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Paraoxonase1 Activity and Its Phenotype in Untreated Hyperthyroid Malay Patients

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Abstract— Paraoxonase 1 (PON 1) is an enzyme synthesized in the liver and is associated with high density lipoprotein (HDL) in the systemic circulation. It is an antioxidant in which prevents oxidative stress and inflammation.Since oxidative stress had been proven to be the complication of hyperthyroidism due to increase metabolic rate, this study is trying to look for the association between PON1 activities and phenotype in untreated hyperthyroid Malay patients. A total of 107 Malays subjects consisting of 55 untreated hyperthyroid patients (TSH<0.01 mU/L) and 52 healthy subjects were included.. The phenotype of the serum was determined by using the ratio of salt stimulation PON 1 activity to the arylesterase activity. The median difference of basal PON 1 activities level between untreated hyperthyroid compared to the healthy control was not statistically significant (p=0.23). There is no correlation between PON 1 activity and TSH (r=0.18, p=0.899) and free T4 (p=0.130). The most common PON 1 phenotype was AB followed by BB and then AA phenotype in Malay population. There is no significant correlation between free T4 and TSH with PON 1 activity in untreated hyperthyroid hence, PON 1 activity is not affected by thyroid status. AB is the most common phenotype in Malay population followed by BB phenotype in Malay population in Kelantan.

Keywords: oxidative stress, hyperthyroid, paraoxonase1, Malay, phenotype

1.Introduction

Hyperthyroidism is one of the commonest endocrine diseases in Malaysia and worldwide. In general, prevalence of overt hyperthyroidism ranges from 0.2% to 1.3% in iodine-sufficient parts of the world [1]. Based on the Endocrine Disorders in Malaysia: Thyroid (MyENDO:Thyroid) Study' initiated by the Malaysian Endocrine Metabolic Society (MEMS) in 2014 which was completed in 2016, the prevalence of overt and subclinical hyperthyroidism being 0.6% and 2.8% respectively in Malaysia[2]. Overproduction of thyroid hormones in hyperthyroidism leads to hyper metabolic states and increased in oxygen consumption leading to increase in production of superoxide and free radical production with consumption of antioxidants to neutralise this effect. Thus, the balance between free radicals and antioxidantswill be disrupted as the disease progress, resulting in oxidative stress andincrease in lipid peroxidation[3-6].

Paraoxonase or aryldialkylphosphatase is a family of three enzymes namely PON 1, PON2 and PON 3.The genes encoding this proteinare in the long arm of chromosome 7. They are located next to each other and share extensive structural homology[7]. Paraoxonaseis synthesized in the liver and once in serum, it will bind to the high-density lipoprotein(HDL) molecules and the activity depends on presence of calcium ion. It was initially discovered due to its ability to detoxify organophosphate toxic metabolite paraoxon and hence the name paraoxonase[8,9]. PON 1 is the most studied among the threebecause of the finding by Mackneset al. that describe the role of HDL associated PON1in reducing lipid peroxidation on LDL, inhibiting LDL oxidation and protecting against atherosclerosis and cardiovascular disease[10-15].

PON1 activity in plasma can be affected by genetic and environmental factors[14]. Environmental factors such as lifestyle, nutritionand drugs (for instance, statins, fats and fatty acids, antioxidant vitamins, nutrients rich in polyphenols content) can affect the circulatory level. In addition, cigarette smoking, acute phase proteins, pregnancy and diet may affect serum PON1 activity aswell [15]. Serum PON 1 activity in proatherogenic diet showed a significant decreasewhile flavonoid antioxidants seem to elevate enzyme activity by as much as 20% [16].To date, PON1 activities has been shown to be reducedin many diseases such as ulcerative colitis, Crohn's disease, chronic renal failure, SLE, Behcet's disease, cancer, Hepatitis B, obesity, metabolic syndrome, Alzheimer's and dementia [17].

Despite of many factors affecting PON 1, the biggest effect on PON 1 activities levels is the genetic polymorphism[14]. Since the discoveries, there were various outcomesobserved between individual with the same duration of exposure to organophosphate. This led to the finding of different polymorphisms in PON1gene betweendifferent individuals. Over 160 polymorphisms have been described so far and one of the polymorphismsis in the codon 192 involving substitution of alanine, (Q or A allele) and glutamine, (R or B allele) where Q allele is more efficient in reducing lipid peroxidation content, thus showing higher antioxidant properties [18].As a result, three phenotypes exist which areAA/QQ, AB/ QR and BB/RR. The Q192R polymorphism is regarded as the chief biomarker of oxidant status, where LDL oxidation is prevented the most in AA/QQ, homozygous patients and the least in BB/RR patients [19].

To date, the exact role of PON1 is still not established and various research have been carried out to explore this promising antioxidant in various clinical conditions associated with oxidative stress. This study was carried out to investigate for the possible association between PON1 activity and in patient with untreated hyperthyroidism. Since PON 1 activity varies between different population, race or ethnic, therefore we decided to observe the activity in Malay population one of the major races in Kelantan.

2. Materials and Methods

2.1 Subjects

This was a case control study which was conducted in Hospital Universiti Sains Malaysia (HUSM), Health Campus, Kelantan to compare PON 1 activity and phenotype between Malay untreated hyperthyroid patient and healthy control. 55 untreated hyperthyroid patients and 52 healthy controls were recruited as subjects.Any Malay patientswho were biochemically hyperthyroiddefined as thyroid stimulating hormone (TSH) less than 0.01 mU/L according to the American Thyroid Association guidelines in 2016, not on any thyroid treatment or defaulted treatment for more than two months, aged above 18 years old that attended outpatient clinic, accident and emergency or admitted in HUSM were recruited as subjects in this study. If the patient was already on treatment for hyperthyroid, pregnant or taking oral contraceptives, smoker, alcoholics, has other medical condition that affect PON 1 activity such as dyslipidaemia, diabetes mellitus (DM), ischaemic heart disease or cerebrovascular accident, and taking medication such as statin or fibrates, were excluded from this study. For healthy control, any voluntarily healthy adults aged 18 years or above, clinically euthyroid, were recruited. Those who smoked, drinks alcohol, obese (BMI>35) and has mixed ethnicity (three previous generation had mixed ethnicity marriage) were excluded. Fivemls of fasting blood sample were drawn once patient has consented. The sample were separated immediately and analysed in batches forfree T4, TSH, PON 1 activity, salt stimulated PON 1, arylesterase and phenotype.

2.2 Biochemical Measurements

Serum thyroxine (fT4) were determined using Cobas analyser e601 using the principle of sandwich immunoassay and serum TSH was determined using the same analyser with the principle of competitive immunoassay.

PON1activities were measured using method described by Eckerson et al. in 1983[20]. The rate of hydrolysis of paraoxon or the rate of formation of p-nitrophenol product of hydrolysis of paraoxon was directly related to the increased in absorbance and paraoxonase activity and measured using spectrophotometer (Shimadzu UV 1800).

Basal activity was determined by mixing calcium chloride, glycine buffer, distilled water, diluted serum (enzyme) and paraoxon (substrate, Sigma Chemical). Nonenzymatic hydrolysis was determined by replacing the enzyme with distilled water. Following the addition of serum, assays were mixed well and the absorbance of p-nitrophenol at 25 degrees Celsius were read at 412 nm wavelength using spectrophotometer and further increased in absorbance were monitored every 1 minute for 4 minutes and the average absorbance per minute will be calculated (A/min).One unit

(U) of PON 1 activity was equal to one micromol of p-nitrophenol formed per minute. Enzyme activity was calculated from the molar absorptivity coefficient, 18290 $M^{-1}cm^{-1}$ (20).

$$\text{Enzyme activity} = \frac{\text{A/min X 1 ml (assay volume)}}{18290\ M^{-1}cm^{-1}\ \text{X}\ 10^{-6}\ \text{X 0.005 ml (sample volume)}}$$

$$= \text{A/min X 10935 nmol/ml}$$

PON 1 phenotype was determined using the ratio of salt stimulated PON 1 activity to the arylesterase activity based on a method described by Eckerson et al. 1983b[21]. Salt stimulated PON 1 activity was determined by adding sodium chloride before adding serum and PON 1. Arylesterase activity was determined by adding calcium chloride, Tris/HCL buffer, distilled water, serumfollowed by phenylacetate. The rate of hydrolysis of phenylacetate was determine at 270 nm, 25 degrees Celsius, using spectrophotometer and further readings were recorded continuously for 2 minutes at one-minute interval. The average of absorbance per minute (A/min) was calculated. The nonezymatic hydrolysis was determined using the same steps but without the addition of serum. One unit (u) of arylesterase activity was defined as one umol of phenylacetate hydrolysed per minute and arylesterase activity was normally expressed as units per ml of serum (U/ml). Enzyme activity was calculated from the molar extinction coefficient, 1310 $M^{-1}cm^{-1}$[21].

$$\text{Enzyme activity} = \frac{\text{A/min X 3 ml (assay volume)}}{1310\ M^{-1}\ \text{X}\ 10^{-3}\ \text{X 0.005 ml (sample volume)}}$$

$$= \text{A/min X 458 µmol/ml}$$

The phenotype of the serum was determined by using the ratio of salt stimulationparaoxonase activity to the arylesterase activity. Based on the ratio calculated, the phenotypes were determined according to the ranges 0.9 -2.5 for AA phenotype, 2.6-7.5 for AB phenotype and 7.6 -12 for BB phenotype published by Adkins et al. , 1993[22].

$$\text{Ratio} = \frac{\text{Paraoxonase activity with 1 M Nacl}}{\text{Arylesterase activity}}$$

2.3 Statistical Analysis

Data analysis was performed using Statistical Package for Social Science (SPSS) version 24. Descriptive statistics was used to summarise the socio-demographic characteristics of subjects and to check for distribution of the data. Numerical data were expressed as median + interquartile range(IQR) for skewed data. Categorical data were expressed as frequency and percentage.

The study was conducted with the approval of the Human Research Ethics Committee USM (HREC: USM/JEPeM/17120730).

3. Results

A total of 107 Malay adult were recruited consisting of 55untreated hyperthyroid patientsand 52 healthy controls. In the untreated hyperthyroid patients, there were 29 female and 26 males while in the healthy control, 24 were male and the remaining 28 subjects were female. For untreated hyperthyroid patients, the age ranged was from eighteen to seventy years old with mean (±standard deviation, SD) age of 41.16(±14.73). In healthy controls, the age ranged from eighteen to fifty-nine years old with mean (±standard deviation, SD) age of 32.77 (±9.80). General characteristic of study subjects and the descriptive statistics of the findings were summarized in Table 1.

Table1: General characteristic and summary of findings.

		Untreated hyperthyroid Patients (n=55)	Healthy Control (n=52)
Age (years)		41.16(±14.73)	32.77 (±9.8)
Sex	Male	26(47.4%)	24(46.2%)
	Female	29 (52.7%)	28 (53.8%)
Basal (U/ml)	Paraoxonase	290.13(±72.65)	278.84 (±80.9)
Salt stimulated (U/ml)		426.32(±114.66)	384.53 (±112.72)
Arylesteraseactivity (U/ml)		78.06(±24.05)	72.64 (±14.73)

The median (±interquartile range) basal PON1 activity in the healthy controls was 247.13(±86.39) U/ml and was slightly lower than the untreated hyperthyroid patients, 274.47(±94.594) U/ml. Salt stimulated PON1 activity shows similar findings where the median (±interquartile range) was higher in the untreated hyperthyroid patient 441.77 (±191.91) U/ml compared with the healthy controls365.83(±221.80) U/ml. The median (±interquartile range) for arylesterase activity, 78.06(±24.05)U/ml was also slightly higher than the healthy subjects 72.64 (±14.73) U/ml.

Table 2 : Basal PON 1, Salt stimulated PON 1 and arylesterase activity between untreated hyperthyroid patients and the healthy controls

	Median (IQR)		Z statistic	P value
	Untreated hyperthyroid	Healthy control		
Basal (U/ml)	274.47(±94.59)	247.13(±86.39)	-1.199	0.230
Salt-stimulated (U/ml)	441.77 (±191.91)	365.83(±221.80)	-1.749	0.080

Arylesterase (U/ml)	68.24 (±39.79)	70.30 (±40.42)	-1.193	0.233

IQR: interquartile range, p < 0.05 considered as significant

There was no significance difference between the median (interquartile range) basal PON activities, 274.47(±94.594)U/ml in untreated hyperthyroid compared to the healthy control, 247.13(±86.39)U/ml since the p value was0.23. The median (±interquartile range) difference between untreated hyperthyroid patients, 441.77 (±191.91)U/ml and normal subjects in salt stimulated PON1 activity, 365.83(±221.80) U/ml also showed no significance statistically since the p value was 0.08.There is also no significance difference between the median arylesterase activities between both groups.

In both groups, spearman rank correlation showed weakpositive correlation between serum TSH and basal PON 1 activity, however this was statistically not significant (r= 0.18, p=0.899). Between serum TSH and salt stimulated PON1 activity, there was also weak positive correlation and statistically not significant (r=0.061, p= 0.660). Free T4 and basal PON1 shows weak negative correlation and statistically not significant(r=-0.207, p=0.130). While between free T4 and salt stimulated PON1 activity, there was weak positive correlation with no significance statistically. (r= 0.022, p= 0.873).

Table 3: Correlation of basal PON1, Salt stimulated PON1 activity and arylesterase activity between the hyperthyroid subjects and the healthy subjects.

Variables	Basal (U/ml)		Salt stimulated (U/ml)		Arylestrase (U/ml)	
	r	p	r	p	r	p
TSH (mU/L)	0.018	0.899	0.061	0.660	-0.272	0.045
T4 (mU/L)	-0.207	0.130	0.022	0.873	0.129	0.350

TSH: Thyroid Stimulating Hormone, T4 : free thyroxine T4, p < 0.05 considered as significant

Based on this study, AB phenotype is the most common polymorphism in Malay population in Kubang Kerian, Kelantan with 67.9 percent in hyperthyroid patient and 73.1 percent in healthy subjects. A phenotype is uncommon in Malay population with only 7.1 percent in hyperthyroid patient and 5.8 percent out of healthy subjects.

Table 4: Proportion of PON 1 Phenotype inuntreated hyperthyroid patient and healthy control

PON1 Phenotype	Untreated hyperthyroid patient	Healthy control
A	4(7.3%)	3(5.8%)
AB	37 (67.3%)	38 (73.1%)
B	14 (25.5%)	11 (21.2%)

Since the majority of the patient and the healthy subjects were AB phenotype, Mann-Whitney test was carried out between the untreated hyperthyroid and healthy control within AB phenotype group to look for the association. However, there is also no significant median difference in basal PON1, salt stimulated and arylesterase activities between these two groups in the AB phenotype patients since the $p > 0.05$.

Table 5: Basal PON 1, Salt stimulated PON 1 and arylesterase activity among the AB phenotype subjects.

	Median (IQR)		Z statistic	P value
	Untreated hyperthyroid patient	Healthy control		
Basal (U/ml)	271.19 (±98.41)	231.82 (±100.60)	-1.035	0.301
Salt-stimulated (U/ml)	403.83 (±231.28)	315.45 (±208.11)	-1.480	0.138
Arylesterase (U/ml)	86.22 (±49.64)	71.68 (±30.11)	-1.691	0.091

IQR; Interquartile range, $p< 0.05$

4. Discussion

In the newly diagnosed hyperthyroid patient, the number of female patients was more than the male patients. This is consistent with the prevalence of hyperthyroidism in women worldwide which is between 0.5 and 2% and is ten times more common in women than in men in iodine-replete communities [23]. The demographic data showed that the mean age in healthy groups was younger compared with the hyperthyroid subjects.All the hyperthyroid patients were randomly selected from the hyperthyroid patient list and were included once the inclusion and exclusion criteria were met.

PON 1 activity showed cardioprotective effect which can prevent atherosclerosis and showed significant association with the dyslipidaemia and cardiovascular mortality [24]. Increase in thyroid hormone showed an association with the alteration in the cardiovascular system including alteration in heart rhythm and arterial wall structure [25-27]. Hyperthyroidism has been linked to an increase in free radicals and cellular metabolic rate [3-5]. Findings from a variety of studies showed that antioxidant enzymes are debatable as it can be increase or decrease [28,29].

The present findings in this study demonstrate that the basal PON 1, salt stimulated PON1 and arylesterase activity wereslightly higher in hyperthyroid patient than in control. However, the median difference was small and statistically not significant. This finding does not coincide with most of previous studies. The differences in finding might be due to racial or ethnicity influences. This is the first study conducted in Malaysia which measure PON 1 activity in untreated hyperthyroid among Malay population. Almost all previous studies were carried out in

Iran and India. This variability of our study subjects is explained by different PON1 gene polymorphism, lifestyle, exercise and diet of Malaysian population [30].

The contradictory finding in this study might also be due to the small number of study subjects. Due to limited time frame and the number of hyperthyroid cases, the number of subjects and the healthy subjects can only fulfil the minimum number required based on the sample size calculation. In addition, some of the subjects had to be rejected due to multiple factors that can affect PON1 activity such as hyperlipidaemia and other comorbidities. Therefore, the small sample size reduces the power of the study and increases the margin of error and it would be appropriate to repeat this study in a larger population-based study.

PON1 activity was believed to be reduced in hyperthyroid patients due to increase in free radical production as a result ofhyper metabolic state which will disrupt the balance between antioxidant and free radical production[3-5]. However, in a newly diagnosed hyperthyroid, there is possibility that the hyper metabolic phase is still in the early phase and the oxidative stress is still not yet establish since there is still balance between free radical production and antioxidant activity. Therefore, during this phase, PON 1 activity might still be in the normal range to counteract or slows down the free radical production.In addition, it is also possible that there is increased in PON 1 expression and production in early phase of hyperthyroid to detoxify the free radical production. To date, there is yet no studies done to show the relationship between the duration of hyperthyroid and severity of oxidative stress. It is difficult to identify and classify the newly diagnosed hyperthyroid patients based on the chronicity. However, such study is requiredin the future to establish and understand the pathophysiology of PON 1 activity in hyperthyroid and when PON 1 activity will be affected during the course of hyperthyroid.

There was still limited number of large population-based study that explore the relationship between untreated hyperthyroid and PON1 activities. In 2004, Raiszadeh et al.showed that PON1 activity was low before treatment and after six-monthtreatment the activity increased. This study used twenty-four hyperthyroid patients and twenty-three age matched controlsubject [31]. D.G Yazuz et el. in 2004 showed that serum paraoxonase activity was significantly lower in the hyperthyroid group before treatment with Propylthiouracil (PTU) and PON 1 activity increased after restoration of the euthyroid which was conducted in twenty patients only[32]. While Baskol et al. in 2012 conducted the study in 30 patients with primary hyperthyroidism found that serum PON 1 activity was lower in patients group compared with control [33]. Another studyby Kale Bhagwat in 2013 used thirtynewly diagnosed hyperthyroid patient and thirty normal subjects showed than PON 1 activity was significantly reduced in hyperthyroid compared to health control and suggested the use of PON 1 as a marker for hyperthyroidism [34]. Other studies conducted in 2014 used thirty-five hyperthyroid patients and thirty-five healthy subjects found that PON 1 activity was decreased and increased the cardiovascular risk in hyperthyroid subjects[35].Another study was carried out in 2017 by Shafeeq N. K to determine the vistafin and PON 1 level in Iraqi hyperthyroid patients with dyslipidaemia. The finding showed that PON

1 activity is reduced in hyperthyroid patients, however the concurrent dyslipidaemia problem in the subjects may contribute the reduction of PON 1 activity [36].

The finding in the study was probably due to the fact that PON1 enzyme is known for its promiscuity meaning that various factors can affect its activity. In this study, the exclusion criteria were met only by a simple questionnaire to the subjects. Since PON 1 function is not fully understood yet, therefore there is possibility that some undiscovered confounding factors exist in our samples that might affect the finding.

Despite of contradictory findings from majority of the studies, there was one study with almost similar findings. The Prevention of Renal and Vascular End Stage Disease, PREVEND Cohort Study conducted in 2017 using 2206 euthyroid subjects discovered that PON 1 activity is positively related to TSH($\beta = -0.045$, $P = .036$) and negatively related to free T4 ($\beta = -0.042$, $P = .050$). However, after adjustment of result for lipids and other confounding factor, TSH lost the significant association [37]. This is the only large cohort study done to investigate PON 1 activity in euthyroid patients. Similarly with this study,our findings also shows a weak positive correlation between PON 1 activity and TSH and weak negative correlation to free T4, however this is not statistically significant.

Majority of the Malay population in this study has heterozygote AB phenotype followed by homozygote BB phenotype. Homozygote AA phenotype is rare in Malay population in Kubang Kerian. This finding is consistent with Poh et al, in 2007 which shown that in Malaysia, Malays and Chinese had a higher frequency of the R or B allele, which is considered a risk factor for cardiovascular disease in a number of populations from previous studies [38].Another study in 2008 which was conducted in National University of Singapore to observe for the distribution of PON 1 polymorphism and possible susceptibility to organophosphate exposure showed similar distribution in PON 1 polymorphism where among Chinese and Malays, the AB phenotype was more prevalent than BB, followed by AA which was present in low numbers[39]. Malays and Chinese therefore have a higher paraoxon activity. Therefore, Malays will be more protected against organophosphate exposure but will have an increased risk of developing atherosclerosis related disease.

There were few limitations in this study that needs to be reviewed and might influence the findings. The small number of participantswas the main limitation in this study. Even though the number for both groupsfulfil the minimum requirement based on sample size calculation, the findings will be more significant with larger sample size. More participants mean that the results will be representative of the population. In addition,to recruit a newly diagnosed hyperthyroid was quite difficult and challenging. This is due to limited number of newly diagnosed hyperthyroid patient from the low prevalence.Since many factors can affect PON1 activities such as DM, overweight, hyperlipidaemia, smoking and etc [40], this will reduce the number of available untreated hyperthyroidpatients available. In addition, the prevalence of hyperthyroidism is higher with increasing age which makes it difficult to recruit subjects as elderly people tend to have hyperlipidaemia, DM and heart disease.

Conclusion

As a conclusion, this study shows no significant difference in PON1 activities between Malay untreated hyperthyroid patients and the healthy controls. There is also no significance correlation between PON1 activities and free T4 and TSH in both untreated hyperthyroid patients and healthy control. This study also shows that phenotype AB is the most common in Malay population.

Conflicts of interest: There is no conflict of interest

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References

[1] GarmendiaMadariaga, A., Santos Palacios, S., Guillén-Grima, F. and Galofré, J.C., 2014. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. *The Journal of Clinical Endocrinology & Metabolism*, 99(3), pp.923-931.https://doi.org/10.1210/jc.2013-2409.

[2] Shahar, Mohammad Arif, Sukor, Norlela, Othman, Hanita &Kamaruddin, Nor Azmi, H, Zanariah, Johari Ali, et al. Prevalence of thyroid disorders and thyroid autoantibodies among coastal communities of Malaysia (part of nationwide study of thyroid disorders in Malaysia), http://www.fmsconference.net/2017/wp-content/uploads/2017/01/SYMPO-15-Prof-Arif_Prevalence-of-thyroid-disease-in-Malaysia.pdf, accessed on 15/8/19.

[3] Marcocci C, Leo M, Altea MA. Oxidative stress in Graves' disease. *European thyroid journa*l. 2012;1(2):80-7. https://doi.org/10.1159/000337976

[4] Venditti P, Di Meo S. Thyroid hormone-induced oxidative stress. *Cellular and Molecular Life Sciences CMLS*. 2006 Feb 1;63(4):414-34. https://doi.org/10.1007/s00018-005-5457-9

[5] Song Y, Driessens N, Costa M, De Deken X, Detours V, Corvilain B, Maenhaut C, Miot F, Van Sande J, Many MC, Dumont JE. Roles of hydrogen peroxide in thyroid physiology and disease. *The Journal of Clinical Endocrinology & Metabolism*. 2007 Oct 1;92(10):3764-73. https://doi.org/10.1210/jc.2007-0660

[6] Žarković M. The role of oxidative stress on the pathogenesis of Graves' disease. *Journal of thyroid research*. 2012;2012. https://doi.org/10.1155/2012/302537

[7] Furlong CE, Marsillach J, Jarvik GP, Costa LG. Paraoxonases-1,-2 and-3: what are their functions?. *Chemico-biological interactions*. 2016 Nov 25;259:51-62. https://doi.org/10.1016/j.cbi.2016.05.036

[8] Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics*. 1996 May 1;33(3):498-507. https://doi.org/10.1006/geno.1996.0225

[9] Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *The Journal of clinical investigation*. 1998 Apr 15;101(8):1581-90. https://doi.org/10.1172/JCI1649.

[10] Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS letters*. 1991 Jul 29;286(1-2):152-4. https://doi.org/10.1016/0014-5793(91)80962-3

[11] Mackness MI, Abbott CA, Arrol S, Durrington PN. The role of high-density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation. *Biochemical Journal*. 1993 Sep 15;294(3):829-34.https://doi.org/10.1042/bj2940829

[12] Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*. 1993 Dec 1;104(1):129-35. https://doi.org/10.1016/0021-9150(93)90183-U

[13] Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. *General Pharmacology: The Vascular System.* 1998 Sep 1;31(3):329-36. https://doi.org/10.1016/S0306-3623(98)00028-7

[14] Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS letters*. 1998 Feb 13;423(1):57-60.

[15] Mackness MI, Mackness B, Durrington PN. Paraoxonase and coronary heart disease. *Atherosclerosis Supplements*. 2002 Dec 1;3(4):49-55. https://doi.org/10.1016/S1567-5688(02)00046-6

[16] Aydin S, Sahin İ, Aydin S, Aksoy A, Citil C. The past and present of paraoxonase enzyme: its role in the cardiovascular system and some diseases. *Journal of Medical Biochemistry*. 2012 Jul 1;31(3):161-73.https://doi.org/10.2478/v10011-012-0006-1

[17] Yilmaz N. Relationship between paraoxonase and homocysteine: crossroads of oxidative diseases. *Archives of medical science*: AMS. 2012 Feb 29;8(1):138. doi: 10.5114/aoms.2012.27294

[18] Shunmoogam N, Naidoo P, Chilton R. Paraoxonase (PON)-1: a brief overview on genetics, structure, polymorphisms and clinical relevance. *Vascular health and risk management*. 2018;14:137. https://doi.org/10.2147/VHRM.S165173

[19] Gan KN, Smolen AN, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. *Drug Metabolism and Disposition*. 1991 Jan 1;19(1):100-6.

[20] Eckerson HW, Romson JO, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. *American journal of human genetics*. 1983 Mar;35(2):214.

[21] Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *American journal of human genetics*. 1983 Nov;35(6):1126

[22] Adkins S, Gan KN, Mody M, La Du BN. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *American journal of human genetics*. 1993 Mar;52(3):598.

[23] Vanderpump MP. The epidemiology of thyroid disease. *British medical bulletin*. 2011 Sep 1;99(1). doi: 10.1093/bmb/ldr030

[24] Grzegorzewska, A. E., Adamska, P., Iwańczyk-Skalska, E., Ostromecka, K., Niepolski, L., Marcinkowski, W., ... &Jagodziński, P. P. (2021). Paraoxonase 1 concerning dyslipidaemia, cardiovascular diseases, and mortality in haemodialysis patients. *Scientific reports*, *11*(1), 1-16.

[25] Danzi, S., & Klein, I. (2012). Thyroid hormone and the cardiovascular system. *Medical Clinics*, *96*(2), 257-268.

[26] Osman, F., Franklyn, J. A., Holder, R. L., Sheppard, M. C., &Gammage, M. D. (2007). Cardiovascular manifestations of hyperthyroidism before and after antithyroid therapy: a matched case-control study. *Journal of the American College of Cardiology*, *49*(1), 71-81.

[27] Völzke, H., Robinson, D. M., Schminke, U., Lüdemann, J., Rettig, R., B. Felix, S., ... & Meng, W. (2004). Thyroid function and carotid wall thickness. *The Journal of Clinical Endocrinology & Metabolism*, *89*(5), 2145-2149.

[28] Asayama, K., DOBASHI, K., HAYASHIBE, H., MEGATA, Y., & KATO, K. (1987). Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. *Endocrinology*, *121*(6), 2112-2118.

[29] Morini, P., Casalino, E., Sblano, C., &Landriscina, C. (1991). The response of rat liver lipid peroxidation, antioxidant enzyme activities and glutathione concentration to the thyroid hormone. *International Journal of Biochemistry*, *23*(10), 1025-1030.

[30] Schrader C, Rimbach G. Determinants of paraoxonase 1 status: genes, drugs and nutrition. *Current medicinal chemistry*. 2011 Dec 1;18(36):5624-43.https://doi.org/10.2174/092986711798347216

[31] Raiszadeh, F., Solati, M., Etemadi, A., & Azizi, F. (2004). Serum paraoxonase activity before and after treatment of thyrotoxicosis. *Clinical endocrinology*, *60*(1), 75-80.

[32] Yavuz DG, Yüksel M, Deyneli O, Ozen Y, Aydin H, Akalin S. Association of serum paraoxonase activity with insulin sensitivity and oxidative stress in hyperthyroid and TSH-suppressed nodular goitre patients. *Clinical Endocrinology*. 2004 Oct;61(4):515-21. https://doi.org/10.1111/j.1365-2265.2004.02123.x.

[33] BAŞKOL G, SEÇKİN KD, Bayram F, Tanriverdi F. Investigation of serum paraoxonase-1 activity and lipid levels in patients with hyperthyroidism. *Turkish Journal of Medical Sciences*. 2012 Nov 21;42(Sup. 1):1166-71. doi:10.3906/sag-1202-87

[34] Bhagwat, K. (2013). Serum paraoxanase-1 activity as a novel marker in diagnosis and prognosis of hyperthyroidism. *International Journal of Biological Research*, *1*, 29-33.

[35] Othman FO. The association between serum paraoxonase-1 activity, thyroid hormones and lipids profile in patients with primary hyperthyroidism. *International Journal of Advanced Research*. 2014.

[36] Shafeeq NK. Visfatin, PON-1 Levels in Iraqi Hyperthyroidism Patient's with Dyslipidemia. *Indian Journal of Clinical Biochemistry*. 2019 Jan 1;34(1):101-7. https://doi.org/10.1007/s12291-017-0717-7

[37] [37] van Tienhoven-Wind LJ, Gruppen EG, James RW, Bakker SJ, Gans RO, Dullaart RP. Serum paraoxonase-1 activity is inversely related to free thyroxine in euthyroid subjects: The PREVEND Cohort Study. *European journal of clinical investigation*. 2018 Jan;48(1):e12860. https://doi.org/10.1111/eci.12860

[38] Poh R, Muniandy S. Ethnic variations in paraoxonase1 polymorphism in the Malaysian population. *Southeast Asian journal of tropical medicine and public health*. 2007;38(2):392-7.

[39] Chia SE, Ali SM, Yap PH, Gan L, Ong YB, Chia KS. Distribution of PON1 polymorphisms—PON1Q192R and PON1L55M among Chinese, Malay and Indian males in Singapore and possible susceptibility to organophosphate exposure. *Neurotoxicology*. 2009 Mar 1;30(2):214-9.https://doi.org/10.1016/j.neuro.2008.12.004

[40] Krzewicka-Romaniuk EL, Siedlecka DA, Warpas A, Wójcicka G. Paraoxonase 1 as an important antiatherogenic agent. *Journal of Education, Health and Sport*. 2019 Jan 17;9(1):133-43. http://dx.doi.org/10.5281/zenodo.2542320