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HUMAN PARAOXONASE 1 (PON1) AND ITS ASSOCIATION WITH CARDIOVASCULAR DISEASE RISK ON A GROUP STUDY OF YOUNG HYPERTENSIVE PATIENTS

Călin POPA[1], Cristina POPESCU[2], Luminiţa PILAT[1,3], Maria PUŞCHIŢĂ[1,3]
[1]Faculty of Medicine "Vasile Goldis" Western University of Arad
[2]Institute of Life Sciences "Vasile Goldis" Western University of Arad
[3]Emergency County Hospital of Arad

ABSTRACT. **Introduction:** The incidence of cardiovascular diseases is increasing every year in our country and hypertension population is leaning to younger ages and for that we need an effective medical tool for prevention. **Aim:** Our objective is to analyse atherogenic risk data for young hypertensive patients. **Method:** comparison of PON1 levels and atherogenic risk calculators on two hypertensive patients groups (20 newly-discovered hypertensive patients and 20 grade I hypertension patients) and a control group (15 subjects). **Results:** The AIP shows important atherogenic risk data with statistically importance for the second hypertensive group rather for the first hypertensive study group but the PON1 values are very relevant for all the groups. **Conclusion:** Determination of PON1 values may be a useful and effective quantification tool for cardiovascular disease risk if we take in consideration the results from our study which shows low levels of PON1 even in asymptomatic patients (control group and the first group of hypertensive patients).

Keywords: PON1, human paraoxonase 1, hypertension, atherogenesis, cardiovascular risk, HDL-c, LDL-c, TC.

INTRODUCTION

Cardiovascular diseases have an important place in the world currently ranking chronic diseases, being the leading cause of death in the world after cancer. According to SEPHAR I and II studies, our country occupies a leading position in the ranking of developing countries with high prevalence of hypertension and dyslipidaemia as a major risk factors for cardiovascular disease. (Dorobantu M, et. al).

Latest genetic studies have shown that from the paraoxonase family (PON1-3) PON1 plays an important role in protecting LDL-c and HDL-c molecules of being oxidized by the free radicals therefore is directly involved in the process of atherogenesis and the association of PON1 with cardiovascular disease. A low serum PON1 activity is correlated with an increased risk of coronary heart disease, myocardial infarction and carotid atherosclerosis. (Aviram M, et. al.)

Our objective is to analyse atherogenic risk data for young hypertensive patients by using the human serum paraoxonase 1(PON1) values and the atherogenic index of plasma (AIP).

MATERIALS AND METHODS

15 clinically healthy subjects (9 females and 6 males; average age: 27.6 ± 3.1; range: 25–35 years) were studied as a control group, the second group (G1) had 20 young newly-discovered hypertensive patients without any antihypertensive treatment (9 females and 11 males; average age: 32.3 ± 3.3; range: 27-38 years) and the third study group (G2) had 20 patients (8 females and 12 males; average age: 39,8± 2.9) with 1st grade hypertension and on single antihypertensive drug treatment (according to the European Society of Cardiology and the European Society of Hypertension guidelines). All participants gave a written consent to participate in our study.

Inclusion criteria was age above 25 years and under 45 years (the average age for female patients was 34.3±3.1 and for male patients was 36.5±4.2) without cardiovascular diseases (history of stenting or bypass, cardiac arrest, or any other cardiac diseases) without tobacco or alcohol consumption. The method used was that of the comparison of AIP (artherogenic index of plasma) and PON1 values.

Blood was taken from vein into commercial vacutainers (Red without additives, Light-yellow with SPS and Purple with EDTA). After centrifugation (10 min at 1500× g) serum was immediately separated and stored in aliquots at –80°C until use. Next step was to use the serum on ELISA kit PON1 for the quantitative determination of human serum paraoxonase 1 concentration. Also we included the determination from venous blood of total cholesterol (CT), triglycerides (TGR), LDL-cholesterol, HDL-cholesterol.

Clinical data of hypertensive patients and control group were selected from anamnestic, clinical and paraclinical exams made to each patient at the moment of inclusion in our study. Blood pressure measurements were performed according to the guidelines of the European Society of hypertension (ESH). (European guidelines on cardiovascular disease prevention in clinical practice, European Society of Cardiology (2012).)

**Correspondence:* *Dr. Calin Popa, 37 George Cosbuc Street, Arad- Romania, Email: calinpopa@msn.com.Tel: +40722 247 246*
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After obtaining the PON1 data and lipid fractions levels we used the AIP (atherogenic index of plasma), which is based on a logarithmic formula that includes values of triglycerides (TG) and HDL-c, Dobiášová et al in their clinical studies have shown that AIP can predict CV risk. (Dobiášová M. et. al.).

All results were expressed as mean±standard deviation (SD). The data was analysed for statistical significance using the computer program Statistica 7. For values of $p<0.05$ was considered significant, for values of $p<0.01$ it was considered distinctive statistically significant, for values of $p<0.001$ was considered very significant statistically and for p values >0.05 were considered non-significant.

RESULTS AND DICUSSIONS

In Table I (data from anamnesis and clinical examination) it can be seen that the age of the subjects in the study groups is significant close, also the BMI (body mass index) isn't very different in hypertensive groups G1 and G2. Regarding the distribution by gender, the female patients had lower values of measured parameters compared to those of the male patients, the level of hypertension in the same groups is significantly different from the control group, indicated by the values of $p< 0.05$ ($p=0.0098$ for male subjects and $p=0.00168$ for female subjects from group G2).

Table I. Clinical data of hypertensive patients and control group

Group	Parameters*					
	Age[1]		BMI[1]		BP[1]	
	♂	♀	♂	♀	♂	♀
Control	28.5±4.3	27.3±3.2	23.3±4.2	20.3±2.4	122±2.1 /74±1.7	112±1.2 /68±1.5
G_1	36.7±4.8	35.3±5.7	**25.9±2.3**	**24.1±2.3**	**148±1.5 /87±1.3**	**142±1.9 /86±2.4**
***p* (G_1 vs. C)[2]**	2.2399	1.5866	0.00372 **	0.00257 **	0.00368 **	0.00258 **
G_2	40.5±2.6	38.9±3.7	**28.4±3.6**	**26.5±1.3**	**152±2.4 /99±1.4**	**147±1.9 /92±1.4**
***p* (G_2 vs. C)[2]**	0.0284 *	0.0258 *	0.01951 ***	0.00256 **	0,00098 ***	0,00168 **

Legend:
[1]= average (mean) and standard deviation; **BMI** = body mass index, **BP** = blood pressure; [2]= ***p*** values for each hypertensive group compared with control group, ***p***<0.05* statistically significant, ***p***<0.01** highly statistically significant and ***p***<0.001*** highly statistically significant.

Further we compared biochemical data that we obtain from the blood sample analysis with the AIP (atherogenic index of plasma- limits presented in Table II) and the PON1 serum values (normal range 75-100mg/ml).

Table II. AIP (atherogenic index of plasma)

Atherogenic risk*	
low	<0,11
intermediate	0.11
increased	>0,21

Legend: *AIP=log (TG/HDL-c)

For the study group 1 patients with hypertension without any antihypertensive treatment (G1) the results were very significant statically with a $p<0.05$ compared with those of control group regarding all measured parameters.

Some subjects from the control group had low values for paraoxonase 1, even if these people have no record of medical diseases, some of the male subjects had a heredocolateral history of diabetes mellitus and stroke and some of the female subjects have taken contraceptive drugs in the last 12 months.

Table III. Biochemical data to study group G_1 compared to the control group

Parameters	Control group		G_1		*P*	
	♀	♂	♀	♂	♀	♂
TC (mg/dl)*	145.1±7.2	157.1±4.2	**202.2±25.8**	**206.5±26.8**	0.0125	0.0118
TG (mg/dl)*	122.8±9.8	145.3±9.6	**144.3±22.4**	**182.6±18.6**	0.0228	0.0214
LDL-c (mg/dl)*	126.4±7.5	124.2±8.4	**154.2±26.8**	**158.5±24.5**	0.0345	0.0278
HDL-c (mg/dl)*	58.1±2.8	52.1±4.4	**46.7±32.3**	**44.8±12.26**	0.0159	0.0147
AIP	**0.037±0.8**	**0.085±0.23**	**$0.139±1.2^{A}$**	**$0.257±1.45^{AA}$**	**0.0025**	**0.0032**
PON1	**68.4±9.3**	**64.5±4.5**	**55.6±3.7**	**52.3±5.8**	**0.0347**	**0.0328**

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 23, issue 4, 2013, pp 477-481

Legend:
* = mean+ DS, the value of p<0.05 considered significant, p>0.05 considered non-significant; [A]- indicates intermediate risk for atherogenesis; [AA]-indicates high risk for atherogenesis.

Table IV. Biochemical data to study group G_2, compared to the control group

Parameters	Control group		G_2		*P*	
	♀	♂	♀	♂	♀	♂
TC (mg/dl)*	186.1±11.2	188.1±10.2	**228.1±54.6**	**264.3±58.5**	0.0036	0.0028
TG (mg/dl)*	122.8±9.8	138.3±9.6	**201.1±25.7**	**235.4±56.2**	0.0068	0.0015
LDL-c (mg/dl)*	126.4±7.5	124.2±8.4	**187.3±25.5**	**198.4±44.3**	0.0145	0.0122
HDL-c (mg/dl)*	58.1±2.8	52.1±4.4	**45.5±34.5**	**38.8±38.5**	0.0224	0.0189
AIP	**0.046±0.8**	**0.064±0.23**	**0.301±1.87[AA]**	**0.453±1.75[AA]**	**0.0025**	**0.0019**
PON1	**68.4±9.3**	**64.5±4.5**	**44.8±2.8**	**39.4±6.7**	**0.0042**	**0.0025**

Legend:
* = mean+ DS, the value of *p*<0.05 considered significant, *p*>0.05 considered non-significant; [AA]-indicates high risk for atherogenesis.

Lipid fractions were all modified for G2 hypertensive patients and over 50% of male patients were overweight as they have a sedentary lifestyle with irregular physical activity and female patients from G2 study group were below 40% overweight and a little more active then male (one or two times/week follow fitness programs).

Table V. PON1 and AIP average data

Groups	Parameters[1]			
	PON1		AIP	
	♀	♂	♀	♂
Control	**68.4±9.3**	**64.5±4.5**	**0.046±0.8**	**0.064±0.23**
G_1	**55.6±3.7**	**52.3±5.8**	**0.139±1.2**	**0.257±1.45**
***p* (G_1 vs. C)[2]**	***	***	**	***
G_2	**44.8±2.8**	**39.4±6.7**	**0.301±1.87**	**0.453±1.75**
***p* (G_2 vs. C)[2]**	***	***	***	***

Legend:
[1] = mean±SD; **AIP** (atherogenic index of plasma); [2]=values of p resulted by comparing each hypertensive group with the control group; *** p<0.0001 (this difference is considered to be extremely statistically significant).

Between hypertension, atherogenesis and PON1 is a complex and still not entirely elucidated connection. The hypertension's pathogenesis is a multifactorial process that implicates the interaction of genetic and environmental factors. Vascular remodelling with decreasing vascular lumen and increased vascular resistance, abnormalities of volume regulation and vasoconstriction contributes to high blood pressure. (Griendling KK et. al.).

On the other hand pathogenesis of the atherosclerotic process is considered to be multi-factorial and characterized by chronic inflammatory response. Although hypertension is known to be one of the most important risk factors for atherosclerosis and many recent studies has indicated that hypertension, through the vasoactive peptides (like angiotensin and endothelin-1) stimulates and speeds up the atherosclerotic process thru inflammatory mechanisms. (Li JJ, et. al.).

Aviram et al proved that human paraoxonase has an important role at the endothelium level by protecting HDL-c and LDL-c molecules to be oxidized by free radicals, and when it's values in blood plasma are high it interferes even with the already installed on atheromatous plaque and reduces its size and also the local inflammation by peroxidase-like activity and lactonase activity (by attenuation of macrophage cholesterol and oxidized lipid accumulation and foam cell formation). (Aviram M. et. al.). These facts are confirmed by Gugliuci and his team and by other scientists, and also admitted that from the family of paraoxonase (PON1, PON2 and PON3) the most active and important in atherogenesis process is the PON1 with all its polymorphism variants in various populations. (Gugliucci A. et. al.).

PON1 values can be modified by food diet, exercises, stress, medication and life style by a small percentage value and for a correct value of our resulting data we made a media between two blood analyses (four weeks between samples taking). According to Blatter et al PON1 values can be raised by red wine, statins and fibrates, exercises, vitamins and aspirin. (Blatter Garin MC et. al.).

If we analyse the total cholesterol (TC) values for the G1 and G2 we can see that the values are not so high (mean value 206.5±26.8 for male patient for G1 and 264.3±58.5 as mean values for male patients for G2 – Table V) and it is not showing us a very high risk for atherogenesis, according to newest cholesterol guidelines the total cholesterol (TC) goal values for

those age 20 and younger are 75-169 mg/dL and 100-199 mg/dL for those over age 21. (Waters D. et. al.). In the past LDL-c levels were considered very conclusive for CV risk, but recently LDL-c is considered as a poor marker of heart health and the logarithmic ratio of HDL-c and TG, also known as AIP (atherogenic index of plasma) can be more accurate than the biochemical data of each lipid fractions (David D. Waters et. al.). Further, the recent studies indicate that the increasing PON1 activity is associated with decreased odds of atherosclerosis (Coombes RH et. al.).

The resulting data indicates that for the G2 study group patients, all the parameters were modified and the atherogenic index of plasma was elevated and that indicates a very high atherogenic risk for these patients. For the male subjects the mean value and standard deviation for AIP was 0.453±1.75 and for the female patients were 0.301±1.87 and PON1 results 39.4±6.7 for male patients and 44.8±2.8 comparing with the normal values of PON1 activity (75-100 mg/dl). Both AIP and PON1 indicate high CV risk for these patients.

In Table IV the mean value of AIP for female subjects was 0.139, meaning a moderate atherogenic risk based on HDL-c values and TG values, and for the male subjects the mean values of AIP was 0.257, a high risk for cardiovascular disease taking in consideration that the blood pressure and the lipid fractions values were higher for the male patients compared to female patients.

CONCLUSIONS

In conclusion, by evaluating the obtained data and linking with the two newly useful medical tools such as AIP and PON1 levels we can estimate patient's risk for eventual CV events for the patient's years to come, being also a personalised investigation. As we can notice that the PON1 is more precise than the AIP for CV risk, for example the low levels of PON1 plasmatic activity for the young patients from our study, which are apparently with no CV risk. Even if PON1 values can be influence by multiple factors (and can change its values by a few percentage) it still can accurately indicate the CV risk, as for further studies it will be interesting to investigate PON1 polymorphism for our region and to measure as well PON1 levels from patients who had stroke or myocardial infarction.

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