The Effect of Age on the Paraoxonase and Arylesterase Activity of Pregnant Mothers and Their Infants

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Aim: The study aimed to research the effect of maternal age at pregnancy on the paraoxonase (PON) and arylesterase activity levels of mothers and infants. **Method:** The study comprised 30 mothers aged more than 35 years and their infants, and 30 mothers aged under 25 years and their infants. All pregnancies had been of 38–42 weeks with normal spontaneous vaginal birth. PON and arylesterase activities were measured using paraoxon and phenylacetate substrates. **Results:** When the PON and arylesterase enzyme activities were compared, the PON and arylesterase activity of the mothers aged more than 35 years was found to be statistically significantly lower compared to that of the mothers aged under 25 years

($P = 0.006$ and $P = 0.03$, respectively), while there was no statistically significant difference in PON and arylesterase enzyme activity between the two groups of infants ($P > 0.05$). **Conclusion:** While the PON and arylesterase enzyme activity of the mothers aged more than 35 years was found to be significantly lower compared to that of the mothers aged under 25 years, there was no statistical significance between the infants. This can be explained by the reduced PON and arylesterase enzyme activity during pregnancies at an older age, although this did not affect the PON and arylesterase enzyme activity of the infants. J. Clin. Lab. Anal. 26:302–306, 2012. © 2012 Wiley Periodicals, Inc.

Key words: older age pregnancy; younger age pregnancy; newborn infant; paraoxonase; arylesterase

INTRODUCTION

Paraoxonase (PON) is localized in the human's seventh chromosome q21–22 region and comprises PON-1, PON-2, and PON-3 (1). Paraoxonase-1 (PON-1), having both paraoxonase and arylesterase activity is a calcium-dependent ester hydrolase. Found in the serum and several tissues, primarily the liver, kidneys, and small intestine, serum PON-1 enzyme expressed into the circulation is a protein with a hydrolytic effect on several substrates including organophosphates, arylesterases, and lactones (2–4).

It is known that there is a relationship between PON-1 and high-density lipoprotein cholesterol (HDL-C) and it inhibits lipid peroxidation by playing a protective role in the oxidative modification of low-density lipoprotein cholesterol (LDL-C), showing antioxidant and anti-inflammatory properties (5–7). PON-1 has been observed to show an antioxidant effect against lipid peroxidation arising from the effects of free radicals on cell membranes and lipoproteins (8).

In previous studies, which have examined the relationship between age and PON-1 enzyme activity, it has been noted that reduced PON-1 enzyme activity is related to increased age (9–11). Moreover, the paraoxonase activity of newborn infants may be as low as approximately half that of adults and reaches the adult level at around the age of 1 year (3).

This study aimed to investigate the relationship with age and whether this was reflected in the infants, of possible variations in serum PON-1 enzyme activity of mothers

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aged more than 35 years and mothers aged under 25 years and their newborn infants.

MATERIAL AND METHOD

For this study, the selection was made from mothers that were being monitored throughout pregnancy at Şanlıurfa Maternity Hospital. The mothers were of similar socio-cultural backgrounds, with no other diseases, aged more than 35 (35–46 years) and under 25 (18–25 years) and had given birth by spontaneous vaginal route after a pregnancy of 38–42 weeks. Approval for the study was granted by Harran University Medical Faculty Ethics Committee and informed consent was obtained from all participants. The study comprised 30 mothers aged more than 35 years and their infants, and 30 mothers aged under 25 years and their infants. The infants had an APGAR score of above 8, no congenital abnormalities, no perinatal or natal hypoxia, were between 2.6 and 4 kg in weight and completely healthy from the results of physical examination and laboratory tests. Mothers who smoked or with diseases such as hypertension, hyperlipidemia, diabetes mellitus, or hypercholesterolemia and infants with an APGAR score below 8, neonatal asphyxia, or with a congenital anomaly were excluded from the study.

Blood Samples

Immediately after the birth, a total blood count was taken using an automatic blood count instrument (Abbot Celldyn 3500 IL) from all the mothers and infants. The blood samples were taken from the study cases for biochemical analysis were centrifuged at 3500 rpm for 10 min then discarded together with the shaped tube and the remaining serum samples were stored at -80°C . On the study day, paraoxonase, arylesterase activity, and lipid parameters were colorimetrically measured using an auto-analyzer (Abbott Aeroset, Abbott Diagnostics, Abbott Park, IL).

Measurement of Lipid Profiles

Plasma triglyceride (TG), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL) were measured by an automated chemistry analyzer using commercial kits of Abbott.

Measurement of Paraoxonase and Arylesterase Activities

Paraoxonase and arylesterase activities were measured using paraoxon and phenylacetate substrates. The rate of paraoxonhydrolysis (diethyl-*p*-nitrophenylphosphate)

was measured by monitoring the increase of absorbance at 412 nm at 37°C . The amount of generated *p*-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was $17,000\text{ M}^{-1}\text{ cm}^{-1}$ (12).

Paraoxonase activity was expressed as U/l serum. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, $1310\text{ M}^{-1}\text{ cm}^{-1}$. One unit of arylesterase activity was defined as 1 μmol phenol generated/min under the above conditions and expressed as U/l serum (13).

Paraoxonase phenotype distribution was determined by a double substrate method that measures the ratio of paraoxonase activity (with 1 M NaCl in the assay) to arylesterase activity, using phenylacetate (12).

Statistical Analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences, version 11.5 for Windows, SPSS® Inc, Chicago, IL). The results were presented as mean $\pm$ standard deviation (SD). The distribution of all data parametric variables was assessed with a one-sample Kolmogorov–Smirnov test variables and was found to be normally distributed. A comparison among repeated laboratory variables was performed by an independent samples *t*-test with the Pearson Chi-square test. Spearman's correlation analysis was used to test the relationships between the parameters. A two-tailed *P* value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of the 30 mothers aged more than 35 years was 37.6 ± 2.6 years (range 35–46 years) and the mean age of the 30 mothers aged under 25 years was 22.6 ± 2.3 years (range 18–25 years). Comparison of the ages of the two groups determined a statistically significant difference ($P < 0.001$). The mean gravida number was 6.3 ± 1.9 (range 3–10) for the mothers aged more than 35 years and 2.13 ± 1.1 (range 1–5) for the mothers aged under 25 years. Comparison of the gravida number of the two groups determined a statistically significant difference ($P < 0.001$).

A comparison of the mean paraoxonase values (118.19 ± 64.08 U/l) and mean arylesterase values (208.52 ± 33.51 U/l) of the mothers aged more than 35 years with the mean paraoxonase values (174.54 ± 87.65 U/l) and mean arylesterase values (228.78 ± 37.92 U/l) of the mothers aged under 25 years showed that the mothers aged more than 35 years were determined to be statistically significantly lower ($P = 0.006$ and $P = 0.03$, respectively).

TABLE 1. Comparison of the Paraoxonase, Arylesterase, and Lipid Profile Values of the Mothers Aged More Than 35 Years and the Mothers Aged Under 25 Years

	Older mothers Mean $\pm$ SD	Yonger mothers Mean $\pm$ SD	<i>P</i>
Arylesterase (U/l)	208.52 $\pm$ 33.51	228.78 $\pm$ 37.92	= 0.03
Paraoxonase (U/l)	118.19 $\pm$ 64.08	174.54 $\pm$ 87.65	= 0.006
TG (mg/dl)	220.51 $\pm$ 73.59	210.70 $\pm$ 83.00	>0.05
Cholesterol (mg/dl)	247.50 $\pm$ 47.37	223.33 $\pm$ 44.91	<0.05
HDL (mg/dl)	53.76 $\pm$ 9.64	59.94 $\pm$ 11.51	<0.05
VLDL (mg/dl)	43.67 $\pm$ 15.64	42.14 $\pm$ 16.60	>0.05
LDL (mg/dl)	128.29 $\pm$ 38.69	145.43 $\pm$ 41.71	>0.05

Older mothers: Mothers more than the age of 35.

Yonger mothers: Mothers under the age of 25.

TG, triglyceride; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein.

Student *t*-test for independent samples was used.

Significance was defined as *P* < 0.05.

When the mean cholesterol levels of the mothers aged more than 35 years (247.50 $\pm$ 47.37 mg/dl) were compared with the mean cholesterol levels of the mothers aged under 25 years (223.33 $\pm$ 44.91 mg/dl), the values of the mothers aged more than 35 years were found to be statistically significantly high (*P* < 0.05). However, in the comparison of HDL values of the mothers aged more than 35 years (53.76 $\pm$ 9.64 mg/dl) with the HDL values of the mothers aged under 25 years (59.94 $\pm$ 11.51 mg/dl), those of the mothers aged more than 35 years were found to be statistically significantly lower (*P* < 0.05). No statistically significant difference was determined in the comparisons of other lipid parameters (*P* > 0.05). The results are shown in Table 1.

In the comparison of the mean paraoxonase values (56.62 $\pm$ 30.90 U/l) and mean arylesterase values (92.66 $\pm$ 26.35 U/l) of the infants of mothers aged more than 35 years with the mean paraoxonase values (72.16 $\pm$ 43.16 U/l) and mean arylesterase values (101.04 $\pm$ 22.92 U/l) of the infants of mothers aged under 25 years, no statistical significance was found (*P* > 0.05 and *P* > 0.05, respectively). In the comparison of the mean lipid profile parameters (TG, cholesterol, HDL, VLDL, and LDL) of the infants of mothers aged more than 35 years with the mean lipid profile parameters of the infants of mothers aged under 25 years, no statistical significance was found (*P* > 0.05). The results are summarized in Table 2.

When all the mothers as one group and all the infants as a separate group were evaluated, the paraoxonase values (64.39 $\pm$ 38.03 U/l) and arylesterase enzyme activity values (96.85 $\pm$ 24.85 U/l) of the infants were found to be at a statistically significant lower level compared to the paraoxonase (146.36 $\pm$ 81.25 U/l) and arylesterase enzyme activity (218.65 $\pm$ 36.92 U/l) values of the mothers (*P* < 0.001). The results are shown in Table 3.

TABLE 2. Comparison of the Paraoxonase, Arylesterase, and Lipid Profile Values of the Infants of Mothers Aged more than 35 Years and the Infants of Mothers Aged Under 25 Years

	Older mothers' infants Mean $\pm$ SD	Yonger mothers' infants Mean $\pm$ SD	<i>P</i>
Arylesterase (U/l)	92.66 $\pm$ 26.35	101.04 $\pm$ 22.92	>0.05
Paraoxonase (U/l)	56.62 $\pm$ 30.90	72.16 $\pm$ 43.16	>0.05
TG (mg/dl)	47.58 $\pm$ 46.33	48.12 $\pm$ 45.23	>0.05
Cholesterol (mg/dl)	76.30 $\pm$ 29.60	77.80 $\pm$ 74.25	>0.05
HDL (mg/dl)	28.87 $\pm$ 11.36	26.69 $\pm$ 7.40	>0.05
VLDL (mg/dl)	10.27 $\pm$ 10.08	9.62 $\pm$ 9.04	>0.05
LDL (mg/dl)	39.70 $\pm$ 18.37	41.48 $\pm$ 75.43	>0.05

Older mothers' infants: Infants of mothers more than the age of 35.

Yonger mothers' infants: Infants of mothers under the age of 25.

TG, triglyceride; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein.

Student *t*-test for independent samples was used.

Significance was defined as *P* < 0.05.

TABLE 3. Comparison of the Paraoxonase and Arylesterase Activity values of all the Mothers and Infants

	All mothers Mean $\pm$ SD	All infants Mean $\pm$ SD	<i>P</i>
Arylesterase (U/l)	218.65 $\pm$ 36.92	96.8535 $\pm$ 24.85	<0.001
Paraoxonase (U/l)	146.36 $\pm$ 81.25	64.39 $\pm$ 38.03	<0.001

Student *t*-test for independent samples was used.

Significance was defined as *P* < 0.05.

Although a correlation was found between maternal age and cholesterol level (*P* < 0.05), there was no correlation between maternal age and other lipid parameters (*P* > 0.05). No correlation was found between gravida number and lipid profile (*P* > 0.05). Although no correlation was found between arylesterase, age, and gravida number, a negative correlation was seen between paraoxonase, age, and gravida number (*r* = -0.292, *P* = 0.05, *r* = -0.302, *P* < 0.05). The results are shown in Table 4.

DISCUSSION

In this study, when the mean paraoxonase and arylesterase values of mothers aged more than 35 years were compared with those of mothers aged under 25 years, the paraoxonase and arylesterase values of the mothers aged more than 35 years were found to be statistically significantly lower. Previous studies have reported that this may be due to the mother's nutrition (14, 15), exposure to environmental pollution (16), and a decrease in PON-1 enzyme activity with increased maternal age (11, 17, 18), and decreased PON-1 enzyme activity associated with increased oxidative stress (19–21) together with increased age (12, 22–26). The low paraoxonase and arylesterase

TABLE 4. Results of the Correlations Between Maternal age and Gravida Number and Arylesterase, Paraoxonase, and Lipid Parameters

	Arylesterase	Paraoxonase	TG	Cholesterol	LDL	HDL	VLDL
Maternal age							
<i>r</i>	-0.178	-0.291	0.145	0.270	-0.164	-0.236	0.139
<i>P</i>	>0.05	<0.05	>0.05	<0.05	>0.05	>0.05	>0.05
Number of births							
<i>r</i>	-0.167	-0.302	0.152	0.185	-0.207	-0.146	0.164
<i>P</i>	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Spearman's correlation analysis was used to test the relationships between parameters.

Significance was defined as $P < 0.05$.

enzyme activity of mothers aged more than 35 years compared to younger mothers in our study is consistent with the literature.

Previous studies have reported the lower levels of paraoxonase and arylesterase enzyme activity in neonates compared to their mothers (27–29). In our study, when all the mothers and infants were evaluated as a separate group, the paraoxonase and arylesterase enzyme activity of the infants was determined as lower compared to the mothers. Previous studies have shown serum PON-1 enzyme activity to be evidently low in patients with liver cirrhosis (30–32). The liver plays a key role in the synthesis of PON-1 enzyme (32). This situation that PON-1 enzyme cannot be synthesized is thought to be associated with the immaturity of the newborn liver.

In this study, when the mean paraoxonase and arylesterase values of the babies of mothers aged more than 35 years were compared with the mean paraoxonase and arylesterase values of the babies of mothers aged under 25 years, no statistical significance was determined. In our study, the mean paraoxonase and arylesterase enzyme activity values of the infants were not shown to be affected by the maternal age. To the best of our knowledge, this is the first study in literature comparing the paraoxonase and arylesterase values of infants of older and younger mothers.

Changes in serum lipid and lipoprotein levels during pregnancy have been reported (33–35). The most conspicuous change in the lipids has been reported to be the increase in triglyceride and total cholesterol. However, in pregnancy, it is not only triglycerides and LDL that increase, but at the same time, HDL increases and the atherogenic effect is inhibited depending on this increase (33–38).

In our study, when the lipid parameters of the mothers aged under 25 years and more than 35 years were examined, the total cholesterol level of the mothers aged more than 35 years was determined as high and HDL as low, compared to the mothers aged under 25 years. These results can be said to be related to maternal age, gravida number (37), nutritional habits, and hormonal changes

(39) as reported in the previous studies. Although, in our study, the total cholesterol was high and the HDL was low in the mothers aged more than 35 years compared to the mothers aged under 25 years, the atherogenic risk of the older mothers is thought to be higher compared to that of the younger mothers. There are studies in literature that establish an association between atherosclerotic vascular disease and low PON-1 values (38–40). As this study showed both HDL and PON-1 enzyme to be lower in the mothers aged more than 35 years, it is thought that more care is required in respect of atherosclerotic diseases.

When the lipid parameters of the infants of the mothers aged more than 35 years were compared with those of the infants of mothers aged under 25 years, no statistical significance was determined in any of the parameters. As there was no difference between the infants of mothers aged more than 35 years and those of mothers aged under 25 years, the age of the mother is thought not to have any effect on the lipid parameters of the infant. This study has the limitations of being a single-center study and the study group was small in number. Larger prospective studies are needed to confirm these findings.

CONCLUSION

While a statistical significance was determined between the older and younger mothers in respect of paraoxonase and arylesterase enzyme activity, there was no significant difference between the infants. These results show that the maternal age did not affect paraoxonase and arylesterase enzyme activity of the infants. However, it can be said that more attention should be paid to older mothers in pregnancy in respect of risks that may be associated with reduced paraoxonase and arylesterase enzyme activity in addition to various other risk factors of pregnancy at an older age.

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