5) | 1 (3.3) | 1 (5.3) | 4 (12.9) | 0.334 |
| **Hyperlipidemia N (%)** | 46 (57.5) | 12 (40.0) | 12 (63.2) | 22 (71.0) | 0.043 |
| **Smoking N (%)** | 32 (40.0) | 8 (26.7) | 8 (42.1) | 16 (51.6) | 0.135 |
| **Rentrop classification N (%)**<br>**0**<br>**1**<br>**2**<br>**3** | <br>49 (61.25)<br>4 (5.0)<br>11 (13.75)<br>16 (20.0) | <br>30 (100)<br>-<br>-<br>- | <br>19 (100)<br>-<br>-<br>- | <br>-<br>4 (12.9)<br>11 (35.5)<br>16 (51.6) | - |
| **SCUBE1, ng/ml**<br>**X ± SS**<br>**Median [Min-Maks]** | 30.61 ± 2, 6<br>31.1 [15.3–32.6] | 30.59 ± 2.8<br>31.2 [17.2–32.6] | 31.25 ± 0.7<br>31.3 [29.3–32.3] | 30.23 ± 3.1<br>30.8 [15.3–32.6] | 0.272 |

The mean blood SCUBE1 levels in the participants according to the groups were given in Chart 1. As is seen in the chart, the SCUBE1 level was 30.59±2.8 ng/ml in Group 1, 31.25±0.7 ng/ml in Group 2, and 30.23±3.1 ng/ml in Group 3.

**Chart 1.** *SCUBE1 measurements by groups*




***Comparison of patients' SCUBE1 levels in terms of demographic and clinical data***

The result of the correlation analysis of SCUBE1 levels of the participants according to Rentrop classification revealed that there was not a significant relationship between their SCUBE1 levels and Rentrop grades (r:-0.176; P=0.119) (Table 2).

**Table 2.** *The result of the correlation analysis of patients' SCUBE1 levels according to Rentrop classification.*

| | | SCUBE1 | Rentrop Class (Grade) |
|---|---|---|---|
| **SCUBE1** | *Spearman rho*<br>*P value* | - | -0.176<br>0.119 |
| **Rentrop Class (Grade)** | *Spearman rho*<br>*P value* | -0.176<br>0.119 | - |

We presented the comparison of SCUBE1 levels according to demographic and clinical characteristics of all the participants in Table 3. As is seen in the table, there was no significant difference between them (P>0.05) (Table 3).

***Results of the ROC analysis of the participants' SCUBE1 values***

In Table 4 and Chart 2, we have shared the outcome of our ROC analysis that was conducted to establish the cut-off point for measuring the presence of coronary artery disease with SCUBE1. The results also include the sensitivity and specificity of this value. The SCUBE1 cut-off value for the presence of CAD was ≤30.94 ng/ml.

The sensitivity and specificity values of this value in predicting the presence of CAD were 46% [31.8 – 60.7] and 66.67% [47.2 – 82.7], respectively (Table 4). According to Chart 2, the AUC for the curve was 0.527 [0.412-0.640]. However, the predictive value of the SCUBE1 level for the presence of CAD was not significant as shown in Table 4 (P = 0.681).

In Table 5 and Chart 3, we showcased the findings of our ROC analysis aimed at identifying the cut-off point for SCUBE1 measurement in detecting the existence of Coronary collateral circulation, along with its sensitivity and specificity. The SCUBE1 cut-off value for the presence of CCC was ≤30.82 ng/ml. The sensitivity and specificity value of this value in predicting the presence of CCC were 54.84% [36.0-72.7], and 66.67%, respectively [47.2 – 82.7] (Table 5).

According to Chart 3, the AUC was 0.599 [0.483-0.707]. However, Table 5 shows that the SCUBE1 level's predictive value for the presence of CCC was not significant (P = 0.155).

**Table 3.** *Comparison of SCUBE1 levels of the participants according to their demographic and clinical characteristics.*

| Characteristics | X ± SD | Median [Min-Max] | P value |
|---|---|---|---|
| **Sex** | | | |
| **Men** | 30.5 ± 3.3 | 31.2 [15.3 – 32.6] | 0.160 |
| **Women** | 30.7 ± 1.2 | 31.0 [27.3 – 32.6] | |
| **Diabetes** | | | |
| **No** | 30.7 ± 2.3 | 31.2 [17.2 – 32.6] | 0.361 |
| **Yes** | 30.4 ± 2.9 | 30.9 [15.3 – 32.5] | |
| **Hypertension** | | | |
| **No** | 30.7 ± 2.7 | 31.1 [15.3 – 32.6] | 0.570 |
| **Yes** | 30.5 ± 2.5 | 31.1 [17.2 – 32.] | |
| **Heart failure** | | | |
| **No** | 30.8 ± 2.3 | 31.2 [17.2 – 32.6] | 0.151 |
| **Yes** | 30.3 ± 3.0 | 30.8 [15.3 – 32.5] | |
| **Renal failure** | | | |
| **No** | 30.6 ± 2.7 | 31.2 [15.3 – 32.6] | 0.152 |
| **Yes** | 30.6 ± 0.8 | 30.4 [29.8 – 32.1] | |
| **Hyperlipidemia** | | | |
| **No** | 30. 4 ± 0.5 | 31.0 [15.3 – 32.6] | 0.459 |
| **Yes** | 30.8 ± 0.3 | 31.2 [17.2 – 32.5] | |
| **Smoking** | | | |
| **No** | 30.6 ± 0.3 | 31.1 [17.2 – 32.6] | 0.482 |
| **Yes** | 30.7 ± 0.5 | 31.1 [15.3 – 32.5] | |
| **Rentrop classification** | | | |
| **0** | 30.8 ± 2.2 | 31.2 [17.2 – 32.6] | 0.334 |
| **1** | 31.3 ± 0.6 | 31.3 [30.6 – 32.1] | |
| **2** | 29.2 ± 4.8 | 30.8 [15.3 – 32.4] | |
| **3** | 30.7 ± 1.4 | 30.6 [27. 9 – 32.6] | |

**Table 4.** *ROC analysis of all the participants' SCUBE1 values to predict the presence of coronary artery disease.*

| Cut-off Point | Sensitivity [%95 GA] | Specificity [%95 CI] | Positive Predictive Value [%95 CI] | Negative Predictive Value [%95 CI] | AUC [%95 CI] | P value |
|---|---|---|---|---|---|---|
| ≤30.94 | 46.00 [31.8- 60.7] | 66.67 [47.2– 82.7] | 69.7 [51.3- 84.4] | 42.6 [28.3-57.8] | 0.527 [0.412-0.640] | 0.681 |

**CI: Confidence Interval, AUC: Area under the Curve*

**Chart 2.** *ROC chart for the SCUBE1 values to predict the presence of CAD*

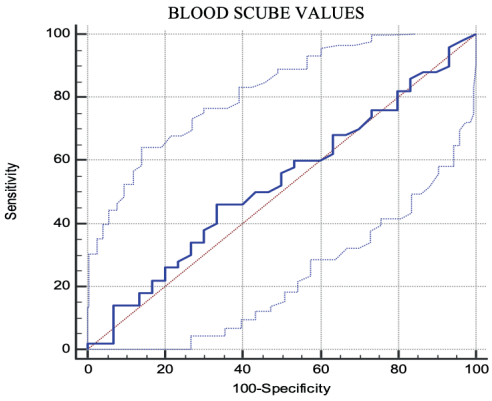



**Chart 3.** *ROC chart for the SCUBE1 values to predict the presence of coronary collateral circulation*

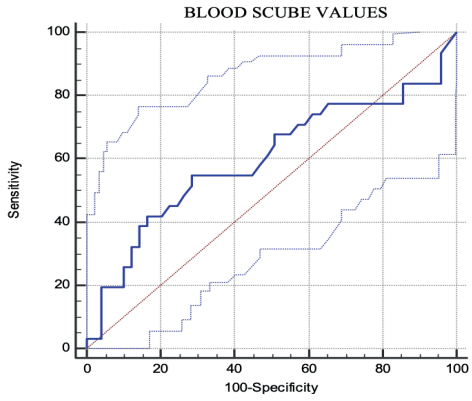


**Table 5.** *ROC analysis of all the participants' SCUBE1 values to predict the presence of coronary collateral circulation.*

| Cut-off Point | Sensitivity [%95 GA] | Specificity [%95 CI] | Positive Predictive Value [%95 CI] | Negative Predictive Value [%95 CI] | AUC [%95 CI] | P value |
|---|---|---|---|---|---|---|
| ≤30.82 | 54.84 [36.0-72.7] | 71.43 [56.7-83.4] | 54.8 [36.0-72.7] | 71.4 [56.7-83.4] | 0.599 [0.483-0.707] | 0.155 |

**CI: Confidence Interval, AUC: Area under the Curve*

## DISCUSSION

In our study, we investigated the serum SCUBE1 levels in patients with CAD who developed CCC by analyzing the relationship between these two parameters.

After the analysis, we determined that there was no significant relationship between their SCUBE1 levels and the presence of CCC, and that the SCUBE1 level was not significant in estimating the presence of CCC in patients.

We did not determine any significant correlation between the Rentrop grades, which are CCC classification, and the mean SCUBE1 level. We also determined that the predictive value of the presence of SCUBE1 level of CCC was not significant. The fact that the sample size of the study was small and that other SCUBE proteins were not included may be the reason why the relationship between SCUBE1 level and CCC could not be revealed. However, researchers of future studies can monitor SCUBE proteins that affect the progressive development of collateral vessels and investigate their relationship with other proteins involved in arteriogenesis mechanisms by conducting multicenter studies with larger samples. Thus, they can better understand the mechanisms of CCC in Patients with CAD and can assess the effectiveness of possible treatments.

Studies have demonstrated that patients with ACS and acute large vessel atherothrombotic stroke have higher levels of plasma SCUBE1, which is sourced from platelets. The release of plasma SCUBE1 is slow and comparable to that of other well-known biomarkers for acute myocardial infarction, like creatine kinase, troponins, lactate dehydrogenases and myoglobin[13]. In patients with acute platelet activation, the plasma SCUBE1 concentration was determined to rise 6 hours after the onset of activation and remained detectable for 3 to 4 days8. Therefore, plasma SCUBE1 can be considered as a new biomarker of platelet activation in acute thrombotic diseases[10]. Our study has found that patients with chronic CAD do not exhibit an increase in plasma SCUBE1 levels. This is due to the different pathogenesis of chronic CAD compared to ACS. While platelets do play a role in atherosclerosis, acute massive platelet activation is less common in chronic stable CAD. Therefore, it is reasonable to expect normal plasma SCUBE1 levels in patients with chronic CAD, unlike ACS. Furthermore, our research shows that there is no significant difference in SCUBE1 levels among patients with CAD without a history of ACS who developed CCC. This can be attributed to the low levels of platelet activation and inflammation involved in the pathogenesis of CAD[14,15].

Through in vitro studies, it has been demonstrated that serum proteases cleave the carboxyl-terminus CUB from the amino-terminal portion of EGF-like repeats in the secreted form of SCUBE1. The potential proteolytic cleavage site has been found to be located within the spacer region, as revealed by various mapping studies using SCUBE1 deletion mutants. The presence of multiple isoforms of SCUBE1 fragments suggests that SCUBE1 cleavage plays a complex role in regulating the pathological formation of ACS and acute ischemic stroke. However, further in vitro and in vivo studies are needed to understand the mechanism of SCUBE1 activation in chronic events such as CAD and CCC[16].

In our study, the SCUBE1 cut-off value was ≤30.94 ng/ml, the sensitivity was 46% [31.8 – 60.7], and the specificity was 66.67% [47.2 – 82.7] for the presence of CAD.On the other hand, as for the presence of CCC, the SCUBE1 cut-off value was ≤30.82 ng/ml, sensitivity was 54.84 [36.0-72.7], and specificity was 71.43 [56.7-83.4]. These results suggest that the predictive value of SCUBE1 level for the presence of CAD or CCC was not significant.

In our study, the sample size was relatively small, which had a limited statistical power to detect minor differences in the analysis of subgroups. In addition, the ELISA method used for plasma SCUBE1 in potential clinical applications has limited sensitivity and specificity. The analysis of SCUBE1 levels at different stages of CCC

development in patients with CAD may enable researchers to use it as a diagnostic or prognostic biomarker and it can be potentially useful in risk stratification of patients with CAD. However, we think that the use of plasma SCUBE1 as a biomarker will be insufficient to confirm its clinical application.

In this study, conducted at a single center, we conducted a comparison between the levels of SCUBE1 in CAD patients who did and did not develop CCC. This is the first study of its kind in the literature. Our findings indicate that relying solely on measuring SCUBE1 levels may not be sufficient to predict the development of CCC in CAD patients. Therefore, we recommend conducting multicenter studies with larger sample sizes to further validate these results.

## REFERENCES


1. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol*, 2011;3: 204-212.

2. Kim EK, Choi JH, Song YB, Hahn JY, Chang SA, Park SJ, Lee SC, Choi SH, Choe YH, Park SW, Gwon HC. A protective role of early collateral blood flow in patients with ST-segment elevation myocardial infarction. *Am Heart J*. 2016 Jan;171(1): 56-63. doi: 10.1016/j.ahj.2015.10.016. Epub 2015 Oct 24. PMID: 26699601.

3. Seiler C, Stoller M, Pitt B, Meier P. The human coronary collateral circulation: development and clinical importance. *Eur Heart J*. 2013 Sep;34(34): 2674-82. doi: 10.1093/eurheartj/eht195. Epub 2013 Jun 5. PMID: 23739241.

4. Stoller M, Seiler C. Salient features of the coronary collateral circulation and its clinical relevance. *Swiss Med Wkly*. 2015 Jul 28;145: w14154. doi: 10.4414/smw.2015.14154. PMID: 26218664.

5. Dai DF, Thajeb P, Tu CF, Chiang FT, Chen CH, Yang RB, Chen JJ. Plasma concentration of SCUBE1, a Novel Platelet Protein, is elevated in patients with acute coronary syndrome and ischemic stroke. *J Am Coll Cardiol*, 2008; 51(22): 2173-2180.

6. Vanpoucke G, Or B, Grace C, Chan R, Ashley GR, Williams K, Franco OE, Hayward SW, Thomson AA. Transcriptional profiling of inductive mesenchyme to identify molecules involved in prostate development and disease. *Genome Biology*, 2007;8: 213.

7. Grimmond S, Larder R, Van Hateren N, Siggers P, Morse S, Hacker T, Arkell R, Greenfield. Expression of a novel mammalian epidermal growth factor related gene during mouse neural development. *Mech Dev*, 2001;102: 209-11.

8. Liao WJ, Wu MY, Peng CC, Tung YC, Yang RB. Epidermal growth factor-like repeats of SCUBE1 derived from platelets are critical for thrombus formation. *Cardiovasc Res*. 2020 Jan 1;116(1): 193-201. doi: 10.1093/cvr/cvz036. PMID: 30722019.

9. Yang RB, Ng CK, Wasserman SM, Kömüves LG, Gerritsen ME, Topper JN. Identification of a novel family of cell-surface proteins expressed in human vascular endothelium. *J Biol Chem*, 2002;277: 46364-46373.

10. Tu CF, Su YH, Huang YN, Tsai MT, Li LT, Chen YL, Cheng CJ, Dai DF, Yang RB. Localization and characterization of a novel secreted protein SCUBE1 in human platelets. *Cardiovasc Res*, 2006;71: 486-495.

11. Ali H, Emoto N, Yagi K, Vignon-Zellweger N, Nakayama K, Hatakeyama K, Asada Y, Rikitake Y, Hirata K. Localization and characterization of a novel secreted protein, SCUBE2, in the development and progression of atherosclerosis. *Kobe J Med Sci*, 2013; 59(4): E122-31.

12. Yang M, Guo M, Hu Y, Jiang Y. Scube regulates synovial angiogenesis-related signaling. *Med Hypotheses*, 2013;81(5): 948-53.

13. Bodor GS. Biochemical Markers of Myocardial Damage. *EJIFCC*. 2016 Apr 20;27(2):95-111. PMID: 27683523; PMCID: PMC4975226.

14. Ozaki Y, Imanishi T, Teraguchi I, Nishiguchi T, Orii M, Shiono Y, Shimamura K, Ishibashi K, Tanimoto T, Yamano T, Ino Y, Yamaguchi T, Kubo T, Akasaka T. Association between P-selectin glycoprotein ligand-1 and pathogenesis in acute coronary syndrome assessed by optical coherence tomography. *Atherosclerosis*, 2014 Apr;233(2): 697-703. doi: 10.1016/j.atherosclerosis.2013.12.052. Epub 2014 Jan 28. PMID: 24583418.

15. Czepluch FS, Kuschicke H, Dellas C, Riggert J, Hasenfuss G, Schäfer K. Increased proatherogenic monocyte-platelet cross-talk in monocyte subpopulations of patients with stable coronary artery disease. *J Intern Med*. 2014 Feb;275(2): 144-54. doi: 10.1111/joim.12145. Epub 2013 Oct 30. PMID: 24118494.

16. Akdogan E, Ayaz T, Kirbas A, Rakici H. Is SCUBE1 helpful to predict the arterial thrombotic risk in patients with multi-ple myeloma: a preliminary study. *Hippokratia*, 2019;23(1): 21-24.