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The role of Visfatin in atherosclerotic peripheral arterial obstructive disease

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ABSTRACT

Visfatin is an adipokine molecule acting as an essential coenzyme in multiple cellular redox reactions. The increased serum levels of Visfatin have been correlated with metabolic syndrome and endothelial homeostasis. In this study we investigate the possible relationship of Visfatin serum levels with the severity and location of atherosclerotic peripheral arterial occlusive disease (PAOD).

Study protocol included 45 consecutive PAOD and 20 Control patients with age >55 years old. Definition of PAOD was based in Rutherord's classification (RC). End-stage PAOD patients (RC-V & -VI) were excluded from study. Data were collected prospectively and included age, gender, atherosclerotic risk factors and the body mass index (BMI). In PAOD patients recorded the PAOD's clinical stage and the presence of carotid stenosis >50%. PAOD patients divided in two subgroups, those with mild (RC-I & -II) and moderate disease (RC-III & -IV). In all serum samples Visfatin was measured, blindly, twice by anosoenzymatic technique. Statistical analysis was performed by non-parametric Mann-Whitney *U* test, Pearson's chi-square, One Way Anova and Kruskall-Wallis tests, as appropriate.

The mean Visfatin value in PAOD and Control groups were 38.5 ± 16.0 and 13.9 ± 3.8 ng/ml respectively ($p < 0.0005$). In-PAOD subgroup of patients the visfatin values were not affected by demographics, BMI and atherosclerotic risk factors ($p > 0.05$). Univariate analysis showed that severity of PAOD (mild vs severe), presence of carotid stenosis >50% and multilevel disease significantly affected outcomes ($p = 0.018$, $p = 0.010$ and $p = 0.006$ respectively). In multivariate regression analysis severity of PAOD was the solely factor with strong correlation with high visfatin values ($p = 0.001$).

High Visfatin levels seem to be strongly correlated with the presence and severity of PAOD. Further and in depth investigation is needed to define the possible role of Visfatin in atherosclerosis and it's value as a potential prognostic biomarker of PAOD.

1. Introduction

Visfatin is an adipokine molecule which displays intrinsic enzymatic activity as a nicotinamide phosphoribosyltransferase (Nampt) [1]. Visfatin/Nampt, in mammals, is involved in synthesis of nicotinamide adenine nucleotide (NAD+) which act as an essential coenzyme in multiple cellular redox reactions [2]. Obesity and type 2 diabetes represent two well-known independent risk factors of inflammation related to atherosclerotic arterial disease. Although, a positive role of Visfatin in these two factors has been proposed, the ongoing investigation shows that this potential role still remains controversial [3]. Additionally, in humans the increased serum levels of Visfatin have been correlated with metabolic syndrome and carotid atherosclerosis [4]. A potentially indirect role of visfatin in cardiovascular diseases has been supposed, through the metabolic syndrome and inflammation. Furthermore, direct actions with smooth muscle cells proliferation and angiogenesis, with increased levels and activity of matrix metalloproteinases (MMP 2 and 9) as well as with promotion of cell adhesion molecules like the ICAM-1, VCAM-1 and E-selectin has also been proposed [2,5]. All these actions, especially in combina-

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tion with other risk factors, might result in atherosclerotic plaque vulnerability and increased incidence of cardiovascular events [6–9]. In this study we attempt to explore the possible relationship between Visfatin serum levels and the severity and location of atherosclerotic peripheral arterial occlusive disease (PAOD), and to investigate the potential role of Visfatin as a biomarker of this disease.

2. Methods

Study protocol included 45 consecutive PAOD and 20 free from any vascular or other inflammatory disease (Control) patients with age >55 years old who were examined between May and June of 2015 at the outpatient department of Division of Vascular Surgery of 2nd Department of Surgery (School of Medicine, Aristotle University of Thessaloniki) at «G. Gennimatas» Hospital. The recorded data were prospectively collected and included the age, gender, four atherosclerotic risk factors (diabetes, dyslipidemia, hypertension, currently smoking or cessation for less than 6 last months) and the body mass index (BMI). According to BMI the patients were classified in 3 subgroups; 1st Subgroup with BMI < 25, 2nd subgroup with BMI from 25 to 30 and 3rd with BMI > 30. Additionally, in PAOD patients we recorded the disease stage according the Rutherford's classification and the coexistence or not of carotid artery stenosis >50%, diagnosed by duplex ultrasound velocity criteria [10,11]. We excluded all end-stage PAOD patients (Rutherford's class V and VI) in order to avoid any potential effect of concomitant inflammation or adjunctive medication in Visfatin levels.

Table 1 shows the recorded demographics and clinical data of all included patients.

Table 1
Demographics, clinical data and Visfatin values of the 2 analyzed groups.

	Patients n = 45	Control n = 20	p
Age (mean ± stdv in years)	69.1 ± 6.8	68.4 ± 4.7	
Median (years)	69	68	0.706*
Range (years)	(56–83)	(61–75)	
Gender male/female ratio	m/f = 33/12	m/f = 14/6	0.782**
Percentage ratio	(73.3%/26.7%)	(70.0%/30.0%)	
Diabetes mellitus (n – %)	n = 31 – 68.9%	n = 7 – 35.0%	**0.006****
Dyslipidaemia (n – %)	n = 38 – 84.4%	n = 10 – 50.0%	**0.004****
Hypertension (n – %)	n = 35 – 77.8%	n = 11 – 55.0%	0.062**
Smoking (n – %)	n = 29 – 64.4%	n = 11 – 55.0%	0.470**
Body Mass Index (mean ± stdv)	28.4 ± 2.5	28.0 ± 2.1	
Median	28.1	28.0	0.644*
Range	(23.1–34.0)	(24.5–32.6)	
BMI classification			
BMI: < 25 (n – %)	n = 3 – 6.7%	n = 3 – 15.0%	
BMI:25 – 30 (n – %)	n = 35 – 77.7%	n = 13 – 65.0%	0.779***
BMI: > 30 (n – %)	n = 7 – 15.6%	n = 4 – 20.0%	
Rutherford classification			
CLASS 0 (n – %)	n = 0 – 0.0%	n = 20 – 100.0%	
CLASS I (n – %)	n = 3 – 6.7%		
CLASS II (n – %)	n = 15 – 33.3%	n = 0 – 0%	N.A.
CLASS III (n – %)	n = 20 – 44.4%		
CLASS IV (n – %)	n = 7 – 15.6 %		
Carotid artery stenosis (n – %)	n = 13 – 28.9%	n = 0 – 0%	N.A.
Visfatin (mean ± stdv in ng/ml)	38.6 ± 16.0	13.9 ± 3.8	
Median (ng/ml)	35.0	13.3	<**0.0005***
Range (ng/ml)	(15.1–84.7)	(8.2–21.1)	

N.A. = Not applicable, all control patients were Rutherford's class 0 and with no evidence of carotid stenosis (absence of PAOD).
* Mann-Whitney *U* test.
** Pearson's chi-square test.
*** One way ANOVA test.

Blood collection was made after overnight fasting and the blood serum was isolated through centrifugation and was frozen at −40 C for storage. For measurement of Visfatin levels we used the Enzyme-linked immunosorbent assay (ELISA by Visfatin EIA, Kit RAB0377, SIGMA/ALDRICH, St-Louis, MI, USA). This in vitro technique allows the quantitative measurement of Visfatin's peptide based on the principals of competitive immunoassay. The kit's microplate is pre-covered with secondary antibodies, which link with the primary antibodies bound to the target antigens, thus catalyzing a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated visfatin peptide and inversely proportional to the amount of endogenous visfatin in the standard or samples. For the quantitative measurement of Visfatin's concentration we used an automated photometer at 450 nm wavelength. Matching of the optical absorption at a final concentration became possible after using a standard curve (Fig. 1) for visfatin samples of known concentration (ng/ml). All measurements were made at the Laboratory of Reference Centre for AIDS of Northern Greece of 1st Department of Microbiology. (School of Medicine, Aristotle University of Thessaloniki), and were performed blindly to the samples grouping. We performed 2 also blind measurements for each sample, in order to assess the agreement between the 2 measurements of the same sample and to validate our measurements technique.

Statistical data were analyzed using IBM SPSS 23.0 Statistics (IBM Corporation, Somers, NY, USA). Continuous data are presented as mean ± stdv as well as median values and range. Categorical data are presented as n and percentage. Agreement between the 2 visfatin measurements of the same sample for all included individuals was assessed by Bland-Altman plot (Fig. 2), which showed good agreement between the 2 measurements. In analysis we used the mean Visfatin value between the 2 measurements of the same sample. Due to skewed distribution of data and the small number of analyzed samples we performed the non-parametric Mann-Whitney *U* test, Pearson's chi-square, One Way Anova and Kruskall-Wallis tests, as appropriate. In all results presented at tables we mention the used test. Statistical significance level was set at $p < 0.05$ and significant differences are highlighted with p values appear bold-typed.

3. Results

Data analysis showed no difference in terms of age, gender and BMI distribution between the two (PAOD and Control) groups

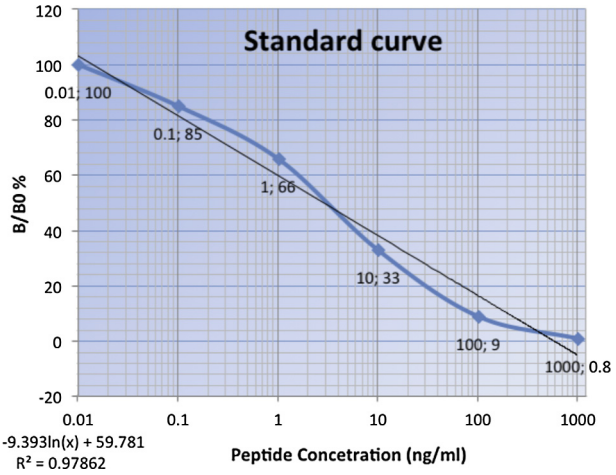

Fig. 1. Standard curve of visfatin concentration that was used for exporting the visfatin values of samples.