Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 31 (3-4)
Sofia ● 2024

Association of Serum Secreted Protein Acidic and Rich in Cysteine (SPARC) Levels with the Severity of Coronary Artery Lesion in Type 2 Diabetic Patients with Coronary Heart Disease among South Indian Population

Divia Paul. Aricatt[1], Pradeep Pereira[2], Ranajit Das[3], Ashwini Prabhu[3], Cleeta Rebeiro[4], Rhema Jacob[2]*

[1] *Department of Anatomy, Fr Muller Medical College, Kankanady, Mangalore, Karnataka*
[2] *Department of Cardiology, Fr Muller Medical College, Kankanady, Mangalore, Karnataka*
[3] *Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, Karnataka*
[4] *Fr Muller Research Centre, Fr Muller Medical College, Kankanady, Mangalore, Karnataka*

*Corresponding author e-mail: drdiviaaricatt@gmail.com, divia_manoj@yahoo.com

Type 2 diabetic patients have high plasma secreted protein acidic and rich in cysteine (SPARC) levels. We aimed to find the association between SPARC levels, type 2 diabetes mellitus (T2DM) and incidence of coronary artery disease with the objectives to investigate SPARC levels with the severity of coronary artery lesion in T2DM patients with and without coronary artery disease (CAD). A single center cross sectional study was conducted with 160 samples. All ethical principles were followed. The patients were categorized in to group A (C), group B (T2DM only), group C (CAD with T2DM), D group (CAD only) for SPARC analysis by Elisa. Gensini score was calculated for coronary stenosis. Patients with liver and kidney dysfunctions were excluded. We found a highly significant difference and association between the serum SPARC level and Gensini score between groups. This study identifies the possibility of SPARC as a new early biomarker for diagnosing CAD in diabetic patients.

Key words: Type 2 Diabetes mellitus; SPARC biomarker; Coronary stenosis; Gensini Score; Biochemical indicators

Introduction

Type 2 diabetes mellitus (T2DM) is on the verge of becoming a pandemic in India [30]. Coronary artery disease (CAD) is a major cause of death and disability among people with T2DM [10, 22]. Statistical predictions denote that by the end of year 2025, 80.9 million people will have diabetes in India, with an evidence of increased

prevalence of CAD among T2DM patients [19, 24]. Secreted protein acidic and rich in cysteine (SPARC), also known as BM-40 and osteonectine, is non-collagenous [21] as well as collagen-binding protein [3]. This molecule plays an important non-structural role in extracellular matrix of bone [5], and has three structural domains with active glycoproteins [17], which were initially reported from bones and named as osteonectine [21].This is an extracellular matrix-related glycoprotein with molecule low molecular weight, which is secreted by heart, brain, kidney, pancreas and skeletal muscle. SPARC in human blood is differentiated mainly from the subcutaneous adipose tissue [23]. Importantly, SPARC protein and gene expression or its serum level changes are during a variety of conditions. Based on its participation in angiogenesis and repair of damaged tissues [1], clinical researches should focus on relationship of SPARC with tumor and wound healing [27].

Recent research has shown that SPARC is involved in the pathophysiological processes of obesity [23], insulin resistance [26] and type 2 diabetes [7, 15]. Newly diagnosed T2DM patients also do possess high plasma SPARC levels [7]. T2DM is an independent risk factor for CAD; hence the close relationship between SPARC, T2DM and its complications suggest that there exists certain relevance between SPARC and the incidence of CAD.

Previous studies have shown that SPARC is highly expressed in T2DM and CAD [10, 30], however, the relationship between SPARC and T2DM combined with CAD has not been reported so far. Quantitative coronary angiography reports investigated by Gensini scoring can explore the quantitative analysis of coronary stenosis. The present study was aimed to investigate the association between serum SPARC levels, T2DM and incidence of CAD with the objectives to assess SPARC levels in T2DM patients with and without CAD and to investigate the correlation of serum SPARC levels with the severity of coronary artery lesion in T2DM patients with coronary heart disease.

Materials and Methods

Study population

A single center cross-sectional study was conducted. All ethical principles for human research were followed and ethical approval was obtained from the Institutional Ethics Committee of the hospital from where the data was collected. The age of the study subjects was given a cut-off at 75 years due to marginal benefits marked during the follow-ups. Hence, a conservative approach is proven to be appropriate for the above-mentioned age, which itself indicates a poor prognosis with an average yearly mortality rate of 33%–35% [2]. The sample size was estimated by consulting a statistician and using the statistical software G* Power 3.0.10. One hundred and sixty subjects were selected for the study by systematic sampling strategy. This consists of 40 healthy controls, 40 patients with T2DM, 40 cases with CAD and diabetes, 40 patients with CAD without diabetes. The above patients were named group A (healthy), group B (T2DM only), group C (T2DM with CAD), D group (CAD only).

The inclusion criteria recruited the participants above 18 years of age and either sex. The patients who report for routine health checkups with no relevant medical history were taken as control group A. Group B (T2DM only) followed WHO

diagnostic criteria of 1999 for diagnosis of diabetes assessment. Diagnosis criteria of CAD were defined according to the stenosis involvement percentages in the involved artery. Exclusion criteria included persons with a previous history of coronary artery bypass graft (CABG), and recanalized normal looking coronary arteries with or without in-stent restenosis coronary arteries as well as patients with severe liver and kidney dysfunction, hyperlipidemia and arthritis. Samples with previous history of myocardial infarction, post-trauma and infection, cancer patients treated with chemo/ radiation therapy were also excluded.

Database Pooling

i. SPARC determination: Fasting blood samples (3mL) were collected from all groups into vacutainers. Samples were held on upright position for 30 min before centrifugation. Samples were centrifuged for 3000 rpm at 25°C for 15 min. After collecting the serum, the SPARC levels *(ug/L)* were quantified using enzyme-linked immunosorbent assay (ELISA), as per manufacturer's instructions. Company: Krishgen Biosystems.

ii. Determination of biochemical indicators*:* Fasting blood samples (3 mL) were collected from all the study participants for biochemical investigations. Fasting blood sugar (FBS) was assessed for group A participants for confirming their blood glucose levels. HbA1c (to assess about active control of diabetes), lipid profile tests (Insulin resistance and T2DM have been constantly associated with high triglyceride and low HDL-cholesterol levels) and C-peptide release test were performed for participants with highly elevated HbA1c using the standard biochemical procedures and tests (Indicative of uncontrolled diabetes) values in groups B and C.

iii. Quantitative analysis of coronary stenosis by Gensini: The Gensini scoring system used an ordinal ranking based on stenosis severity in 11 coronary segments (score range, 0 to 72)[11]. Non-stenosed arteries were recorded with 0 point. 1% to 25% stenosis was recorded with 1 point. 26%-50% stenosis was recorded with 2 points. 51% to 75% stenosis was recorded with 4 points. Patients with 76% to 90% were recorded with 6 points. 91% to 99% stenosis was recorded as 16 points. 100 percent stenosed arteries were recorded with 32 points. LMCA with CVD were recorded as five points. LAD or LCx proximal segment lesion was recorded with 2.5 points. The middle of the LAD artery lesion was recorded with 1.5 points. The LAD artery distal lesions were recorded with 1 point. Lesions from middle and distal sections of LCx after obtuse marginal branch were recorded with 1 score. RCA lesions were recorded 1 score. Small branch lesions of RCA were recorded with 0.5 score. Total score of coronary artery lesions were the sum of each segment.

Clinical end point definitions

WHO diagnostic criteria of 1999 were used for the diagnosis of diabetes: the symptoms of diabetes combined with plasma glucose≥11.1mmol/L (200mg/dL) at any time, or FPG≥7.0mmol/l (126mg/dL),or OGTT 2h PG≥11.1mmol/L (200mg/dL), and it is necessary to be repeated to confirm with diabetes [6]. Diagnosis criteria of CAD were defined according to the stenosis involvement percentages in the involved artery. A stenosis diameter of 50% or >50% in one or more sites of the coronary artery was regarded as CAD. In this system, we observed three major coronary arteries including the left anterior descending (LAD) with its diagonal (DIAG) branch, left circumflex (LCx) with its obtuse marginal (OM) branch and RCA with its PDA and PLB branches [11].

Statistical analysis of the present study was performed using the GraphPad Prism v9. The participants were named group A (healthy), group B (T2DM only), group C (T2DM with CAD), group D (CAD only). Descriptive statistics was used to summarize the data and results were expressed in mean difference. One way ANOVA, followed by Tukey's multiple comparisons was employed to compare the serum SPARC levels among the different groups. The association between the biochemical indices (HbA1C) and serum SPARC level for Group B and C was statistically assessed using Pearson's correlation. Further, the association between the Gensini score and serum SPARC level for Group C and D was statistically assessed using Pearson's correlation. Multiple linear regression analysis was performed to assess the relevance between SPARC levels and T2DM combined with age and gender. $P<0.05$ was considered statistically significant.

Results

The healthy controls- Group A, T2DM- Type 2 diabetic mellitus- Group B, T2DM with CAD- Type 2 diabetic mellitus with coronary artery disease- Group C, CAD-coronary artery disease- Group D differed significantly among each other in terms of SPARC level (ug/L) (One-way ANOVA, $p<0.05$). While comparing the groups pair-wise, we found a highly significant difference in the serum SPARC levels (ug/L) between the A vs C and D groups (Tukey's multiple comparison, $p<0.0001$), and the B vs C and D groups (Tukey's multiple comparison, $p<0.0001$ and $p=0.003$, respectively). Overall, the highest mean serum SPARC level (ug/L) was observed in C group (x=9.94), followed by D group (x=8.3).The mean SPARC level (ug/L) was found to be discernibly low among B (x=4.90) and A (x=3.14) groups (**Table 1, Fig. 1**).

Table 1. Multiple comparison based on Elisa reports among four group of samples (n=160).

Groups	Group 1 (V1)	Group 2 (V2)	Samples			
			Mean V1	**Mean V2**	**MD**	**p-value**
Healthy Controls	A	T2DM (B)	3.141	4.903	-1.762	0.2579
	A	T2DM with CAD (C)	3.141	9.941	-6.799	<0.0001****
	A	CAD (D)	3.141	8.3	-5.159	<0.0001****
Diabetic	T2DM (B)	T2DM with CAD (C)	4.903	9.941	-5.038	<0.0001****
	T2DM(B)	CAD (D)	4.903	8.3	-3.397	0.0028***
	T2DM with CAD (C)	CAD (D)	9.941	8.3	1.64	0.3195

The results based on SPARC Elisa Kit reports expressed in (ug/L) for each group
Statistical test used: *Post Hoc (Tukeys test), $p<0.01$**** indicates highly significant difference, $p<0.05$*** indicates significant difference, $p>0.05$ indicates non significant difference between dependant variable and comparison variables.*
Abbreviations: *MD-Mean difference, A- Healthy Controls, B-T2DM – Type 2 diabetic mellitus, C-T2DM with CAD – Type 2 diabetic mellitus with coronary artery disease, D- CAD – coronary artery disease*

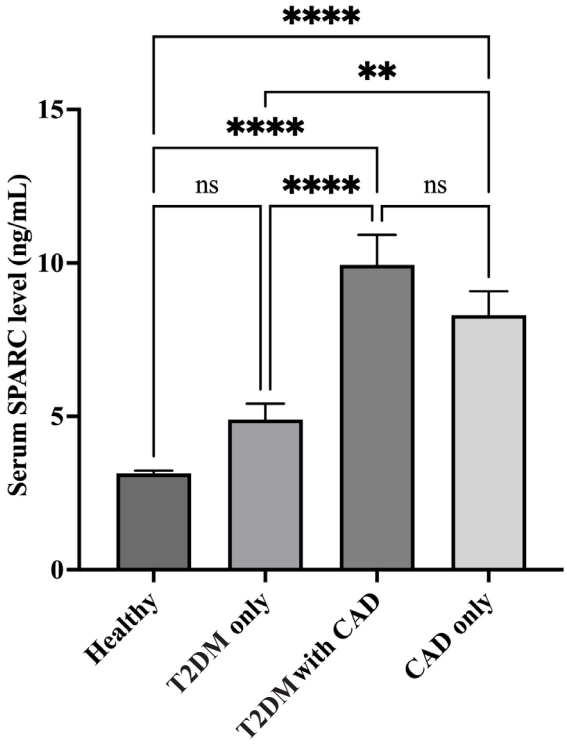

Fig.1 SPARC level in four groups.

We found a significant association between serum SPARC levels (ug/L) and Gensini score for both C (Pearson's correlation, r=0.45, p=0.004) and D (Pearson's correlation, r=0.34, p=0.029) groups (**Table 2**). We also observed a significant correlation between serum SPARC level (ug/L) and HbA1C both in B group (Pearson's correlation, r=0.66, p<0.0001), and C group (Pearson's correlation, r=0.38, p=0.016), the association was found to be discernibly higher in the former (**Table 3, Figs. 2, 3**).

Table 2. Correlation of Elisa reports with Gensini score among group C and D samples (n=80).

	Groups	**Correlation analysis**	
ELISA REPORTS Vs. GENSINI SCORE	T2DM with CAD (C)	r	0.4482
		p-value	0.0037***
	CAD (D)	r	0.344
		p-value	0.0298***

The results based on SPARC Elisa Kit reports expressed in (ug/L) for each group
Statistical test used: *Pearson correlation test. p<0.01**** *indicates highly significant difference, p<0.05*** *indicates significant difference, p>0.05 indicates non significant difference between Elisa and Gensini score among group C and D samples.*
Abbreviations: *C – T2DM with CAD – Type 2 diabetic mellitus with coronary artery disease, D – CAD-coronary artery disease*

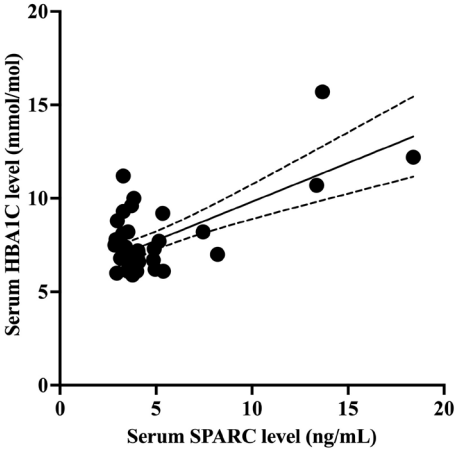

Fig. 2. SPARC levels vs biochemical indices (HbA1C) in T2DM cases.

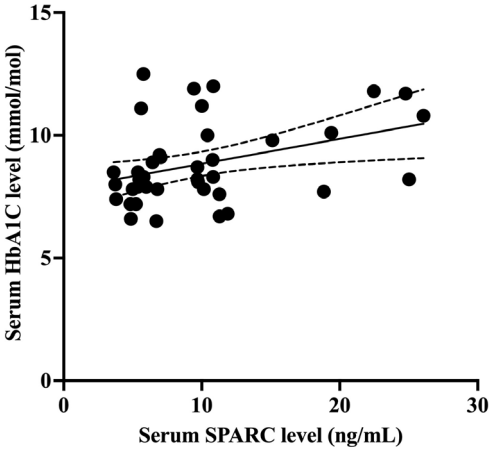

Fig. 3. SPARC levels vs biochemical indices (HbA1C) in T2DM with CAD.

Table 3. Correlation of Elisa reports with HBA1C among group B and C samples (n=80).

	Groups	**Correlation analysis**	
ELISA REPORTS Vs. HBA1C	T2DM (B)	r	0.6603
		p-value	<0.0001****
	T2DM with CAD (C)	r	0.38
		p-value	0.0156***

The results based on SPARC Elisa Kit reports expressed in (ug/L) for each group
*Statistical test used: Pearson correlation test. p<0.01****indicates highly significant difference, p<0.05***indicates significant difference, p>0.05 indicates non significant difference Elisa and HBA1C among group B and C samples*
Abbreviations: B-T2DM- Type 2 diabetic mellitus, C - T2DM with CAD- Type 2 diabetic mellitus with coronary artery disease,

To assess the effect of age and gender on the serum SPARC levels (ug/L), we investigated the association in a multiple linear regression framework. Serum SPARC level (ug/L) was highly elevated among males in the B group (|t|=4.75, p=0.009); but not in C and D groups. Further, older age was found to be significantly associated with higher serum SPARC levels (ug/L) in the B (|t|=7.37, p=0.002) and D (|t|=2.15, p=0.038) groups, but not in the C group. While the length of years being diabetic has significant association with serum SPARC level (ug/L) among patients with C (|t|=2.56, p=0.02), no such association was observed among B group patients (|t|=1.42, p=0.23). Congruent with the correlation analysis, serum HbA1C level was found to be significantly associated with serum SPARC level (ug/L)in both B and C groups (|t|=10.1, p=0.0005, |t|=2.28, p=0.034 respectively). However, as depicted by the correlation analysis, the association was found to be discernibly higher in the former.

Discussion

The healthy controls- Group A, T2DM – Type 2 diabetic mellitus- Group B, T2DM with CAD – Type 2 diabetic mellitus with coronary artery disease- Group C, CAD-coronary artery disease- Group D. Diabetic subjects have higher prevalence as well as increased risk of CAD than the non-diabetic counterparts [19, 24]. Left main coronary artery (LMCA) and its branches were observed as narrower in diabetic patients than in non-diabetics, when the diameters of both were compared using qualitative comparative analysis (QCA). Evidence for the narrowing of lumen diameter of coronary arteries in patients with diabetics and several factors affecting the lumen diameters have been studied previously in different countries on different populations [1, 6-7, 15-16]. The improved awareness and enhanced treatment options can control the cardiovascular risk factors in participants with known diabetes [4].

SPARC is involved in insulin resistance by activating PI3K/AKT pathway, which is the major insulin transduction pathway. SPARC and inflammatory factors during CAD causes severe vascular stress reactions. SPARC can lead to changes in the structure of extracellular matrix by affecting the deposition of fibronectin and laminin [18], can regulate cell migration, and has anti-cell adhesive effect. Studies have shown that SPARC is closely related to inflammatory response factors and adipose factors [13]. In the present study, we observed significant association between serum SPARC levels and HbA1C both in group Band group C, the association was found to be discernibly higher in the former.

The possible mechanism of SPARC is the occurrence and development of insulin resistance. Related research has shown that SPARC can inhibit the proliferation and differentiation of VEGF, PDGF and FGF stimulated fibroblasts, smooth muscle cells and endothelial cells to impede the vascular repair process. This phenomenon can damage the blood vessel barrier to induce atherosclerosis as time advances, and promotes the deposition of smooth muscle cells in the intima to a rapid progression of vascular endothelial atherosclerosis [25]. In our study, while the length of years being diabetic had significant association with serum SPARC levels among patients with group C, no such association was observed among group B patients. We could relate that adiponectin can relieve the inflammation of endothelial cells, protect vascular endothelial and increase insulin sensitivity. When oxidative stress occurs, SPARC secretion increases in the body. SPARC is positively correlated with adiponectin and leptin, which can activate the tyrosine kinase (JAK) signaling system, which are the relative factors to be involved in the formation of atherosclerosis [12].

SPARC damages the vascular barrier and thus participates in the formation of coronary atherosclerosis [14, 28]; thus SPARC levels can be use as predictor of coronary atherosclerosis. In the present study, the mean SPARC level was found to be discernibly low among group A and was increased in group B. The highest mean serum SPARC level was observed in group C followed by group D and group B. Increased SPARC in obese and T2DM subjects suggested that SPARC may play a role in pathogenesis of both obesity and diabetes [31].SPARC can cause fatty fibrosis, increase excessive lipids in circulation by re-locating it from liver, pancreas, blood vessels and other non-adipose tissues [25]. This process causes increased levels of triglycerides in the circulating blood, thereby leading to the development of insulin resistance and is gender specific.

Authors have reported that inhibition of adipose tissue build-up along with the increased consumption of dietary lipids contributes majorly to systemic hyperlipidemia and influx of triglycerides to the vital organs, ultimately leading to insulin resistance [20]. The discovery of a potential novel function for SPARC in the control of insulin secretion relevant to the onset of Type 2 diabetes is described by Harries et al. [8]. By reducing the expression of RGS4, SPARC was able to control insulin secretion, demonstrating that SPARC was important for both G-protein signaling in beta cells as well as insulin secretion physiology [9]. Since inflammatory cytokine itself can cause atherosclerosis, SPARC can promote the occurrence of CAD through this synergistic effect. We could relate these findings to the present study as the association in a multiple linear regression framework with effect of age and gender on the serum SPARC level, the male gender is highly significantly associated with higher serum SPARC level in the group B patients but not in the group C and D patients.

Different "jeopardy scores" were developed to quantitate plaque burden, to predict patient-based clinical outcomes and to identify the risk factors for the presence of atherosclerosis and its progression. Serum SPARC level is elevated in T2DM patients with coronary heart disease, which can be correlated with the severity of coronary artery disease significantly[29].The Gensini scoring system used an ordinal ranking based on stenosis severity in 11 coronary segments (score range, 0 to 72) [11]. In our study, we found significant association between serum SPARC level and Gensini score for participants from both groups C and D. The scoring indices were higher in group C patients compared to group D. Thus, we can correlate that the SPARC values and Gensini scores were interrelated and has higher predictive value.

Conclusion

SPARC levels can be used as predictor of coronary atherosclerosis. It is hypothesized that if we can reduce SPARC content during the onset of T2DM, risk of` CAD could be delayed. This study identifies the possibility of SPARC as a new early biomarker for diagnosing CAD in diabetic patients. This study may enable better life in patients diagnosed with diabetes and to deliver improved outcomes for diabetic patients when combined with clinical diagnostics.

Acknowledgements: All authors appreciate the great effort of Dr. Ranajit Das, Yenepoya Research Centre, Mangalore, Karnataka, India for analysing and verifying the data of this study. We acknowledge Father Muller Medical College, Kakanady, Mangalore, India for the Institutional Grant (Grant Number: FMRC/FMMC/ST/04/2021) towards the smooth execution of the study.

Ethical Approval

All ethical principles for human research were followed and ethical approval was obtained from the Institutional ethics committee of the hospital from where the data was collected [FMMC/FMIEC/l46/2022]. We have obtained informed consents from sample population involved in this study.

References

1. **Adil, M., M. Nadeem, M. Hafizullah, H. Jan.** Comparison of left coronary artery diameter among diabetics and non-diabetics. – *J. Postgrad. Med. Inst*., **26**(4), 2012, 369-376
2. **Azad, N., G. Lemay.** Management of chronic heart failure in the older population. – *JGC*., **11**(4), 2014,329.
3. **Bradshaw, A. D.** The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? – *J. Mol. Cell. Cardiol.,* **93,** 2016,156-161.
4. **Brown, T. M., R. M. Tanner, A. P. Carson, H. Yun, R. S. Rosenson, et al.** Awareness, treatment, and control of LDL cholesterol are lower among US adults with undiagnosed diabetes versus diagnosed diabetes. – *Diabetes Care*, **36**(9), 2013, 2734-2740.
5. **Chlenski, A., L. J. Guerrero, H. R. Salwen, Q. Yang, Y. Tian, et al.** Secreted protein acidic and rich in cysteine is a matrix scavenger chaperone. – *PloS one*, **6**(9), 2011, e23880.
6. **Gabir, M. M., R. L. Hanson, D. Dabelea, G. I. Imperatore, J. A. Roumain, et al.** Plasma glucose and prediction of microvascular disease and mortality: evaluation of 1997 American Diabetes Association and 1999 World Health Organization criteria for diagnosis of diabetes. – *Diabetes care*, **23**(8), 2000, 1113-1118.
7. **Gui, M. H., G. Y. Qin, G. Ning, J. Hong, X. Y. Li, et al.** The comparison of coronary angiographic profiles between diabetic and nondiabetic patients with coronary artery disease in a Chinese population. – *Diabetes Res. Clin. Pract.*, **85**(2), 2009,213-219.
8. **Harries, L. W., L. J. McCulloch, J. E. Holley, T. J. Rawling, H. J. Welters, et al.** A role for SPARC in the moderation of human insulin secretion. – *PloS one*, **8**(6), 2013, e68253.
9. **Hu, L., F. He, M. Huang, Q. Zhao, L. Cheng, et al**. SPARC promotes insulin secretion through down-regulation of RGS4 protein in pancreatic β cells. – *Sci. Rep.*, **10**(1), 2020, 17581.
10. International Diabetes Federation. *IDF Diabetes Atlas,* 7th Edition, Brussels, Belgium, International Diabetes Federation, 2015.
11. **Mann, D. L., D. P. Zipes, P. Libby, R. O. Bonow.** *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*, Part 1, vol. 10 (Ed. E. Braunwald), Elsevier Health Sciences, 2017, 392-428.
12. **Kang, Y. J., A. K. Stevenson, P. M. Yau, R. Kollmar.** Sparc protein is required for normal growth of zebrafish otoliths. – *ARO.*, ***9*,** 2008, 436-451.
13. **Kos, K., S. Wong, B. Tan, A. Gummesson, M. Jernas, et al.** Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. – *Diabetes*, **58**(8), 2009, 1780-1788.
14. **Matsuzawa, Y., T. Funahashi, S. Kihara, I. Shimomura.** Adiponectin and metabolic syndrome. – *ATVB.*, **24**(1), 2004, 29-33.
15. **Melidonis, A., V. Dimopoulos, E. Lempidakis, J. Hatzissavas, G. Kouvaras, et. al.** Angiographic study of coronary artery disease in diabetic patients in comparison with nondiabetic patients. – *Angiology*, **50**(12), 1999, 997-1006.

16. **Mosseri, M., M. Nahir, Y. Rozenman, C. Lotan, D. Admon, et al.** Diffuse narrowing of coronary arteries in diabetic patients: the earliest phase of coronary artery disease. – *Cardiol.*, **89**(2), 1998, 103-110.
17. **Motamed, K**. SPARC (osteonectin/BM-40). – *Int. J. Biochem. Cell. Biol.*, **31**(12), 1999, 1363-1366.
18. **Nie, J., E. H. Sage.** SPARC inhibits adipogenesis by its enhancement of β-catenin signaling. – *JBC.*, **284**(2), 2009, 1279-1290.
19. **Reddy, K. S., S. Yusuf.** Emerging epidemic of cardiovascular disease in developing countries. – *Circ.*, **97**(6), 1998, 596-601.
20. **Riboulet-Chavey, A., A. Pierron, I. Durand, J. Murdaca, J. Giudicelli, et al.** Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. – *Diabetes*, **55**(5), 2006, 1289-1299.
21. **Rosset, E. M., A.D. Bradshaw.** SPARC/osteonectin in mineralized tissue. – *Matrix Biol.*, ***52***, 2016, 78-87.
22. **Sarwar, N., P. Gao, S. R. Kondapally Seshasai, R. Gobin, S. Kaptoge, et al.** Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. – *The Lancet*, **375**(9733), 2010, 2215-2222.
23. **Schwartz, L., K. E. Kip, E. Alderman, J. Lu.** BARI 2D Study Group. Baseline coronary angiographic findings in the bypass angioplasty revascularization investigation 2 Diabetes trial (BARI 2D). – *Am. J. Cardiol.*, **103**(5),2009, 632-638.
24. **Sicree, R.** Diabetes and impaired glucose tolerance. *Diabetes atlas*, **15,** 2006, 109.
25. **Song, H., Y. Guan, L. Zhang, K. Li, C. Dong.** SPARC interacts with AMPK and regulates GLUT4 expression. – *Biochem. Biophys. Res. Commun.,* **396**(4), 2010, 961-966.
26. **Standring, S., N. R. Borley, P. Collins, A. R. Crossman, M. A. Gatzoulis, J. C. Healy.** Gray's Anatomy. 40th*ed. Spain: Churchill Livingstone Elsevier*, **487**, 2008,725-726
27. **Stein, B., W. S. Weintraub, S. S. Gebhart, C. L. Cohen-Bernstein, R. Grosswald, et.al.** Influence of diabetes mellitus on early and late outcome after percutaneous transluminal coronary angioplasty. – *Circulation*, **91**(4), 1995, 979-989.
28. **Valsamakis, G., R. Chetty, P. G. McTernan, N. M. Al-Daghri, A. H. Barnett, et.al**. Fasting serum adiponectin concentration is reduced in Indo-Asian subjects and is related to HDL cholesterol. – *DOM.*, **5**(2), 2003, 131-135.
29. **Wang, Z., H. Y. Song, M. M. An, L. L. Zhu.** Association of serum SPARC level with severity of coronary artery lesion in type 2 diabetic patients with coronary heart disease. – *IJCEM.*, **8**(10), 2015, 19290.
30. **Wild, S., G. Roglic, A. Green, R. Sicree, H. King.** Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. – *Diabetes care*, **27**(5), 2004, 1047-1053.
31. **Wu, D., L. Li, M. Yang, H. Liu, G. Yang.** Elevated plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus. – *Eur. J. Endocrinol.,* **165**(4), 2011, 597-601.