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Research Article

Polycyclic Aromatic Hydrocarbon Pollutants in Relation to Idiopathic Recurrent Spontaneous Abortion

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Abstract

Objectives: The objective of the present study was endeavored to assessment of occurrence and distribution of PAHs in mussels collected from eastern harbor, Alex coast, and the possible association between exposure to PAHs in the environment and female idiopathic recurrent spontaneous abortion.

Materials and Methods: The study population consisted of 76 women attending an idiopathic recurrent spontaneous All the women were healthy and under 45 years of age. All participants were interviewed. The interview included questions concerning demographics, socio-economic status, medical history related to past diseases which may have an impact on fertility, lifestyle factors and occupational information. Concentrations of α-β-naphtholin the urine samples were analyzed using high performance liquid chromatography (HPLC), 8OH-guanisene was analysed by elisa kite.

Results: There were high concentration of many PAHs in the tissues of two species of mussels collected from Alex coast. Also, Among the study participants the level of α-naphthol and β-naphthol were high in aborted groups than that of the cross ponding control group. The participant suffered from elevated level of lipid peroxide with low level of antioxidant. PAHs induced panic oxidative DNA damage which in turn produce high significant levels of (8-OHDG)

Conclusions: Presented findings indicate that the environmental level of PAHs exposure affects female reproduction. The future large-scale studies should incorporate different biomarkers to generate a more accurate and full assessment of the effects of PAHs exposure on idiopathic recurrent spontaneous abortion

Keywords: Polyaromatic hydrocarbon PAHs; α-naphthol; β-naphthol; DNA damage (8-OHDG)

Introduction

Pregnancy is a complex, heterogenous, biological phenomenon in which the embryo develops into a fetus within the female uterus. The duration of gestation is divided into three trimesters of nearly three months each. nevertheless, due to various etiological factors, the growing embryo incapable to survive is expelled from the pregnant mother at different gestational ages and this is referred to as pregnancy loss or abortion. Pregnancy loss occurs in 10 to 15% of all pregnancies, of which 1-2% are recurrent [1]. Recurrent Pregnancy Loss (RPL) was initially defined as the loss of three or more clinically recognized pregnancies spontaneously during early gestation. However, the modern definition back to the spontaneous loss of two or more consecutive pregnancies before twenty weeks of gestation [2]. The World Health Organization (WHO) has defined miscarriage as the loss of a fetus weighing ≤500g, which would at 20-22complete weeks of gestation [3].

Recurrent pregnancy loss affects 0.5-3% of women in the reproductive age group and approximately from 50% to 60% of recurrent pregnancy losses are idiopathic. One considering factor of recurrent pregnancy losses environmental pollution [4]. The strongest testimony of environmental contaminant exposures interfere with healthy reproductive function in adult female is xenobiotic [4]. There is a strong association between miscarriage and pollutant compounds such as PAHs, heavy metals, solvents, phthalates, polychlorinated biphenyls and pesticides, Women are exposed to these compounds from the environment everyday often without knowing [5].

Polycyclic Aromatic Hydrocarbons (PAHs) are an important class of Persistent Organic Pollutant (POPs). However, oxygenation of PAHs often generates electrophilic arene oxide, dioepoxide and other reactive species which damage the DNA and/or affect protein functions, leading to adverse effects. Reactive Oxygen Species (ROS), which are strongly linked with oxidative stress, are oxygen-derived free radicals that include superoxide anions, hydroxyl, peroxyl, alkoxyl radicals, and hydrogen peroxide [6]. ROS can be generated either from endogenous physical processes such as mitochondrial respiration [7] or from various environmental factors, which include drugs, pollution, toxins, smoking, radiation, and diet [8]. Therefore, scavenging excess ROS is mandatory for normal fertility and health.

Antioxidant response element (NRF2/ARE) signaling pathway and its regulated antioxidant enzymes GST, Catalase have been shown to play crucial roles in cellular oxidative stress defense during fertilization [9,10].

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Table 1: Concentration of polycyclic aromatic hydrocarbon (PAHs ng/g) in the tissues of the two species of mussels collected from eastern harbor, Alex, Coast.

PAH compound	Andaradulofii	Donaxtrunculus
Acenaphtylene	3.2	3.4
Fluorene	16.5	62.3
Phenanthrene	311.4	597
Anthracene	7.2	7.1
Pyrene	22	12.9
Benzo(a) anthracene	8.1	5.5
Chrysene	10.7	7.5
Benzo(b)fluoranthene	6.3	4.4
Benzo(k)fluoranthene	6.4	4
Benzo(a)pyrene	3.6	8.8
Indeno(1,2,3-cd)pyrene	2.7	2.6
Dibenzo(a,h)anthracene	2.2	3
Benzo(g,h,i)perylene	2.8	3.1
Total PAHsconc.	403.1	721.6

Thus, the objective of the present study was endeavored to assessment of occurrence and distribution of PAHs in mussels collected from eastern harbor, Alex coast, and the possible association between exposure to PAHs in the environment and female idiopathic recurrent spontaneous abortion.

Methods and Patients

1-naphthol, 2-naphthol, β-Glucuronidase from Helix pomatia (G7017), acetonittril (HPLC grade), Methanol (HPLC grade), n-hexane (HPLC grade) were purchased from sigma chemical CO; (St; Louis, MO, U.S.A). The concentration of progesterone and FSH were measured by monobinede ELISA kit (from Italy). Kits for the determination of Reduced glutathione, Glutathione-S-Transferase, Catalase&Malondialdehyde were purchased from Biodiagnostic CO: (29 Taahreerst; Dokki Giza (Egypt)). Kit for determination of creatinine was purchased from Diamond diagnostic co; (30175 Hannover, Germany).

Donaxtrunculus and Andaraduloii were collected from Mediterranean Sea "Alex Beach." for determination of polycyclic aromatic hydrocarbons (PAHs).

After the agreement of the ethics committee on this study, the volunteer subjects consent is taken before they were included in the study by the ethics committee of Alexandria University (US Department of Health and Human Services (HHS), Registration of an Institutional Review Board (IRB), IORG0008812 Medical Research Institute, Expires 4/8/2019, OMB No: 0990-0279).

A total of seventy six (76) female with an average age 20:35 years at El-Shatby Maternity Hospital, Alexandria University. The subjects in this study were divided into four groups A) Group I (Control) Comprised of 18 normal healthy fertile women who had no history of recurrent spontaneous abortion. All had at least one living child and their pregnancy proceed successfully giving full term healthy newborn. B) Group II, III and IV: Contained 24, 18 and 16 women respectively with medically unexplained recurrent spontaneous abortion, aborted women have at least a two successive spontaneous abortion up to 20 weeks gestational age.

A volume of 10ml Urine were collected from all patients & control stored at -20°C until used for detection of urinary metabolites of PAHs "1-naphthol, 2-naphthol".

8ml venous blood samples were withdrawn from all subjects, 3ml of blood was taken on anticoagulant (EDTA) for determination of GSH and catalase enzyme. Another 5ml of venous blood samples was taken without anticoagulant and allowed to coagulate for 20 minutes, and centrifuge at 4000rpm for 10 minutes. The aspirated serum was stored at -20°C till assay of malonedialdehyde, progesterone, follicle stimulating hormone, and 8-hydroxy-2-deoxyguanosine. The red cells washed once with 10 volumes of cold saline then the red cell pellets lysates by adding 4 volumes of cold deionized water. Red cell storm removed by centrifugation (4000rpm for 10 minutes at 4°C). The resulting clarified supernatant was collected, stored at -70°C until used for determination of glutathione s-transferase.

Environmental study

According to Andral et al. [11,12] the extracted polycyclic

Table 2: Statistical analysis of urinary α-naphthol (nmol/mmol creatinine) in the 4 studied groups.

	Group I (control) (n=18)	Group II (2 Abortions)	Group III (3 Abortions)	Group IV (> 3 Abortions)
		(n=24)	(n=18)	(n=16)
Mean	0.7	4.1	4.3	5.4
Medium	0.03	2.83	2.89	4.88
Min. – Max.	0.001 – 6.40	0.001 – 18.30	0.001 – 15.10	0.001 – 17.60
S.D.	1.61	5.21	4.72	5.46
S.E.	0.38	1.063	1.113	1.365
p1		0.042	0.186	0.09
p2			1	1
p3				1

Group II, two abortion; Group III, three abortion; Group IV more than three abortion.
The mean differences is significant when *P1*, *P2*, *P3*< 0.05.
The comparison between groups were done by Kruskal-Wales test.
The comparisons between every two groups were done by Mann Whitney with Bonforni correction.
P1 is the *p* value when comparing group II, group III and group IV with group I.
P2 is the *p* value when comparing group III and group IV with group II.
P3 is the *p* value when comparing group IV with group III.

Table 3: Statistical analysis of urinary β-naphthol (nmol/mmol creatinine) in the 4 studied groups.

	Group I (control)	Group II	Group III	Group IV
		(2 Abortions)	(3 Abortions)	(> 3 Abortions)
	(n=18)	(n=24)	(n=18)	(n=16)
Mean	1	5.3	6.6	8.5
Medium	0.01	4.95	3.75	7.84
Min. – Max.	0.001 – 5.50	0.001 – 18.22	0.001 – 24.10	0.001 – 29.80
S.D.	1.6	5.01	7.68	8.39
S.E.	0.376	1.023	1.811	2.098
p1		0.024	0.186	0.018
p2			1	1
p3				1

The mean differences is significant when *P1, P2, P3*< 0.05.
The comparison between groups were done by Kruskal-Wales test.
The comparisons between every two groups were done by Mann Whitney with Bonforni correction.
P1 is the *p* value when comparing group II, group III and group IV with group I.
P2 is the *p* value when comparing group III and group IV with group II.
P3 is the *p* value when comparing group IV with group III.

aromatic hydrocarbons from mussels sample were identified and quantified using Gas chromatography, (Agielent technologies 1200 series), National Institute of Oceanography &Fisheries, Alexandria.

Biochemical studies

Colorimetric determination of reduced Glutathione (GSH) Content (kits) [13]: The method based on the reduction of 5, 5'dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produces a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405nm.

Determination of serum catalase enzyme (kits) [14,15]: Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase (HRP), remaining H_2O_2 reacts with 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample.

Determination of Serum Malondialdehyde (MDA) Concentration (kits) [16,17]: Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534nm.

Determination of serum 8-hydroxy-2-deoxyguanosine level (ELISA Kits) [18,19]: The concentration of (8-OHDG) measured by Stat Fax ELISA unit.

HPLC identification and quantification of 1-naphthol and 2-naphthol in urine: According to Heon Kim [20] the urine Hydrolyzed enzymatically with 30µl of β-glucuronidase and sulfatase, for 16h at 37°C in a shaking water bath.

Determination of creatinine in urine: (Kits): [21]: This assay is based on the reaction of creatinine with sodium picrate as described by Jaffe. creatinine reacts with alkaline picrate forming a complex.

Table 4: Statistical analysis of serum MDA (nmol/ml) in the 4 studied groups.

	Group I (control)	Group II	Group III	Group IV
		(2 Abortions)	(3 Abortions)	(> 3 Abortions)
	(n=18)	(n=24)	(n=18)	(n=16)
Mean	3.03	9.28	9.22	9.46
Medium	3.35	9.3	8.25	7.9
Min. – Max.	1.2 – 4.7	4.3 – 14.5	5.3 – 13.8	6.4 – 14.5
S.D.	1.21	3.06	3	2.9
S.E.	0.28	0.62	0.7	0.72
p1		0.006	0.006	0.006
p2			0.845	0.811
p3				0.591

The mean differences is significant when *P1, P2, P3*< 0.05.
The comparison between groups were done by Kruskal-Wales test.
The comparisons between every two groups were done by Mann Whitney with Bonforni correction.
P1 is the *p* value when comparing group II, group III and group IV with group I.
P2 is the *p* value when comparing group III and group IV with group II.
P3 is the *p* value when comparing group IV with group III.

The time interval chosen for measurements avoids interferences from other serum constituents. The intensity of the color formed is proportional to the creatinine concentration in the sample.

Results and Discussion

The present results revealed that there were high concentration of many PAHs in the tissues of two species of mussels collected from Alex coast (Table1,2). Where, phenanthrene recorded high concentration in two sp. (311.4, and 597ng/g). Also, pyrene present in high concentration in the two sp. (22.0, and 12.9ng/g). Meanwhile, Donaxtrunculus have high concentration of fluorine in comparing with Andaradulofii. In this context, the use of sentinel organisms (mussels) to measure the levels of bioavailable contaminants has been established by various pollution monitoring programs. The advantage of using these sentinel organisms is the ability to concentrate many organic contaminants by a factor of 10 above the ambient sea water levels and even higher than sediments, providing a direct representation of pollutants bioavailability [22].

α-naphthol, and β-naphthol concentration in a urine sample were chosen as a biomarker of exposure to PAHs. Among the study participants the level of α-naphthol and β-naphthol were high in aborted groups than that of the cross ponding control group. Where, the concentration of α-naphthol ranged 0.001 - 6.40 in control group and from 0.001 - 18.30 in group ii, 0.001 - 15.10 in group iii, 0.001 - 17.60 in group iv. Meanwhile, β-naphthol recorded the concentration of range from 0.001 - 5.50 in control group, 0.001 - 18.22 in group ii, 0.001 - 24.10 in group iii, 0.001 - 29.80nmol/mmol creatinine in group iv. This finding agreement with many literatures that identify PAHs as environmental endocrine disruptors. PAHs may act as antiestrogens by binding with the aryl hydrocarbon receptor, leading to induction of aryl hydrocarbon-responsive genes that result in a broad spectrum of antiestrogenic responses, or PAHs may act as antiestrogens by antagonistically binding the estrogen receptor (ER) [23].

On the other hand, the results of the herein study shows that circulating levels of oxidative stress biomarker (MDA) was strikingly

Table 5: Statistical analysis of whole blood glutathione content (mg/dl) in the 4 studied groups.

	Group I (control)	Group II	Group III	Group IV
		(2 Abortions)	(3 Abortions)	(> 3 Abortions)
	(n=18)	(n=24)	(n=18)	(n=16)
Mean	27.8	25.6	24.6	20.7
Medium	28.4	25.9	25.1	20.6
Min. – Max.	22.4 – 32.9	19.4 – 31.3	17.9 – 29.6	16.2 – 27.1
S.D.	3.08	3.28	3.34	2.46
S.E.	0.77	0.67	0.78	0.61
p1		0.133	0.03	0.001
p2			0.795	0.001
p3				0.003

The mean differences is significant when *P1, P2, P3*< 0.05.
The comparison between groups was done by one way ANOVA test.
The comparison between every two groups was done by Games- Howell post hoc test.
P1 is the value when comparing group II, group III and group IV with group I.
P2 is the value when comparing group III and group IV with group II.
P3 is the value when comparing group IV with group III.

Table 6: Statistical analysis of plasma catalase enzyme activity level (U/l) in the 4 studied groups.

	Group I (control)	Group II	Group III	Group IV
		(2 Abortions)	(3 Abortions)	(> 3 Abortions)
	(n=18)	(n=24)	(n=18)	(n=16)
Mean	449.7	187.6	126.3	118.5
Medium	231.2 – 741.5	78.7 – 397.6	55.1 – 259.8	51.1 – 217.5
Min. – Max.	457.58	144.45	108.4	106
S.D.	128.07	92.66	51.38	43.96
S.E.	30.18	18.91	12.11	10.99
p1		0.006	0.006	0.006
p2			0.01	0.006
p3				0.805

The mean differences is significant when *P1, P2, P3* < 0.05.
The comparison between groups were done by Kruskal-Wales test.
The comparisons between every two groups were done by Mann Whitney with Bonforni correction.
P1 is the value when comparing group II, group III and group IV with group I.
P2 is the value when comparing group III and group IV with group II.
P3 is the value when comparing group IV with group III.

significantly higher in aborted women as compared to control group (Table 4). The statistical analysis of the variance between groups using Kruskal Wales test elucidated that there were high significant differences between the three aborted women groups when compared with the control group. The present results showed that systemic oxidative stress, of which lipid peroxidation represents a major manifestation, plays an important role in recurrent spontaneous abortion. Since, MDA is a byproduct of lipid peroxidation, thus an elevation in MDA levels may reflects an overproduction of lipid peroxides and/or impaired antioxidant defense mechanism which associated with panic decrease in the GSH and catalase enzymatic levels in the aborted women group as compared with control group (Table 5,6). Another study showed significantly low levels of the antioxidant enzymes GPx, SOD, and catalase in patients with idiopathic RPL, in addition to increased MDA levels [24]. From

Table 7: Statistical analysis of serum 8-hydroxy-2-deoxyguanosine level (8-OHDG) (ng/ml) in the 4 studied groups.

	Group I (control)	Group II	Group III	Group IV
		(2 Abortions)	(3 Abortions)	(> 3 Abortions)
	(n=18)	(n=24)	(n=18)	(n=16)
Mean	14.8	17.4	23.4	27.2
Medium	14.755	15.94	20.34	24.05
Min. – Max.	10.12 – 20.20	13.56 – 28.04	16.59 – 46.34	18.08 – 50.79
S.D.	2.6	4.12	7.65	9.47
S.E.	0.613	0.879	1.804	2.368
p1		0.126	0.006	0.006
p2			0.006	0.006
p3				0.924

The mean differences is significant when *P1, P2, P3*< 0.05.
The comparison between groups were done by Kruskal-Wales test.
The comparisons between every two groups were done by Mann Whitney with Bonforni correction.
P1 is the *p* value when comparing group II, group III and group IV with group I.
P2 is the *p* value when comparing group III and group IV with group II.
P3 is the *p* value when comparing group IV with group III.

(Table 5). The statistical analysis of the variance between groups using one way ANOVA test demonstrated that there were no significant differences between the control and groups II but there were high significance between the control and group III and IV. Meanwhile, (Table 6) showed. The statistical analysis of mean difference showed that there was a sharply significant decrease in the plasma catalase enzyme activity of group II, group III and group IV when compared with that of group I.

At the same time there was a statistically significant decrease in the plasma catalase enzyme activity when group III and group IV compared with group II. While there was no significant difference in the enzyme activity between group IV and group III.

Oxidative stress has also been implicated as an important cause of recurrent pregnancy loss. Loss of antioxidant defenses has been shown to be associated with recurrent pregnancy loss. Biochemical markers of ROS-induced membrane damage such as lipid peroxidation products, reach high levels immediately before abortion. It has been proposed that an oxidant/antioxidant imbalance is associated with pregnancy loss [25]. Oxidative stress is implicated in first trimester miscarriage from premature placental perfusion of maternal oxygenated blood and accompanying ROS into the early embryonic environment [26]. Early embryo development occurs in a low oxygen state, and it is not until the tenth to twelfth week of gestation that maternal blood begins to gradually infiltrate the intervillous space of the yet fully developed placenta [27].

On the other hand, the findings of the present study proved that the aborted women were exposed to high levels of PAHs induced panic oxidative DNA damage which in turn produce high significant levels of (8-OHDG) (Table 7). The statistical analysis of the variance between groups using Kruskal-Wales test proved that there was no significant differences between the control and groups II while there were highly significant increase in (8-OHDG) between the control group and group III and IV (Table 3,4). Oxidation of DNA occurs normally but increases with exposure to oxidizing agents.

Guanosines are susceptible to oxidation, and this reaction can lead to G: C → T: A mutations that could have serious consequences. These oxidized bases are recognized and excised by DNA repair machinery. 8-OHdG is excreted in urine and, as such, provides an assessment of general oxidative stress throughout the body. So 8-hydroxy-2′-deoxyguanosine (8-OHdG) becomes a biomarker for oxidative stress [28].

Conclusion

PAHs act as environmental endocrine disruptors, PAHs may act as antiestrogens by binding with the aryl hydrocarbon receptor, leading to induction of aryl hydrocarbon-responsive genes that result in a broad spectrum of antiestrogenic responses, or PAHs may act as antiestrogens by antagonistically binding the estrogen receptor (ER).

Several PAH metabolites present in the urine of aborted women and low level of antioxidant.

Oxidative stress has also been implicated as an important cause of recurrent pregnancy loss. 8-OHdG is excreted in urine and, as such, provides an assessment of general oxidative stress throughout the body. So 8-hydroxy-2′-deoxyguanosine (8-OHdG) becomes a biomarker for oxidative stress.

The future large-scale studies should incorporate different biomarkers to generate a more accurate and full assessment of the effects of PAHs exposure on idiopathic recurrent spontaneous abortion.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from the patient included in the study.

Authors' Contributions

Elagwany had done the diagnoses and surgery along with writing the article.

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