Research Article

iMedPub Journals
www.imedpub.com

Journal of Anatomical Science and Research

2024
Vol.7 No.1:001

Effect of Cardiovascular Risk Factors on Expression of Vascular Endothelial Growth Factors-A (VEGF-A) during Obstructive Coronary Heart Disease: A Autopsy-Based Study

Sheeja Balakrishnan[1*] and Senthil Kumar B[2]

[1]Department of Anatomy, Government Medical College (Institute of Integrated Medical Sciences), Palakkad, Kerala, India

[2]Department of Anatomy, Vinayaka Mission's Kirupananda Variyar Medical College and Hospitals, Vinayaka Missions Research Foundation (DU), Salem, Tamil Nadu, India

[*]**Corresponding author:** Sheeja Balakrishnan, Department of Anatomy, Government Medical College (Institute o f Integrated Medical Sciences), Palakkad, Kerala, India; E-mail: sheejabkrishna@gmail.com

Received date: May 17, 2023, Manuscript No. IPJASR-23-17026; **Editor assigned date:** May 22, 2023, PreQC No. IPJASR-23-17026 (PQ); **Reviewed date:** June 06, 2023, QC No. IPJASR-23-17026; **Revised date:** December 27, 2023, Manuscript No. IPJASR-23-17026 (R); **Published date:** January 03, 2024, DOI: 10.36648/IPJASR.7.1.001

Citation: Balakrishnan S, Kumar SB (2024) Effect of Cardiovascular Risk Factors on Expression of Vascular Endothelial Growth Factors-A (VEGF-A) during Obstructive Coronary Heart Disease: A Autopsy-Based Study. J Anat Sci Res Vol:7 No:1

Abstract

Vascular Endothelial Growth Factor A (VEGF-A) acts as a specific mitogen for endothelial cells inducing its migration and proliferation which in turn increases the vascular permeability of blood vessels under occlusion. VEGF has been reported as an important factor promoting collateral formation in ischemic cardiac diseases. When the large dose of VEGF was infused into the proximal stump of an occluded artery an increase in the collateral flow was noticed. However, the complete mechanism and factors affecting VEGF expression during acute myocardial infarction are not fully understood. Selective coronary angiography in parallel with the autopsy of 35 (26 male and 9 female) subjects was included in the study. Selective coronary angiography of both right and left coronary arteries was done to know the grade of occlusion and severity of the disease parallel to autopsy. Only the specimens of decedents with complete clinical data which was taken within one month of death were included in the study. The study was done after getting written consent from the relatives and from the department of forensic medicine, government medical college Palakkad. Excluding the heart specimens without any coronary occlusion 5 cm slice, of coronary artery segments were taken at the level of the highest grade of stenosis. For the heart without any occlusion, segments were taken from the proximal part of both right and left coronary arteries. Sections of 6 µm thickness were stained with haematoxylin and eosin. Immunohistochemical staining was done in all sections to know the VEGF expression of endothelial cells. VEGF expression was correlated based on the grade of stenosis and the presence of cardiovascular risk factors like diabetes mellites and hypertension from the case report. The coronary artery with stenosis with less than 90% showed no substantial VEGF expression. A statistical significance was found when the VEGF expression was correlated with the grade of stenosis and the presence of cardiovascular risk factors like diabetes mellitus. Considerable variation was shown in the expression of the VEGF in the endothelial cells of the coronary artery with stenosis greater than 90%. In the present study variation in the expression of VEGF-A was found in subjects with and without diabetes mellitus in higher grades of stenosis. Impaired monocyte migration might explain the reduced arteriogenic potential in diabetic patients.

Keywords: Coronary artery disease; Collateralization; Coronary angiogram; VEGF (Vascular Endothelial Growth Factor); Angiogenesis; Diabetes mellitus

Introduction

The vascular endothelial growth factor is an angiogenic factor which causes migration and proliferation of endothelial cells, enhances vascular permeability and modulates thrombogenicity [1]. It promotes the formation of new blood vessels (arteriogenesis) which plays an essential role in the maintenance of homeostasis of the internal environment, between blood vessels and target organs [2]. The shear stress on the endothelium increases monocyte aggregation and releases inflammatory cytokines such as VEGF (Vascular Endothelial Growth Factors) [3]. Coronary artery disease decreases the arterial pressure distal to the stenosis and increases shear stress, causing marked activation of endothelium which in turn causes several morphological changes and vascular re-modeling [4]. The degree of coronary artery stenosis is described as a positive determining the extent of collateral formation. The present study was conducted to know the effect of cardiovascular risk factors like age, gender, type 2 diabetes mellitus and hypertension on the VEGF expression of endothelial cells during coronary artery stenosis.

Materials and Methods

Subjects for the study were selected through consecutive sampling from the department of forensic medicine, government medical college, Palakkad (District Hospital Palakkad) recommended for autopsy. Only the subjects with complete clinical history taken within one month of death were

included in the study. Decedents with a history of malignancy, pulmonary disease, previous history of PCI and renal disease were excluded from the study. Post-mortem selective coronary CT angiography of the isolated heart was done in 40 subjects parallel with the autopsy. Of these 30 decedents were with a history of sudden cardiac death and 10 subjects taken as control, with a cause of death other than coronary artery disease. Written informed consent was taken according to the rates of the institutional ethics committee which approved the study. The heart was separated at the level of ascending aorta from the body and selective coronary angiography was done separately in both the coronary arteries. After performing selective coronary CT angiography of 10 controls only 5 were found with normal coronary arteries (without coronary occlusion) and 30 with a history of Sudden Cardiac Death (SCD) had occlusion in any one of the coronary arteries.

Selective coronary angiography

The heart was removed and selective angiography was performed. The heart was isolated with aorta 2-3 cm above the coronary ostium. The tissue surrounding the coronary artery was removed and 6 Fr coronary angio catheters which are shortened to 5 cm were inserted into the right and left coronary ostium respectively and secured with the ligature at the side of the aorta. After ligature, the catheter was pulled inside to a specific point. High viscosity contrast material composed of 15 ml on opaque 350 in 500 ml of polyethene glycol 250 is made. The solution was infused into an empty drip infusion set and put in a pressurized bag and the bag was pressurized to 120-150 mm of Hg. CT was performed by continuous injection for 3 min (enhanced CT was performed without stopping perfusion) [5].

Left and right coronary angiography was performed separately. Primage review and 3D image were built up. The level of the maximum coronary lesion was noted from the 3D built-up image. It was then compared to dissection findings made during the autopsy. A 5 mm slice of the coronary artery was removed at the site of the lesion (comparing both dissection findings of autopsy and angiogram). The heart was then handed over to the forensic department after the evaluation within the time frame stipulated for completion of the autopsy (Figure 1).

Figure 1: Coronary CT angiography in autopsy samples.

The solution was infused into an empty drip infusion set and put in a pressurized bag, the bag was pressurized to 120-150 mm of Hg and CT was performed by continuous.

Documentation of the demographic factors and clinical history

Baseline data regarding clinical history, age, weight, and gender were obtained from the medical history after getting consent from the relatives of the patients. Diabetes Mellitus (DM) was defined as a history of DM, which is the use of anti-diabetic drugs or fasting plasma glucose level of ≥ 7 mol/L. Hypertension (HTN) was defined as a history of Hypertension (HTN) or use of antihypertensive drugs, or blood pressure ≥ 140/90 mm of Hg [6].

Histopathological examination and grading of stenosis

Specimen size and fixation: The section of 5 mm size was taken at the level of maximum occlusion of the coronary artery correlating the angiographic and forensic findings. Sections are then transferred to a tissue processing cassette. Each cassette was separately labeled with a diamond pencil. Fixation is done using 10% buffered formaldehyde solution. And routine histo techniques were done.

Grading of stenosis in coronary arteries

The grading of coronary artery lesions was done by the two experienced pathologists who were blind to the study protocol, clinical history and medical report of the patients. Microscopic assessment of the stenosis after taking digital photographs of coronary stenosis.

Grading of coronary stenosis was done under a four-point scale. Grade 0: Normal coronary artery, grade I: Hypercellular atherosclerotic plaque, grade II: Advanced atherosclerotic plaque and grade III: Total occlusion in the lumen of the coronary artery [7].

Immunohistochemistry manual staining

For immune histochemistry, the slides were deparaffinised before staining, with xylene I and xylenes II. The slides were then placed in 90% and 70% ethanol for 5 minutes. After deparaffinisation, the slides were washed with distilled water for 5 minutes. The washed slides were kept in Tris EDTA buffer and were allowed to cool. After cooling, the slides were kept in Tri-EDTA buffer (pH 8.5-9.5) autoclaved (15 minutes) and were allowed to cool. After cooling, the slides were incubated in 3% Hydrogen Peroxide (H_2O_2) for ten minutes. The tissue on the slide was marked after washing in Tris buffered saline (pH 7.4-7.6) for 3-5 minutes, followed by incubation with primary antibody for 1 hour at room temperature VEGF-A antibody (ImmunoTag-Cat Ltt05513) 1:200 was used as the primary antibody. The slides were washed twice in Iris buffered saline (3 minutes each). Tissues in the slides were flooded with secondary antibodies (HRP conjugate polymer horseradish peroxidase anti-rabbit) and were incubated for 30 minutes at room temperature. After incubation, the slides were washed with Tris buffered saline four times followed by the addition of development chromogen (DAB-3,3'-diaminobenzidine) to visualize the protein. The slides were further washed in distilled water four

times. The slides were counterstained with haematoxylin for one minute and were washed with distilled water, hydrated, cleaned and mounted using D.P.X. The slides were finally observed under a phase contrast microscope (Olympus CKX 41 with Optika Pro5 CCD) camera and microscopic observations were captured.

Quantification of VEGF expression on endothelial cells

The surface area containing VEGF positive endothelial cells was quantified by the use of computer aided planimetry and expressed as a percentage of the total surface area of endothelial cells. The area is termed as VEGF positive endothelial area [8].

$$\text{Positive endothelial cell area} = \frac{\text{Area occupied by the VEGF+endothelial cells}}{\text{Area occupied by the endothelial cells}} \times 100$$

Statistical analyses

The Statistical Package for Social Science (SPSS) 21 for windows was used for statistical analysis. All the p values are considered with a significant level <0.05.

Of the total number of 35 subjects, 30 were with occlusion in any one of the coronary arteries and in the remaining 5 subjects (control) no significant occlusion was found in angiogram (with normal coronary artery).

Continuous variables are presented as mean ± standard deviation and categorical data are summarized as frequencies or percentages. The differences between groups were analysed by *Chi-square* test for categorical clinical variables and independent sample students test for continuous variables. The relationship between coronary collateral circulation and clinical and angiographic variables was done by multivariate logistic regression analysis. Correlation between VEGF expression and morphological and cardiovascular risk factors on patients with various grades of stenosis was done by ANOVA (Analysis of Variance). All p-values are considered with a significant level of <0.05.

Results

Grading of coronary stenosis in coronary artery segments

Sections of the coronary arteries were taken from both case and control groups. In the case study group sections were taken from the proximal part of coronary occlusion. Of the 35 sections taken 5 had grade 0 stenosis, 7 sections had grade 1 stenosis and 15 had grade 2 stenosis and 8 had grade 3 stenosis (Figure 2).

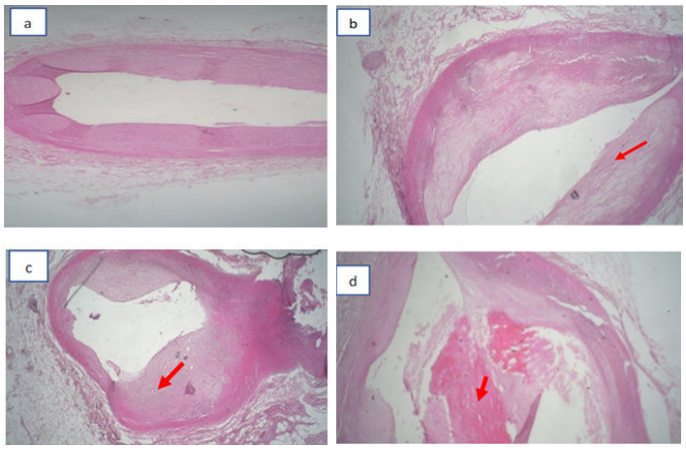

Figure 2: a) Grade 0: Normal coronary arteries, b) Grade I: Hypercellular atherosclerotic plaque well defined lipid core with the luminal surface covered by intima (arrow show atherosclerotic plaque in the tunica intima, c) Grade II: Adavanced atherosclerotic plaque lipid core with fibrous cape (shows red arrow), d) Grade III: Total occlusion fibroatheroma with haemorrhage or thrombosis occluding the arterial lumen (red arrow thrombus in the lumen of the artery).

Quantification of VEGF-positive endothelial cell area

Area of quantification of VEGF-positive endothelial cells was quantified using computer aided planimetry and expressed as a percentage of the total surface area occupied by VEGF positive endothelial cells [9].

$$\text{VEGF-positive endothelial cell area} = \frac{\text{Area occupied by the VEGF+ebndothelial cells}}{\text{Area occupied by the endothelial cells}} \times 100$$

$$\text{VEGF-positive cell area} = \frac{\text{Area occupied by the VEGF+cells in tunica intima}}{\text{Area occupied by the endothelial cells}} \times 100$$

VEGF positivity in endothelial cells of normal coronary artery sections (Figure 3).

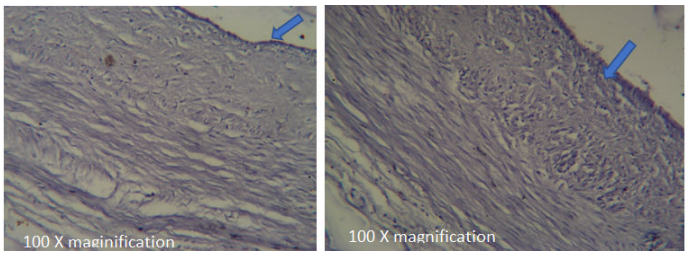

Figure 3: Normal coronary arteries-grade 0 (in control group) n=5.

In the above Figure 3, the blue arrow shows the endothelium lining the blood vessels. The VEGF staining was found virtually negative in almost all 5 sections of the coronary artery.

VEGF+endothelial cells area of 5 samples were taken and the mean was taken as the true value.

Endothelial cells and smooth muscle cells were virtually negative for VEGF staining. The VEGF+endothelial cell area was 0.350 ± 0.196%. And VEGF+cell area was 0.551 ± 0.06% VEGF

positivity in endothelial cells of coronary artery sections with hyper cellular atherosclerotic lesion (Figure 4).

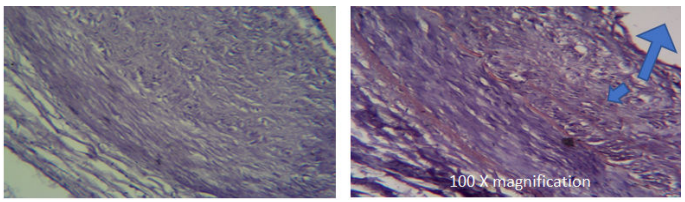

Figure 4: Hyper cellular atherosclerotic lesion (grade 1 lesion) n=7.

In the above Figure 4, macrophages scattered throughout the intima showed no VEGF positivity. VEGF positive staining was seen in some of the endothelial cells (occasionaly) and in some macrophages and smooth muscle of tunica media. (VEGF positive cells are shown by the blue arrow).

The EGF+endothelial cell area was=23.08 ± 1.720% and VEGF +cell area was 25.532 ± 0.26% (Figure 5).

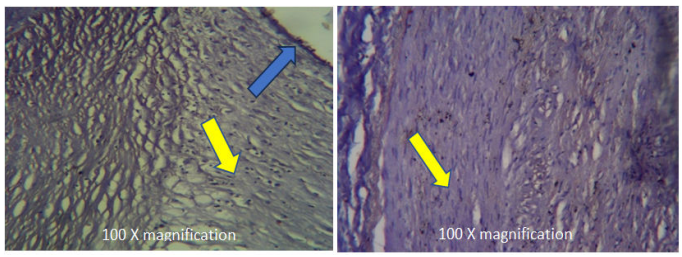

Figure 5: Advanced atherosclerotic plaques.

Advanced atheromatous plaque with a distinct core of lipid and fibrous cap. A distinct VEGF positive endothelial cells with VEGF positive macrophages and smooth muscle cells (blue arrow shows VEGF positive endothelial cell).

The VEGF+endothelial cell area was 27.48 ± 3.382%. And VEGF+cell area was 30.132 ± 0.26 %

VEGF positivity in endothelial cells of coronary artery sections with total occlusion (Figure 6).

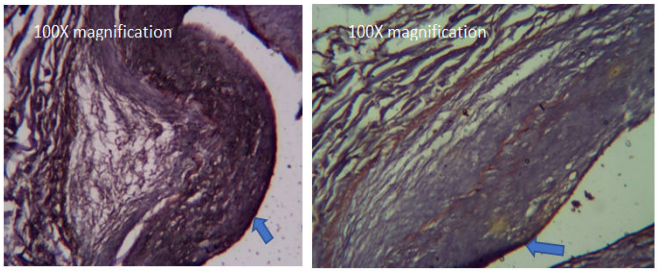

Figure 6: Total occlusion (>90% of occlusion) n=8.

The VEGF+endothelial cell area was=33.217 ± 1.2746% VEGF +cell area was 34.22 ± 0.56 % (Figure 7).

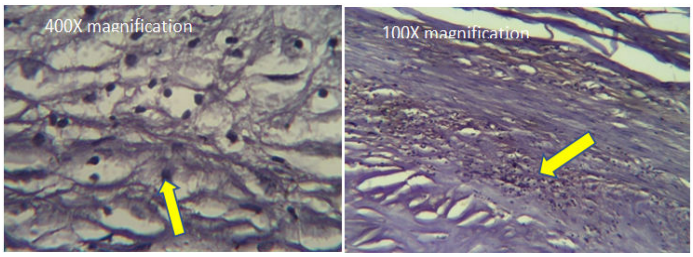

Figure 7: In iltrated macrophage cells of tunica intima and tunica media (yellow arrow shows the macrophage cells).

The above Figure 7 shows the VEGF positivity of macrophage cells in iltrated in the tunica intima of occluded coronary artery.

Correlation of VEGF expression with cardiovascular risk factors in various grades of stenosis

Effect of demographic factors on VEGF expression on various grades of stenosis: When the VEGF expression is correlated with grades of stenosis in old age and middle age group no statistical p-value was obtained, which shows age has no correlation with VEGF expression in various grades of stenosis ($p<0.05$ was considered statistically significant) (Table 1).

Table 1: Effect of age on VEGF expression in various grades of coronary stenosis.

Grade of stenosis	Age	n	Mean	Std. deviation	p-value
0	<60	3	0.387	0.197	0.564
	≥ 60	2	0.2945	0.258	
1	<60	5	23.28	1.765	0.699
	≥ 60	2	22.6	2.156	
2	<60	7	27.105	3.2413	0.291
	≥ 60	8	27.81	3.685	
3	<60	6	32.86	1.272	0.131
	≥ 60	2	34.27	0.565	

Effect of gender on VEGF expression in various grades of coronary stenosis: When the grade of stenosis is correlated with the VEGF expression with gender no statistically significant correlation was found ($p>0.05$ was non-significant, and a value of $p<0.05$ was considered statistically significant) (Table 2).

Table 2: Effect of gender on VEGF expression in various grades of coronary stenosis.

Grade of stenosis	Gender	n	Mean	Std. deviation	p-value
0	Male	6	0.35	0.1966	-
	Female	0	-	-	
1	Male	5	22.59	1.397	0.245
	Female	2	24.315	2.397	
2	Male	10	27.69	3.804	0.853
	Female	5	24.315	2.669	
3	Male	6	33.148	1.277	0.502
	Female	2	33.425	1.76	

Effect of diabetes mellitus on VEGF expression in different grades of stenosis: Among the 35 patients, 5 patients had normal coronary arteries without any occlusion. In these 5 patients, 4 are without diabetes mellitus and 1 with diabetes mellitus. In the grade one lesion, 4 are without DM and 3 with DM. In grade 2 stenosis group, 2 are without diabetes mellitus and 13 with DM. In 8 patients with grade 3 stenosis, 3 are without diabetes mellitus and 5 were with DM (Table 3).

Table 3: Effect of diabetes mellitus on VEGF expression in different grades of stenosis.

Grade of stenosis	Mean ± SD	Median (mini-Max)	p-value
0 NDM (4) DM (1)	0.393 ± 0.1974 -	0.447 (0.112-0.567) -	0.48
1 NDM (4) DM (3)	23.23 ± 0.989 22.90 ± 2.706	22.31 (22.16-24.130) 21.61 (21.08-26.01)	0.48
2 NDM (2) DM (13)	28.15 ± 0.663 27.37 ± 3.25	28.15 (24.15-32.16) 26.13 (23.14-32.16)	0.864
3 NDM (3) DM (5)	32.73 ± 0.981 26.738 ± 2.62	32.18 (32.16-33.87) 26.13 (24.13-31.17)	0.022
Note: A statistical significance in p-value is obtained when the expression of VEGF in the endothelial cells is correlated with diabetes mellitus in grade 3 stenosis. ($p<0.05$ was considered statistically significant) (NDN-Non Diabetes mellite-Diabetes mellitus)			

In 35 patients 5 were the control group with normal coronary arteries of which no patients had hypertension. In patients with grade 1 stenosis 6 had normal blood pressure and 1 had high blood pressure. Of patients with grade 2 stenosis five had normal blood pressure and nine with high blood pressure. In patients with grade 3 stenosis, 5 patients had normal blood pressure and 3 had high blood pressure.

Correlation diabetes with VEGF expression of infiltrated monocyte in the tunica intima in different grades of stenosis (Table 4).

Table 4: Effect of hypertension on VEGF expression in different grades of stenosis.

Grade of stenosis	Mean ± SD	Median (mini-Max)	p-value
0 NBP (5) HTN (0)	0.350 ± 0.196 -	0.418 (0.112-0.567) -	
1 NBP (6) HTN (1)	22.60 ± 1.250 -	23.91 (21.08-24.130) -	0.134
2 NBP (5) HTN (9)	28.08 ± 3.79 27.62 ± 3.19	26.13 (24.15-32.16) 26.13 (24.13-32.16)	0.737
3 NBP (5) HTN (3)	28.34 ± 3.05 30.06 ± 5.20	26.13 (26.13-32.160) 32.18 (24.13-33.87)	0.445
Note: When the presence of hypertension is correlated with the VEGF expression in the endothelial cell at different levels of stenosis, no statistically significant value was found. ($p<0.05$ was considered statistically significant) (NBP-Normal Blood Pressure, HTN-Hypertension))			

The above study on live patients shows that morphological factors like proximal location of the lesion in RCA, higher grade of lesion and severity of the lesion affect collateral formation. Diabetes mellitus causes low collateralization. Studies in serum level VEGF shows high collateral formation is directly related to high serum VEGF level. Autopsy samples show low collateral scores compared to live patients. VEGF expression in endothelial cells was found to correlate with the grade of stenosis and severity of disease and artery affected. Impaired VEGF expression was found in the diabetic patient with higher grade of stenosis (Table 5).

Table 5: Effect of diabetes mellitus on VEGF expression in macrophages in tunica intima in different grades of stenosis.

Grade of stenosis	Mean ± SD	Median (mini-Max)	p-value
2 NDM (4) DM (1)	0.551 ± 0.06 -	0.467 (0.212-0.801) -	0.46
1 NDM (4) DM (3)	25.69 ± 0.929 24.78 ± 0.030	24.32 (24.16-27.27) 23.26 (22.28-28.21)	0.95
4 NDM (2) DM (13)	31.32 ± 0.68 28.90 ± 3.25	31.32 (27.38-35.26) 27.74 (27.13-34.24)	0.028
5 NDM (3) DM (5)	35.18 ± 0.621 33.64 ± 0.62	35.18 (34.26-36.87) 33.64 (32.18-35.29)	0.018
Note: A significant p-value was found in VEGF expression and VEGF-positive cells infiltrated in the tunica intima of blood vessels when correlated in diabetic and non-diabetic subjects in grade 2 and 3 stenoses.			

Univariate analysis of variance independent effect of factors on VEGF expression in the endothelial cells of the occluded artery (Table 6).

Table 6: Univariate analysis of variance the independent effect of factors on VEGF expression in the endothelium (VEGF endothelial area).

Source	Type III sum of squares	df	Mean square	F	Signature
Intercept	9449.902	1	9449.902	1686.303	0.001
Grades of stenosis	740.793	3	246.931	44.064	0.001

DM	29.641	1	29.641	5.289	0.039
HTN	2.222	1	2.222	0.397	0.54

Note: A higher grade of stenosis showed a significant p-value 0.001 (p-value <0.05) when correlated with the expression of VEGF on endothelial cells. Cardiovascular risk factor diabetes mellitus also showed a significant p-value 0.039 when correlated with the VEGF expression of endothelial cells.

From the above it was understood that the VEGF expression of the endothelial cells are mainly depended on factors like grade of stenosis, diabetes mellitus. Thus, diabetes mellitus can be considered as an important variable which effect the collateral formation in coronary artery disease.

Discussion

The autopsy study provides a means of better understanding the basic process which sets the stage for clinically significant atherosclerotic cardiovascular disease [10]. There is no valid method of sampling the living population. The study of the effect of VEGF as a mitogen in the endothelial cells is an extremely difficult task in living subjects and an autopsy study is the best possible way to work on it.

Correlation of ischemic heart disease in autopsy study

The study conducted on post-mortem specimens by Vandana, et al., concluded that males are more prone to cardiovascular disease than females and the common type of atherosclerosis was type III. It was also concluded that the left anterior descending artery was the most frequently involved vessel. In the present study, the number of male patients was more when compared to female patients. In comparing the artery most affected by CAD it was LAD which was similar to the previous studies.

Inukochi, et al., studied the utility of post-mortem computerized angiography as supportive evidence for finding ischemic heart disease during autopsy. In the present study, the same method was found to be an effective technique to detect the level of coronary occlusion.

Expression of vascular endothelial growth factor in atherosclerotic lesion

Studies done on 38 coronary artery segments by Inoue, et al., concluded that a distinct expression of VEGF and its receptors was found in various grades of atherosclerotic lesions in coronary arteries. In the present study also an increase in the expression of VEGF in the endothelial cells was found in various grades of stenosis. Endothelial cells are the prime targets of VEGF.

Yu, et al., in their study concluded that VEGF-A receptors are not found in normal coronary artery segments, but their expression increases in endothelial cells of micro capillaries in atherosclerotic lesions. Chronic stress induces atherosclerosis in tunica intima and plaque instability which promotes angiogenesis this in turn is related to an increase in serum VEGF-A level. In the present study also a potent increase in the expression of VEGF-A in endothelial cells as observed when the grade of stenosis increased.

VEGF-A has shown to be an inducer of enhanced collateral blood flow as it is considered to be an inducer of monocyte migration. In the present study on autopsy samples, great variation in the expression of VEGF-A in the endothelial cells was found between the subjects with and without diabetes mellitus, especially in the cases of high grade stenosis and severity of the disease. These data support the hypothesis that impaired monocyte migration might explain a reduced arteriogenic potential in the diabetic heart when compared to the non-diabetic heart which causes reduced expression of VEGF-A in the endothelial cells. As diabetes mellitus is associated with impairment of collateral vessel formation as well as an impairment of VEGF-A-induced monocyte function, any treatment strategy using VEGF-would likely be less effective in diabetic individuals compared to non-diabetic individuals.

Conclusion

In sections of the coronary artery, VEGF-A expressions and monocyte recruitment was found impaired in diabetic patients when compared with non-diabetic. This may be the reason for the low collateral score in diabetic patients and thus treatment strategies with VEGF-A are found less effective in diabetic patients.

References

1. Futrakul N, Futrakul P (2011) Vascular homeostasis and angiogenesis determine therapeutic effectiveness in type 2 diabetes. Int J Vasc Med 2011:971524
2. Renault MA, Losordo DW (2007) Therapeutic myocardial angiogenesis. Microvasc Res 74:159-171
3. Fujita M, Tambara K (2004) Recent insights into human coronary collateral development. Heart 90:246-250
4. Bexell D, Setser RM, Schoenhagen P, Lieber ML, Brener SJ, et al. (2008) Influence of coronary artery stenosis severity and coronary collateralization on extent of chronic myocardial scar: Insights from quantitative coronary angiography and delayed-enhancement MRI. Open Cardiovasc Med J 2:79-86
5. Inokuchi G, Yajima D, Hayakawa M, Motomura A, Chiba F, et al. (2013) The utility of postmortem computed tomography selective coronary angiography in parallel with autopsy. Forensic Sci Med Pathol 9:506-514
6. Inoue M, Itoh H, Ueda M, Naruko T, Kojima A, et al. (1998) Vascular endothelial growth factor (VEGF) expression in human

coronary atherosclerotic lesions: Possible pathophysiological significance of VEGF in progression of atherosclerosis. Circulation 98:2108-2116

7. Lanzino G, Rabinstein AA, Brown Jr RD (2009) Treatment of carotid artery stenosis: Medical therapy, surgery, or stenting? Mayo Clin Proc 84:362–368
8. Abedinzadeh N, Pedram B, Sadeghian Y, Nodushan SMHT, Gilasgar M, et al. A histopathological analysis of the epidemiology of coronary atherosclerosis: An autopsy study. Diagnpathol 3:87-91
9. Porwal V, Khandelwal S, Jain D, Gupta S (2016) Histological classification of atherosclerosis and correlation with ischemic heart disease: A autopsy based study. Annals pathol Lab Med 3:100-104
10. Ferrara N (1996) Vascular endothelial growth factor. Eur J Cancer 32:2413-2422