Comparison of serum paraoxonase and arylesterase activities between iron deficiency anemia patients and chronic kidney disease patients with anemia

Yildiz Okuturlar a, Nilgul Akalin b, Ozlem Harmankaya Kaptanogullari b, Nurten Turan Guner c, Deniz Yilmaz a, Asuman Gedikbasi d, Ozlem Soyluk e, Meral Mert e, Sibel Ocak Serin f, Hakan Kocoglu a, Mehmet Hursitoglu a and Abdulbaki Kumbasar a

aDepartment of Internal Medicine, Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey; bDepartment of Nephrology, Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey; cDepartment of Radiology, Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey; dDepartment of Biochemistry, Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey; eDepartment of Endocrinology and Metabolism, Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey; fDepartment of Internal Medicine, Umraniye Education and Research Hospital, Istanbul, Turkey

ABSTRACT

Objective: Altered paraoxonase (PON) and arylesterase (ARE) activities have been shown in anemic chronic kidney disease (CKD) patients and in iron deficiency anemia (IDA) patients. Whether accompanying anemia alone is responsible for this diminished PON and ARE activities in CKD patients or an additive factor for this is not well studied. Therefore, we tried to clarify this issue here. Methods: A total of 82 subjects that consisted of 19 patients with IDA (group 1), 23 anemic CKD patients (group 2), and 40 age and sex matched healthy subjects (group 3) were enrolled. Carotid intima media thickness (CIMT), serum total thiol (–SH), PON, and ARE activities of the participants were analyzed. Results: Group 2 patients had significantly lowest serum levels of Total –SH, PON and ARE. Further comparison showed that total –SH, PON and ARE levels were lower in group 1 than group 3 (p = 0.0001 in both). Regarding comparison of group 1 and 2, only serum ARE levels were significantly lower in group 2 (p = 0.001). PON activity was not different between group 1 and group 2 whereas ARE activity was lower in group 2 than groups 1 and 3. In addition, correlation analysis showed that CIMT was negatively correlated with PON and ARE. Conclusions: This markedly decreased ARE activity in CKD patients, which could not be explained by the anemia alone, may have a role in the pathogenesis of increased atherosclerosis in such patients. Still further studies are needed to certain this.

ARTICLE HISTORY
Received 28 August 2015
Revised 16 February 2016
Accepted 25 February 2016
Published online 23 March 2016

KEYWORDS
Anemia; arylesterase; atherosclerosis; chronic kidney disease; paraoxonase

Introduction

It is well known that anemia is a risk factor for atherosclerosis and cardiovascular disease (CVD). Anemia commonly occurs in people with chronic kidney disease (CKD). It might begin to develop in the early stages of CKD, when creatinine clearance falls to 70 mL/min or lower among males and to 50 mL/min or lower among females. Previous studies have shown that anemic CKD patients have higher risk for CVD when compared to patients with only anemia. One reason could be that anemic CKD patients have more risks for atherosclerosis than patients with only anemia, such as reduced GFR, microalbuminuria, concomitant diseases (hypertension, diabetes, etc.), and abnormal calcium and phosphate metabolism. The paraoxonase (PON) gen family consists of PON1, PON2 and PON3 which are located next to each other on the long arm of chromosome 7q21.3–22.1. Altered serum PON1 activity has been shown to be associated with atherosclerosis and CVD. The enzymatic components of PON1 system, which act as a single enzyme, are PON, arylesterase (ARE), and diazoxonase. Decreasing of the plasma total thiol (–SH) level is also a good reflector of generated free radicals. Diminished PON and ARE activities have been shown in CKD patients and in iron deficiency anemia (IDA) patients.

Carotid intima media thickness (CIMT) is an early marker of the atherosclerotic process and is currently used to assess the presence and the progression of atherosclerosis. Studies showed that CIMT was positively correlated with cardiovascular risk factors and it was associated with increased incidence of cardiovascular disease.

As mentioned earlier, both anemic CKD patients and IDA patients have decreased PON and ARE activities but
their risks for atherosclerosis are different. Therefore, we tried to compare PON and ARE activities between both groups in order to determine whether diminished PON and ARE activities in anemic CKD patients are caused by only anemia or not. Also, we tried to determine the relationship between PON, ARE activities, and atherosclerotic status in both groups by measuring CIMT.

Methods

After the approval of the local ethic committee (No: 2014/18), 82 individuals (23 IDA patients, 19 anemic CKD patients [but not received dialysys yet] and 40 healthy control subjects) were enrolled in the study. Patients with known malignancy and ischemic heart disease or any symptoms which could be related to ischemic heart disease were excluded. Healthy control subjects (group 3) were established three groups which consisted of IDA patients (group 1), anemic CKD patients (group 2), and healthy subjects (group 3). The reason of selecting patients with iron deficiency anemia was the other types of anemia which could have an underlying disease (e.g., megaloblastic anemia or anemia of chronic disease) may alter the PON and ARE levels.

Group 1 consisted of IDA patients with hemoglobin (Hb) level lower than 10 g/dL and who have no evidence of any other disease (such as malignancy in their upper and lower endoscopic investigations, diabetes, hyperlipidemia, chronic kidney disease, chronic liver disease, etc.). The etiology of IDA in this group was menstrual bleeding or hemorrhoids in females and bleeding due to hemorrhoids in males. Group 2 consisted of chronic renal failure patients with also Hb levels lower than 10 g/dL. Patients who received renal replacement therapy and/or hemodialysis were excluded. Healthy control subjects without anemia have formed the Group 3.

Carotid intima media thicknesses (CIMT) of all the groups were measured. Blood samples were taken after overnight fasting and processed within 1 h of collection. The samples were obtained from antecubital vein using sterile needle and blood was allowed to flow freely into vacutainer tubes containing EDTA (to analyze complete blood count) or no additive (to analyze serum parameters). Serum was separated from the cells by centrifugation at 1500 g for 10 min. and the serum samples were stored at −80 °C until the PON, ARE, and total-SH analyses. Serum creatinine, LDL-C, HDL-C, iron and total iron binding capacity (TIBC), and other biochemical parameters were determined by Abbott Architect C16200 Integrated System and using commercial kits (Abbott Laboratories, Abbott Park, IL). Complete blood count was determined in a Coulter LH 750 auto analyzer (Beckman Coulter, Brea, CA).

Assay of total PON and ARE

Paraoxonase (PON) and ARE activities were determined using a novel automated measurement method developed by Erel (Relassy, Turkey). Briefly, the rate of paraoxon hydrolysis was measured by the increased absorbance at 412 nm at 25 °C. The PON activity is expressed as U/L serum. The coefficient of variation (CV) for individual samples was 1.8%. ARE activity was measured spectrophotometrically using phenyl acetate. The reaction was started by the addition of the serum; the increase in absorbance was read at 270 nm. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol. One unit of ARE activity was defined as 1 µmol phenol generated/min under the defined assay conditions and expressed as U/L serum. The CV for individual serum samples was 3.3%.

Determination of serum total thiol levels

Serum total –SH content was measured by using dithio-nitrobenzoic acid (DTNB). The results were expressed in terms of µmol/L. The coefficients of intra- and inter-assay variations were 5.8 and 7.6%, respectively.

Measurement of carotid intima-media thickness

Subjects were evaluated for CIMT and plaque occurrence by using high resolution grey-scale Doppler ultrasonography (General Electric, Logiq 9, Fairfield, CT) with 10 MHz linear transducer used. In a semi dark room, all subjects lay supine with their necks slightly hyperextended and rotated away from the imaging transducer. CIMT was defined as the distance between the leading edge of the lumen intimal interface and the leading edge of the media adventitia interface of the far wall. The measurements were taken from far of the plaque areas. Three measurements were taken proximal to the carotid bifurcation from both carotid arteries and was derived the mean CIMT.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS, Inc., Chicago, IL) statistical package for Windows. The distributions of variables were checked with the Kolmogorov–Smirnov test. Continuous data were expressed as mean standard deviation. Descriptive statistics were expressed as a median (interquartile range) for numerical variables that did not display normal distribution. Differences between groups were assessed with ANOVA (parametric). For significant variables, post hoc Tukey HSD or Tamhane’s T2 test was applied to
The mean ages of groups 1, 2, and 3 were 53.69 ± 13.64, 55.31 ± 14.63, and 49.95 ± 10.28 years, respectively (p = 0.244). The age range of patients enrolled in this study was between 30 and 86 years. Female/male ratios of groups 1, 2, and 3 were 21/2, 8/11, and 29/11, respectively. There was a significant difference in body mass index (BMI) among groups (p = 0.004). Group 1 and 2 had a significant difference in BMI (30.33 ± 5.60 vs. 25.02 ± 5.32, respectively, p = 0.003). There was a significantly lower Hb level between group 1 and 2 (p = 0.22) but both groups’ Hb levels were lower than group 3 (p = 0.0001). Iron levels were different between all groups (p = 0.0001) and its levels were below normal in groups 1 and 2. Transferrin saturation \([\text{Fe/TIBC} \times 100]\) in group 2 was different from groups 1 and 3 (p = 0.002 in both). It was also significantly low in groups 1 than group 3 (p = 0.0001) (Table 1).

As expected, the creatinine levels of the CKD patients were significantly higher compared to IDA patients and control subjects. HDL-C levels in CKD patients were significantly lower than groups 1 and 3 (p = 0.003) and 0.0001 respectively. Total cholesterol, triglyceride, and LDL-cholesterol levels were not different between groups (p = 0.252, 0.527, and 0.14; respectively) (Table 1).

The total –SH, PON, ARE levels, and PON/ARE ratio were different between all groups (p = 0.0001 in all). CIMT was also different in all groups (p = 0.019) (Table 2). Post hoc analysis showed that total –SH, PON, and ARE were lower in group 2 than group 3 but the CIMT and PON/ARE ratio were higher in group 2 than group 3 (p = 0.0001, 0.042, 0.0001, 0.026, and 0.01, respectively). Also comparing group 1 with group 3 showed that total –SH, PON, and ARE levels were lower in group 1.

### Table 1. The clinical and biochemical characteristics of all groups.

| Groups | IDA patients | CKD patients | Healthy controls | p   |
|--------|--------------|--------------|------------------|-----|
| Group I | 333.48 ± 71.01 | 300.58 ± 80.78 | 530.04 ± 168.55 | 0.0001 |
| Group II | 108.78 ± 19.80 | 122.48 ± 54.09 | 157.36 ± 26.43 | 0.0001 |
| Group III | 168.64 ± 13.67 | 122.54 ± 30.69 | 256.10 ± 24.62 | 0.0001 |

Bold values represent that p values ≤ 0.05 are statistically significant.

### Table 2. Comparison of antioxidant levels and Carotid Intima Media Thickness between groups.

| Group 1 | Group 2 | Group 3 | p   |
|---------|---------|---------|-----|
| Total –SH (μmol/L) | 333.48 ± 71.01 | 300.58 ± 80.78 | 530.04 ± 168.55 | 0.0001 |
| PON (U/L) | 108.78 ± 19.80 | 122.48 ± 54.09 | 157.36 ± 26.43 | 0.0001 |
| ARE (U/L) | 168.64 ± 13.67 | 122.54 ± 30.69 | 256.10 ± 24.62 | 0.0001 |

Bold values represent that p values ≤ 0.05 are statistically significant.

**Results**

The mean ages of groups 1, 2, and 3 were 53.69 ± 13.64, 55.31 ± 14.63, and 49.95 ± 10.28 years, respectively (p = 0.244). The age range of patients enrolled in this study was between 30 and 86 years. Female/male ratios of groups 1, 2, and 3 were 21/2, 8/11, and 29/11, respectively. There was a significant difference in body mass index (BMI) among groups (p = 0.004). Group 1 and 2 had a significant difference in BMI (30.33 ± 5.60 vs. 25.02 ± 5.32, respectively, p = 0.003). There was no significant difference in Hb levels between group 1 and 2 (p = 0.22) but both groups’ Hb levels were lower than group 3 (p = 0.0001). Iron levels were different between all groups (p = 0.0001) and its levels were below normal in groups 1 and 2. Transferrin saturation [Fe/TIBC] in group 2 was different from groups 1 and 3 (p = 0.002 in both). It was also significantly low in groups 1 than group 3 (p = 0.0001) (Table 1).

As expected, the creatinine levels of the CKD patients were significantly higher compared to IDA patients and control subjects. HDL-C levels in CKD patients were significantly lower than groups 1 and 3 (p = 0.003). Total cholesterol, triglyceride, and LDL-cholesterol levels were not different between groups (p = 0.252, 0.527, and 0.14; respectively) (Table 1).
(p = 0.0001 in all). On the other hand, comparison of groups 1 and 2 showed that only ARE and PON/ARE ratio were significantly different in group 2 (p = 0.001) (Table 2).

The correlation analyses of total –SH, PON, ARE, PON/ARE, and other parameters are shown in Table 3. Total –SH, PON, and ARE levels were positively correlated with each other. CIMT was negatively correlated with PON and ARE levels, but not with total –SH level and PON/ARE ratio. CRP, hemoglobin and iron levels were correlated with total –SH, PON, and ARE levels, but not with PON/ARE ratio. LDL was correlated with PON level and PON/ARE ratio, while HDL was correlated with total –SH and ARE levels (for $r$ and $p$ values, refer Table 3).

### Discussion

Our study results, which are consistent with previous studies, showed that both PON and ARE activities are diminished in IDA patients and in anemic CKD patients when compared to healthy subjects. And our findings, which are also consistent with previous studies, showed that PON and ARE have negative correlations with CIMT. However, our study results also showed that PON activity was not different between IDA patients and anemic CKD patients, whereas ARE activity was significantly lower in anemic CKD patients than IDA patients. One reason could be that diminished PON activity in anemic CKD patients may be caused by only anemia, whereas decreased ARE activity in those patients may be caused by both anemia and other endogenous free radicals that may contribute the atherosclerotic process. This hypothesis also correlates with Kennedy et al.'s study. In that study, they have shown an association between ARE – not PON- and long-term adverse cardiovascular events in CKD patients. Although further studies are needed to clarify this point, we can speculate that because of anemic CKD patients have more risks than IDA patients, ARE – but not PON – can be used to predict atherosclerotic risks in those patients. ARE also had been shown as a good predictor for early atherosclerosis in other diseases. A study performed by Okuturlar et al., in which participants were diabetic and/or hypothyroidic patients, stated that ARE may be superior to brachial artery intima-media thickness in detecting early atherosclerosis in those patients.

Michalak et al. emphasized the importance and usefulness of PON/ARE ratio in predicting risk of ischemic stroke in their study. Therefore, we also tried to compare PON/ARE ratio between groups but we could not have determined a correlation between CIMT and PON/ARE ratio.

Also in our study, CIMT was significantly different only between CKD patients and healthy subjects. There was no significant difference between CKD patients and IDA patients. Also, there was no significant difference between healthy subjects and IDA patients even though CIMT was slightly higher in IDA patients than healthy subjects. This finding could be resulted from inadequate sample size. Also, there are controversial studies about the relationship between body iron status and CIMT. Kiechl et al. have stated in their study that iron stores were related to progression of carotid atherosclerosis, whereas other studies could not confirmed this finding. CIMT values of CKD group were significantly higher than the healthy controls ($p = 0.026$) (Table 2). This finding may also support the atherogenic role of ARE in CKD.

In our study, the female patients were less than male patients in group 2 when compared to groups 1 and 3. Sumegova et al. reported in their study that there were no differences in PON1 paraoxonase and PON1 arylerase activities and PON/ARE ratio between the genders ($p > 0.05$). In conclusion, PON activity was not different between IDA patients and anemic CKD patients, whereas ARE activity was lower in anemic CKD patients than IDA patients. These lowest ARE activity and concomitant higher CIMT values in CKD patients (in compare to the healthy controls) show that ARE activity may have a role in the pathogenesis of atherosclerosis in such patients.

### Table 3. Correlation analyzes between Total –SH, PON, ARE and other parameters.

|          | PON  | ARE  | CIMT | CRP  | Hb   | Hct  | Iron | LDL | HDL | Cr  |
|----------|------|------|------|------|------|------|------|-----|-----|-----|
| Total –SH| 0.539| 0.631| 0.225| 0.278| 0.603| 0.617| 0.426| 0.034| 0.262| 0.379|
| $r$ value| 0.0001| 0.0001| 0.058| 0.015| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001|
| $p$ values| 0.0001| 0.0001| 0.058| 0.015| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001|
| PON | 0.448| 0.307| 0.29 | 0.626| 0.625| 0.371| 0.449| 0.122| 0.253|
| $r$ value| 0.0001| 0.0001| 0.007| 0.009| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001|
| $p$ values| 0.0001| 0.0001| 0.007| 0.009| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001|
| ARE | 0.287| 0.236| 0.746| 0.781| 0.594| 0.073| 0.337| 0.602|
| $r$ value| 0.013| 0.034| 0.0001| 0.0001| 0.0001| 0.051| 0.002| 0.0001|
| $p$ values| 0.013| 0.034| 0.0001| 0.0001| 0.0001| 0.051| 0.002| 0.0001|
| PON/ARE | 0.115| 0.018| 0.124| 0.16| 0.11| 0.302| 0.193| 0.263|
| $r$ value| 0.328| 0.873| 0.268| 0.15| 0.335| 0.006| 0.082| 0.017|
| $p$ values| 0.006| 0.002| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001| 0.0001|

Bold values represent that $p$ values < 0.05 are statistically significant.

Total –SH: total thiol; PON: Paraoxonase; ARE: Arylesterase; CIMT: Carotid intima media thickness; CRP: C-reactive protein; Hb: hemoglobin; Hct: hematocrit; LDL: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein cholesterol; BUN: Blood urea nitrogen; Cr: Creatinine.
Further detailed studies are needed to confirm this issue.

Limitations

Small numbers of samples are one of the limitation of our study. Also we could not observe and analyze the effect of treatment (iron, erythropoietin, etc.) on these biochemical parameters.

Acknowledgements

We would like to thank Mr Bulent Altundal who gave us support in writing and English editing. Also we thank Mrs Feride Kalafat and Mrs Gulsun Can for their technical support.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Sarnak MJ, Tighiouart H, Manjunath G, et al. Anemia as a risk factor for cardiovascular disease in The Atherosclerosis Risk in Communities (ARIC) study. J Am Coll Cardiol. 2002;40:27–33.
2. Eschbach JW, Adamson JW. Anemia of end-stage renal disease (ESRD). Kidney Int. 1985;28:1–5.
3. Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: Results from the Third National Health and Nutrition Examination Survey. J Am Soc Nephrol. 2002;13:504–510.
4. Druke TB, Locatelli F, Clyne N, et al. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. N Engl J Med. 2006;355:2071–2084.
5. Jurkovitz C, Abramson J, McClellan WM. Anemia and cardiovascular and kidney disease. Curr Opin Nephrop Hypertens. 2006;15:117–122.
6. Phrommintikul A, Haas SJ, Elsk M, Krum H. Mortality and target haemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: A meta-analysis. Lancet. 2007;369:381–388.
7. Ritz E, Laville M, Bilous RW, et al. Target level for hemoglobin correction in patients with diabetes and CKD: Primary results of the Anemia Correction in Diabetes (ACORD) Study. Am J Kidney Dis. 2007;49:194–207.
8. Jurkovitz CT, Abramson JL, Vaccarino LV, Weintrob WS, McClellan WM. Association of high serum creatinine and anemia increases the risk of coronary events: Results from the prospective community-based atherosclerosis risk in communities (ARIC) study. J Am Soc Nephrol. 2003;14:2919–2925.
9. Brenner BM. Cardiovascular and renal progression factors in chronic kidney disease: A colloquium in honor of John H. Dirks ISN World Congress of Nephrology Singapore, June 26, 2005. Kidney Int. 2005;68:1411–1412.
10. Jun M, Lv J, Perkovic V, Jardine MJ. Managing cardiovascular risk in people with chronic kidney disease: A review of the evidence from randomized controlled trials. Ther Adv Chronic Dis. 2011;2:265–278.
11. Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res. 2005;46:1239–1247.
12. Watson AD, Berliner JA, Hama SY, et al. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. J Clin Invest. 1995;96:2882–2891.
13. Sanghara DK, Aston CE, Saha N, Kamboh MI. DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. Am J Hum Genet. 1998;62:36–44.
14. Schmidt H, Schmidt R, Niederkorn K, et al. Paraoxonase PON1 polymorphism leu-Met54 is associated with carotid atherosclerosis: Results of the Austrian Stroke Prevention Study. Stroke. 1998;29:2043–2048.
15. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol. 2001;21:473–480.
16. Mackness MI, Mackness B, Durrington PN, et al. Paraoxonase and coronary heart disease. Curr Opin Lipidol. 1998;9:319–324.
17. Onat A, Yuksel M, Koroglu B, et al. Turkish Adult Risk Factor Study survey 2012: Overall and coronary mortality and trends in the prevalence of metabolic syndrome. Turk Kardiyoloji Dernemi Arsi. 2013;41:373–378.
18. Ashlan M, Kosecik M, Horoz M, Selek S, Celik H, Erel O. Assessment of paraoxonase and arylesterase activities in patients with iron deficiency anemia. Atherosclerosis. 2007;191:397–402.
19. Prakash M, Phani NM, Kavya R, Supriya M. Paraoxonase: Its antiatherogenic role in chronic renal failure. Indian J Nephrol. 2010;20:9–14.
20. Saeed SA, Elsharkawy M, Elsaeed K, Fooda O. Paraoxonase-1 (PON1) activity as a risk factor for atherosclerosis in chronic renal failure patients. Hemodial Int. 2008;12:471–479.
21. Dantoine TF, Debord J, Charmes JP, et al. Decrease of serum paraoxonase activity in chronic renal failure. J Am Soc Nephrol. 1998;9:2082–2088.
22. Paragh G, Seres I, Balogh Z, et al. The serum paraoxonase activity in patients with chronic renal failure and hyperlipidemia. Nephron. 1998;80:166–170.
23. Salonen R, Salonen JT. Progression of carotid atherosclerosis and its determinants: A population-based ultrasound study. Arteriosclerosis. 1990;8:133–40.
24. Druke T, Witko-Sarsat V, Massy Z, et al. Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. Circulation. 2002;106:2212–2217.
25. Burke GL, Evans GW, Riley WA, et al. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke. 1995;26:386–391.
26. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of
stroke and myocardial infarction: The Rotterdam Study. *Circulation*. 1997;96:1432–1437.

27. Aycicek A, Erel O. Total oxidant/antioxidant status in jaundiced newborns before and after phototherapy. *J Pediatr*. 2007;83:319–322.

28. Gungor O, Kircelli F, Toz H. Paraoxonase 1, atherosclerosis and arterial stiffness in renal patients. *Int Urol Nephrol*. 2013;45:441–447.

29. Cece H, Yazgan P, Karakas E, et al. Carotid intima-media thickness and paraoxonase activity in patients with ankylosing spondylitis. *Clin Invest Med*. 2011;34:E225.

30. Kennedy DJ, Tang WH, Fan Y, et al. Diminished antioxidant activity of high-density lipoprotein-associated proteins in chronic kidney disease. *J Am Heart Assoc*. 2013;2:e000104.

31. Okuturlar Y, Mert M, Karakaya P, et al. Paraoxonase-1 activity in different patient groups with high risk of atherosclerosis. *Acta Med*. 2015;31:351.

32. Michalak S, Kazmierski R, Hellmann A, et al. Serum paraoxonase/arylesterase activity affects outcome in ischemic stroke patients. *Cerebrovasc Dis*. 2011;32:124–132.

33. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: Prospective results from the Bruneck study. *Circulation*. 1997;96:3300–3307.

34. Moore M, Folsom AR, Barnes RW, Eckfeldt JH. No association between serum ferritin and asymptomatic carotid atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol*. 1995;141:719–723.

35. Engberink MF, Geleijnse JM, Durga J, et al. Blood donation, body iron status and carotid intima-media thickness. *Atherosclerosis*. 2008;196:856–862.

36. Sumegová K, Blažiček P, Fuhrman B, Waczkliková I, Duračková Z. Paraoxonase 1 (PON1) and its relationship to lipid variables, age and gender in healthy volunteers. *Biologia*. 2006;61:699–704.