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Determination of Paraoxonase 1 Activity and Phenotype Distribution in Cervical Disk Herniation Patients

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ABSTRACT

Objectives: Cervical disk herniation (CDH) is a common disease that usually develops as a result of intervertebral disk degeneration (IVDD) or trauma, which can cause pain or neurological deficiency by nevre root or spinal cord compression. Paraoxonase 1 (PON1) has antioxidant qualities, and its function may vary based on genetic variations and ethnic background. This study aims to compare PON1 activity and phenotype distribution in CDH patients and individuals without the condition. **Materials and Methods:** This study involved 70 CDH patients and 70 individuals in good health. Spectrophotometric tests were conducted to measure the serum PON1 and arylesterase (ARE) activities. The PON1 ratio, which indicates the salt-stimulated PON/ARE level, showed a three-peak distribution. This ratio was utilized to determine the various phenotypes; QQ, QR, and RR for each participant.**Results:** The PON1 activity was lower in CDH patients compared to the healthy individuals ($p < 0.05$). CDH patients exhibited a statistically significant QQ phenotype in comparison to the healthy participants ($p < 0.05$).**Conclusion:** Patients with CDH exhibited significantly reduced PON1 activity, indicating that low PON1 activity and the PON1 QQ phenotype could potentially be a risk factor for the development of CDH.

Keywords: Cervical disk herniation, Paraoxonase, Phenotype, PON1.

Servikal Disk Herniasyonlu Hastalarda Paraoksonaz 1 Aktivitesinin ve Fenotip Dağılımının Belirlenmesi

ÖZ

Amaç: Servikal disk herniasyonu (SDH), genellikle intervertebral disk dejenerasyonu (IVDD) veya travmanın bir sonucu olarak gelişen yaygın bir hastalıktır ve sinir kökü veya omurilik sıkışması nedeniyle ağrı veya nörolojik bozukluklara yol açabilir. Paraoksonaz1 (PON1), antioksidan özelliklere sahiptir ve genetik varyasyonlara ve etnik kökene bağlı olarak fonksiyonu değişebilir. Bu çalışmanın amacı, servikal disk herniasyonlu hastalar ve sağlıklı bireylerde PON1 aktivitesini ve fenotip dağılımını karşılaştırmaktır. **Gereç ve Yöntem:** Bu çalışmada 70 SDH hastası ve 70 sağlıklı birey yer aldı. Serum PON1 ve arilesteraz (ARE) aktivitelerini ölçmek için spektrofotometrik testler yapıldı. Tuz uyaranlı PON/ARE seviyesini gösteren PON1 oranı, üç tepe dağılımını gösterdi. Bu oran, her katılımcı için farklı fenotipleri belirlemek için kullanıldı; QQ, QR ve RR. **Bulgular:** PON1 aktivitesi, SDH hastalarında sağlıklı bireylere göre daha düşüktü ($p <0.05$). SDH hastaları, sağlıklı katılımcılara göre istatistiksel olarak anlamlı bir QQ fenotipi sergiledi ($p <0.05$). **Sonuç:** SDH hastalar, belirgin şekilde azalmış PON1 aktivitesi sergiledi, bu da düşük PON1 aktivitesinin ve PON1 QQ fenotipinin potansiyel olarak SDH gelişimi için bir risk faktörü olabileceğini göstermektedir.

Anahtar Kelimeler: Servikal disk herniasyonu, Paraoksonaz, Fenotip, PON1.

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INTRODUCTION

Cervical disk herniation (CDH) is a common disease that usually develops as a result of intervertebral disk degeneration (IVDD) or trauma, which can cause pain or neurological deficiency by nevre root or spinal cord compression. Recent investigations have demonstrated that specific inflammation, in addition to mechanical problems, is an important cause of disk degeneration and disk herniation (Bailey & Badgley, 1960; Boger et al., 1986). It has been shown that there are changes in inflammatory enzyme levels in the blood in cases of cervical disc herniation (Ethemoğlu et al., 2020). While oxidative stress is known to cause chronic inflammation, we have limited information about its effect on disk degeneration and CDH.

Paraoxonase 1 (PON1, EC.3.1.8.1) and arylesterase (ARE) are two enzymes that derive from the same gene and possess analogous active sites. PON1 an antioxidant enzyme linked to plasma high-density lipoproteins (HDL), has been shown to protect low-density lipoprotein (LDL) and HDL from oxidation by free radical sand reduce oxidative stres (Draganov et al., 2004; Balcı et al., 2004). The PON1 enzyme is found in many tissues and serums, including liver, kidneys, thin cartilage and the brain primarily, where the activity of the enzym is influenced by genetic and environmental factors (Sarioglu et al., 2015). PON 1 was associated with intervertebral disk degeneration (Chen et al., 2019).

Prior research has explored the PON1 activity in individuals with CDH, yet the PON1 phenotypes and their influence on CDH development are still undetermined. This research investigated the occurrence and activitiy of PON1 phenotypes in CDH patients and a control group. Three distinct PON1 phenotypes were identified by comparing the PON and ARE activities of PON1

MATERIALS AND METHODS

Study groups.

This study involved 70 patients diagnosed with CDH (mean age: 45.48 ±6.5 years, age range: 25–70 years) who were treated at the Balıkesir University Neurosurgery Department, along with 70 healthy individuals. The inclusion criteria for CDH patients encompassed ipsilateral radicular pain and MRI confirmation of extruded or sequestered CDH. Exclusion criteria for subject selection included degenerative spondylolisthesis, ossified posterior longitudinal ligament availability, the presence of accompanying inflammatory diseases such as infectious diseases and autoimmune disorders, neoplastic diseases, familial hypercholesterolemia, as well as liver, pulmonary, kidney, and heart diseases. Smoking habit was also an exclusion criterion.

The control group for this study comprised 70 healthy individuals (mean age: 42.26 ± 5.6 years, age range: 24–70 years) with no history of CDH or radicular pain, and who did not meet any of the exclusion criteria. Consistent strenuous manual labor and heavy lifting are recognized as risk factors for CDH in humans. To ensure a valid comparison, we selected a control group consisting of individuals with similar lifestyles and work statuses as the CDH patients.

Blood samples

Venous blood samples (6 mL) were obtained from 70 CDH patients and 70 control subjects who had fasted overnight. The samples were then centrifuged (10 min at 3,000 xg), and the resulting sera were stored in tubes at −30°C until further analyses.

Measurement of PON1 and ARE activities

The PON1 enzyme activity was evaluated using the method established by Eckerson et al. (Eckerson et al., 1983). This method involved measuring the hydrolysis rate of PON1 spectrophotometrically by monitoring the increase in absorbance at 412 nm resulting from the generation of p-nitrophenol, using paraoxon (p-nitrophenyl phosphate, Sigma Chemical Co.) as a substrate at 37°C for 1 minute. The PON1 enzyme activity was determined based on the molar extinction coefficient of 17100 M−1 cm−1. One unit (U) of PON activity was defined as 1 µmol of p-nitrophenol formed in 1 minute. Fresh paraoxon substrate was prepared daily. For phenotype distribution, PON catalysis was determined in a pH 10.5 phosphate buffer containing 1M NaCl.

The activity of ARE was evaluated through spectrophotometric analysis, which involved measuring the rise in absorbance at 270 nm for a duration of 1 minute at 25°C, using a phenylacetate substrate. The enzyme activity was determined using a molar extinction coefficient of 1310 M−1 cm−1. One unit (U) of ARE activity was defined as the formation of 1 µmol of phenylacetate in 1 minute. . Fresh phenylacetate substrate was prepared on a daily basis.

PON1 phenotype distribution

The dual-substrate technique was employed to establish the phenotypic distribution of PON1 (La Du & Eckerson, 1984). The PON1 enzyme activity is determined by the 192 Q/R polymorphism, which affects the phenotypic distribution of the enzyme. Individuals with the Q allele exhibit lower PON1 enzyme activity compared to those with the R allele. Blood paraoxon hydrolysis capacity is used to assess PON1 enzyme activity, which reflects the 192 Q/R polymorphism and changes in the concentration of the PON1 enzyme.

The genetic polymorphism at codon 192Q/R exists in two forms: Q (low activity) and R (high activity). The ratio of PON catalysis in a 1 M NaCl-containing buffer to phenylacetate catalysis was utilized to determine the presence of the three phenotypes (QQ, QR, and RR). The paraoxon hydrolysis activity associated with the R allele of PON1 is eight times greater than that associated with the Q allele. The PON Q/R polymorphism has been shown to impact serum concentration and enzyme activity.

A single alteration in the amino acid sequence determines the enzyme's structure and its activity. Individuals with the Q (glutamine) rather than R (arginine) at position 192 exhibit reduced serum PON

enzyme activity. Homozygous carriers of the R allele have a higher enzyme concentration compared to homozygous Q individuals. The R allele is linked to elevated PON activity, while the Q polymorphism is associated with lower PON activity. Q/Q is correlated with reduced activity, whereas R/R and Q/R are linked to heightened activity.

The cut-off values for the various phenotypes are as follows: low enzyme activity, QQ type, ratio<3.0; moderate enzyme activity, QR type, ratio 3.0–7.0; high enzyme activity, RR type, ratio >7.0 (La Du &Eckerson, 1984)

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the parametric variables. A comparison of the patients' ages between the two study groups was conducted using an independent sample t-test. The gender distribution among the groups was assessed using a Chi-square test. PON1 and ARE activities in the CDH and control groups were compared using the Mann–Whitney U-test. The distribution of PON1 phenotypes in patient and control subjects was also evaluated using a Chi-square test. Statistical significance was determined using a significance level of $p< 0.05$. All statistical analyses were conducted using SP-SS 20.0 statistical software.

Ethical considerations

The study received approval from the Balıkesir University Clinical Research Ethics Committee (Decision No. 2022/93, Date: 07/09/2022). All CDH patients and control individuals provided verbal and written consent before participating in the study.

RESULTS

The research involved 70 individuals with CDH and 70 healthy participants. Table 1 presents the age, gender, and clinical characteristics of the CDH and control groups.

Table 1. Clinical parameters of subjects.

Variable	CDH (n=70)	Control (n=70)	*P**
Sex (M/F)	33/37	32/38	>0.05
Age	45.48±6.5	42.26±5.6	>0.05
CDH Level			
C4-C5			8
C5-C6			35
C6-C7			27

* Independent sample t test, chi-square test , *p < 0.05, statistically significant.

There were no statistically significant variances in the mean age and gender distribution between the CDH patients and the healty group, and none of the participants met the exclusion criteria.

The PON1 activity in CDH patients was significantly lower compared to that of the control subjects (26.27 ± 10.7 vs. 61.17 ± 12.8 IU) ($p < 0.05$).

Table 2. Biochemical parameters of subjects.

Parameters	CDH (n=70)	Control (n=70)	*P value
PON1 activity (U ml^{-1})	26.27 ± 10.7	61.17 ± 12.8	p<0.05
ARE activity (U ml^{-1})	84.78 ± 12.4	96.25 ± 14.3	p>0.05

*Mann-Whitney U test, PON1: Paraoxonase, ARE: Arylesterase *p < 0.05, statistically significant

Table 3. PON1 phenotype distribution in CDH and control groups.

		Phenotypes			Total
		QQ	QR	RR	
Groups	CDH	37 (52.9%)	16 (22.9%)	17 (24.2%)	70 (100%)
	Control	19 (27.1%)	28 (40%)	23 (32%)	70 (100%
Total		56 (40%)	44 (31.4%)	40 (28.6%)	140 (100%)

*Chi-square test, QQ phenotype distribution was more common in CDH group than in control group *(p<0.05)*

Additionally, the ARE activity was assessed to determine the phenotype frequency in both the CDH and control groups, respectively (84.78 ± 12.4 vs. 96.25 ± 14.3 IU) ($p > 0.05$) (Table 2). CDH patients exhibited a notably higher frequency of the QQ phenotype in comparison to the control subjects, and this difference was statistically significant ($p < 0.05$) (Table 3) (Figure 1).

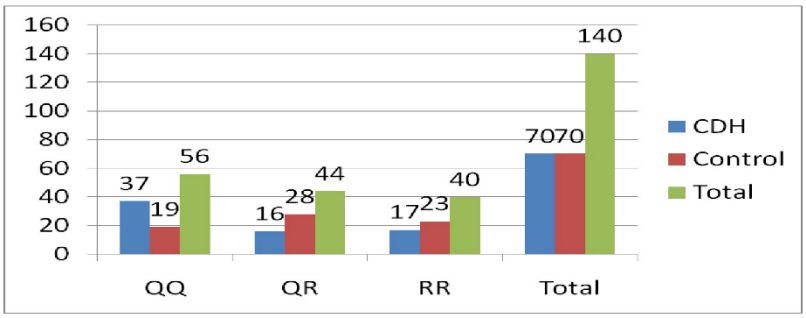

Figure 1. PON1 phenotype distribution in CDH and control groups.

DISCUSSION

Cervical disk herniation is a prevalent condition associated with neck pain, stemming from IVDD, and it is a primary cause of radiculopathy in adults. Disc degeneration is a process characterized by biochemical, vascular and anatomical changes in the intervertebral disk, where extrinsink, intrinsink and genetic factors play a role. Although the underlying physio pathological mechanisms have not been fully clarified, degenerative cervical disc disease is an anatomical adaptation process of the spine to load and mechanical stress, and is characterized by clinical syndrome ssuch as disc hernia or spondylosis. Cervical spondylosis is a term generally used to describe developing vertebral changes in the degenerative back ground, and cervical disk herniation is often part of this degenerate phenomen on (Borovkova et al., 2017). Past studies have identified that inflammatory reactions and oxidative stress are the leading causes of extracellular matrix degradation and a reduction in nucleus pulposus cells. Alleviating inflammatory responses and oxidative stress could potentially mitigate the advancement of IVDD and CDH (Chen et al., 2019).

The most important production site of PON1 in the body is the liver and it is distributed throughout the body from there (Deakin et al., 2002). PON1 activity may be impacted by smoking and dietary habits (Mouhamed et al., 2012). Research suggests that the consumption of butter, certain fruits, and lifestyle factors such as moderate alcohol consumption can elevate PON1 activity (Costa et al., 2011). The study excluded individuals using medication for atherosclerosis, diabetes mellitus, coronary heart disease (Mirdamadi et al., 2008), hypertension, rheumatoid arthritis, neurological, liver conditions. Participants with a history of cancer or hepatitis, and those using antipsychotic, serum lipid-lowering, cigarette, and antioxidant medications were also not included in the study.

Several research studies have documented the anti-inflammatory or antioxidant characteristics of PON1. Recent research has found that inflammation and oxidative stress can change the microenvironment of nucleus pulposus cells, resulting in IVDD. (Risbud & Shapiro, 2014; Dimozi et al., 2015). In addition, multiple research studies have explored the association between PON1 phenotype and various disease (Zhao et al., 2012; Bassu et al., 2023; Ayan et al., 2019). While there are investigations into the link between PON1 activity and IVDD or LDH (Chen et al., 2019; Karabağ & Sezen, 2016), no study has specifically examined the relationship between PON1 phenotype distribution and CDH.

Chen et al. reported that PON1 expression is indicative of severe IVDD; PON1 has been demonstrated to have a crucial role in preserving the homeostatic balance of intervertebral discs. (Chen et al., 2019). Chen et al demonstrated a robust correlation between the anti-inflammatory and antioxidant characteristics of PON1 and the process of IVDD. Our findings indicate that PON1 expression is considerably diminished in severely degenerated cervical disks, and there is an inverse relationship between PON1 expression and IVDD.

Chen et al. (Chen et al., 2019) conducted a study to explore the role of PON1 in the gene expression process related to IVDD. They identified several variations in the PON1 gene, including two common polymorphisms at amino acid codons 55 and 192 within the coding region. Additionally, they identified five single nucleotide polymorphisms in the promoter region at positions −108, −126, −162, −832, and −909. The researchers used PCR analysis to investigate the relationship between IVDD and PON1 in their study. On the other hand, Karabag et al. (Karabağ and Sezen, 2016) focused on examining the association between PON1 and LDH. They also investigated the correlation between levels of lipid hydroperoxide (LOOH), total oxidative status, total antioxidative status markers, and LDH. The authors utilized the Eckerson method (Eckerson et al., 1983) to measure PON1 activity. However, they did not perform phenotype

classification and did not compare the relationship with LDH.

In our study, we compared the PON1 activity between the patient and control groups using the Gan method. This involved measuring the increase in absorbance at 412 nm using paraoxon as a substrate. Additionally, we determined the PON1 phenotype distribution in both groups using the dual-substrate method. This method utilizes phenyl acetate and paraoxon as substrates, and the equation provided in the material and methods section was used for calculation. It is worth noting that no previous study has examined the correlation between PON1 phenotype distribution and CDH using the method proposed in our study.

Therefore, we hypothesized that there may be a relationship between PON1 activity, ARE activity/phenotype distribution, and CDH disease. In our study, we compared the activities of PON1 and ARE, as well as the phenotype distribution of PON1, between individuals with CDH and healthy individuals. PON1 is known for its anti-inflammatory and anti-oxidative properties (Borovkova et al., 2017; Furlong et al., 2016; Mackness & Mackness, 2015). Our findings showed that CDH patients had significantly lower PON1 activity compared to control individuals, suggesting a link between low PON1 activity and CDH. Furthermore, we conducted a comparison of the ARE activity in order to assess the distribution of PON1 phenotype between individuals with CDH and those who are healthy.. We measured and compared the three PON1 phenotypes (QQ, QR, RR) in CDH and healthy subjects, and observed that the CDH group had a significantly higher frequency of the QQ phenotype compared to the control group. .This study is the first to investigate PON1 phenotypes in CDH, and our findings demonstrate that the CDH group has a significantly higher frequency of the QQ phenotype compared to control subjects.

CONCLUSION

Our research revealed that the CDH patient group exhibited reduced PON activity compared to the control group, and the Q allele was more prevalent in the CDH patient group. Previous literature has indicated that individuals with Q alleles have diminished PON1 activity, which corroborates our findings. In addition, our study revealed a significantly higher prevalence of the QQ phenotype in the CDH group compared to the control group.However, further investigation across multiple centers is required to validate whether the QQ phenotype is a risk factor for CDH disease. One of the strengths of our research is that it is the first to investigate PON1 phenotypes in both individuals with CDH and those who are healthy. Nevertheless, it is crucial to acknowledge that this research was carried out at a solitary facility with a restricted number of participants, thus restricting the applicability of our results

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Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: UA, SK; **Material, methods and data collection:**UA, SK, NG; **Data analysis and comments:** NG, KÇ; **Writing and corrections:**UA, SK.

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Ethical considerations

The study received approval from the Balıkesir University Clinical Research Ethics Committee (Decision No. 2022/93, Date: 07/09/2022). All CDH patients and control individuals provided verbal and written consent before participating in the study**.**

REFERENCES

Ayan, D., Senes, M., Caycı, A. B., Söylemez, S., Eren, N., Altuntas, Y., & Oztürk, F. Y. (2019). Evaluation of paraoxonase, aryylesterase and homocysteine thiolactonase activities in patients with diabetes and incipient diabetic nephropathy. *Journal of Medical Biochemistry*, *38*, 481–488. https://doi.org/10.2478/jomb-2019-0014

Bailey, R. W., & Badgley, C. E. (1960). Stabilization of the cervical spine by anterior fusion. *Journal of Bone and Joint Surgery*, 42, 565-59

Balcı, Ö.E., Donma, O., & Ekmekçi, H. (2004). Paraokonaz. *Cerrahpaşa Tıp Derg*, *35*, 78-82.

Bassu, S., Mangoni, A. A., Argiolas, D., Carru, C., Pirina, P., Fois, A. G., & Zinell, A. (2022). A systematic review and meta-analysis of paraoxonase 1 activity in asthma. *Clinical and Experimental Medicine*, *23*, 1067–1074. https://doi.org/10.1007/s10238-022-00930-0

Boger, D. C. (1986). Traction device to improve CT imaging of lower cervical spine. *AJNR*, *7*, 719.

Borovkova, E. I., Antipova, N. V., Komeenko, T. V., Shakparonov, M. I., & Borovkov, I. M. (2017). Paraoxonase: The universal factor of antioxidant defense in human body. *Vestn Ross Akad Med Nauk*, *72*(1), 5–10. https://doi.org/10.15690/vramn764

Chen, F., Liu, H., Li, Z., Pei, Y., Wang, H., Zhang, J., Wang, J., & Zheng, Z. (2019). Paraoxonase 1 was negatively associated with intervertebral disc degeneration. *Spine (PhilaPa 1976)*, *44*(18), E1053-E1062. https://doi.org/10.1097/brs.0000000000003059

Costa, L. G., Giordano, G., & Furlong, C. E. (2011). Pharmacological and dietary modulators of paraoxonase 1 (PON1) activity and expression: the hunt goes on. *Biochem Pharmacol*, *81*(3), 337-344. https://doi.org/10.1016/j.bcp.2010.11.008

Deakin, S., Leviev, I., Gomaraschi, M., Calabresi, L., Francesshini, G., & James, R. W. (2002). Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high-affinity, saturable, desorption mechanism. Journal of Biological Chemistry, *277*, 4301–4308. https://doi.org/10.1074/jbc.M107440200

Dimozi, A., Mavrogonatou, E., & Sklirou, A. (2015). Oxidative stress inhibits the proliferation, induces premature senescence and promotes a catabolic phenotype in human nucleus pulposus intervertebral disc cells. *European Cell Materials*, *30*, 89-102. https://doi.org/10.22203/ecm.v030a07

Draganov, D. I., & La Du, B. N. (2004). Pharmacogenetics of paraoxonases: A brief review. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *369*, 78-88. https://doi.org/10.1007/s00210-003-0833-1

Eckerson, H. W., Wyte, C. M., & La Du, B. N. (1983). The human serum paraoxonase/arylesterase polymorphism. *American Journal of Human Genetics*, *35*, 1126-1138.

Ethemoğlu, K. B., & Erkoç, Y. S. (2020). Is there any relationship between cervical disc herniation and blood inflammatory response? *Cureus*, *12*(8), e10161. https://doi.org/10.7759/cureus.10161

Furlong, C. E., Marsillach, J., Jarvik, G. P., & Costa, L. G. (2016). Paraoxonases-1, -2, and -3: What are their functions? *Chemical Biology & Interactions*, *259*, 51-62. https://doi.org/10.1016%2Fj.cbi.2016.05.036

Karabağ, H., & Sezen, H. (2016). The relation of lumbar disc herniation with increased lipid hydroperoxide, paraoxonase 1, and total oxidative status. *Crescent Journal of Medical and Biological Sciences*, *3*(3), 86-90. https://sid.ir/paper/344940/en

La Du, B. N., & Eckerson, H. W. (1984). The polymorphic paraoxonase/arylesterase isozymes of human serum. *Federation Proceedings*, *43*(8), 2338-2341.

Mackness, M., & Mackness, B. (2015). Human paraoxonase-1 (PON1): Gene structure and expression, promiscuous activities and multiple physiological roles. *Gene*, (1), 12–21. https://doi.org/10.1016/j.gene.2015.04.088

Mirdamadi, H. Z., Sztanek, F., Derdak, Z., Seres, Z., Seres, I., Harangi, M., & Paragh, G. (2008). The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters. *British Journal of Clinical Pharmacology*, *66*(3), 366-374. https://doi.org/10.1111/j.1365-2125.2008.03213.x

Mouhamed, D., Ezzaher, A., Mechri, A., Neffati, F., Omezzine, A., Bouslama, A., Gaha, L., Douki, W., & Najjar, M. (2012). Effect of cigarette smoking on paraoxonase 1 activity according to PON1 L55M and PON1 Q192R gene polymorphisms. *Environmental Health and Preventive Medicine*, *17*(4), 316–321. https://doi.org/10.1007%2Fs12199-011-0256-4

Risbud, M. V., & Shapiro, I. M. (2014). Role of cytokines in intervertebral disc degeneration: Pain and disc content. *Nature Reviews Rheumatology*, *10*(1), 44-56. https://doi.org/10.1038/nrrheum.2013.160

Sarioglu, N., Hismiogullari, A. A., Erel, F., Demir, D., & Gencer, D. (2015). Paraoxonase 1 phenotype and paraoxonase activity. *Iranian Journal of Allergy, Asthma, and Immunology*, *14*(1), 60-66.

Zhao, Y., Ma, Y., Fang, Y., Liu, L., Wu, S., Fu, D., & Wang, X. (2012). Association between PON1 activity and coronary heart disease risk: A meta-analysis based on 43 studies. *Molecular Genetics and Metabolism*, *105*, 141–148. https://doi.org/10.1016/j.ymgme.2011.09.018