Biochemical Effects of Passive Maternal Smoking as Measured by Serum Cotinine on Birth Weight, Oxidative Stress, Some Toxic Metals and Trace Elements

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ABSTRACT

Prenatal exposure to passive smoking has consequences both in childhood and in adulthood. Cigarette smoking during pregnancy, preoxidant/antioxidant imbalance might have pathomorphological and pathophysiological effect on fetus. Therefore ***the aim of the present study*** *was to estimate the effect of tobacco smoking during pregnancy on activity of superoxide dismutase, and the levels of lipid peroxidation marker, lead, cadmium, copper and zinc.* ***The subjects*** *of the study consisted of sixty three parturient mothers and their neonates. Urine and serum samples were collected from mothers and their neonates. Our results reveled that there was a significant positive correlation between cotinine levels in meconium and both maternal urinary and serum cotinine levels.* ***It could be concluded that*** *preventing and reducing passive maternal smoking during pregnancy might have a beneficial impact on infant size at birth.*

INTRODUCTION

Cigarette smoking during pregnancy is associated with numerous obstetrical, fetal, and developmental complications, as well as an increased risk of adverse health consequences in the adult offspring [(1)]. It has been well documented that cigarette smoking during pregnancy is associated with a number of adverse obstetrical outcomes including: spontaneous abortion[(2)], placenta previa[(3,4,5)], placental abruption[(6)], preterm birth[(7,8)], stillbirth[(9,10)], fetal growth restriction[(11,12)], low birth weight [(13,14)], and sudden infant death syndrome (SIDS) [(15)] as well as long-term behavioral and psychiatric disorders..

Smoking cessation or at least reduction of cigarette smoking during pregnancy can ameliorate damage to the developing fetus[(16,17)]. Indeed, smoking cessation programs based on behavioral therapy, which are implemented during pregnancy, have been shown to reduce the incidence of low birth weight and preterm birth [(18)].

Environmental tobacco smoke is a recognized factor of morbidity and mortality. The first victims are children, sometimes starting from conception[(19)]. However, the underlying physiological mechanisms for these ill-effects are not fully understood [(20)]. Moreover, recently

conducted studies also indicate that prenatal exposure to tobacco smoke is a risk factor for respiratory infections, asthma, allergy, childhood cancer, and it has neurobehavioral consequences regarding children's health [(21)].

Oxidative stress is a condition in which production of oxidative species overcomes antioxidant defenses, resulting in oxidative damage. Oxidative stress is thought to underlie many conditions related to tobacco exposure and it is thought that hypoxic conditions in the placenta can lead to oxidative stress. Studies of the acute effects of exposure to tobacco smoke on oxidative stress suggest that smoking results in increased products of lipid peroxidation and degradation products of extracellular matrix proteins[(22)]. Smokers have evidence of greater oxidative damage to DNA than non-smokers, as measured by 8-hydroxydeoxyguanosine (8-OH-dG), regardless of the biologic material studied. Exposure of human plasma to cigarette smoke results in increased damage to proteins, as evidenced by elevated levels of protein carbonyls. In addition, there is evidence that smokers have reduced blood levels of antioxidant micronutrients (such as vitamin C, α-carotene, β-carotene, and cryptoxanthin) compared with nonsmokers.

From another perspective, a significant flux of toxic metals, along with other toxins, reaches the lungs through smoking[(23)]. Also, cigarette smoking is a source of radiation exposure due to the concentrations of natural radionucleotides in the tobacco leaves[(24)]. In this respect, **Galazyn-Sidorczuk**[(25)] estimated exposure to cadmium (Cd) and lead (Pb) through cigarette smoking, the concentrations of both metals in the blood or/and urine of smokers and their non-smoking counterparts inhabiting an environmentally unpolluted area. The results give clear evidence that in the case of inhabitants of areas unpolluted with Cd and Pb habitual cigarette smoking, due to tobacco contamination, creates a serious source of chronic exposure to these metals, especially to Cd. Moreover, **Massadeh et al.**[(26)] estimate the average quantity of Cd and Pb inhaled from smoking one packet of 20 cigarettes to be in the range of 3.65-7.30 μg, and 0.74-2.22 μg, respectively.

There are some other trace elements, like heavy metals, that may cause damage to the mechanisms involved in fetal growth. Placenta acts as a barrier for some toxic elements, thus avoiding transference of them to the fetus (e.g. Cd), it does not have a restrictive barrier to protect against other heavy metals (e.g. Pb) transference.

Aim of the study: The aim of the study was to assess the effect of maternal passive smoking on birth weight, Apgar score, and on maternal and fetal serum proteins and oxidative stress markers.

SUBJECTS & METHODS

Subjects:

The study subjects consisted of 63 parturient mothers and their neonates admitted to Assiut University Hospital for delivery from October 2008 till July 2010. The inclusion criteria were being in labor, whether primigravida or multigravida,

not active smoker, and having a currently smoking husband. Women age ranged between 18 and 40 years, with a mean 24.9±5.6 years.

Data collection tools: Data collection tools consisted of an interview questionnaire form and a physical assessment and lab sheet.

- Interview questionnaire form: This form was designed by the researchers to collect the following data:
  - Socio-demographic data: such as mother age, education, residence, and consanguinity.
  - Exposure to passive smoking: details of husband smoking history as the number of cigarettes and the duration of smoking in years, which were used to calculate the smoking index; it also included data about other sources of exposure to passive smoking at home, work, or outside, as well as habits related to smoking at home, e.g. opening windows, etc.
  - Obstetric history: such as gravidity, parity, history of abortions, as well as the details of current pregnancy, antenatal care attendance, and admission details.
- Physical assessment and lab sheet: This sheet was used to record the following:
  - Infant birth weight and Apgar score at the first and fifth minutes.
  - Lab results, which included the levels of lead, cadmium, and cotinine in maternal urine, as well as the levels of albumin, proteins, lipid, iron, zinc and superoxide dismutase activity in maternal and fetal serum.

Methods:

Urine was collected from mothers at the time of delivery by a catheter to avoid contamination with blood. A 10-20 ml sample of urine was placed in a plastic container and frozen at -15 to -20°C until the time of the assay. Blood samples were collected from mothers and their neonates. They were centrifuged and the sera were kept frozen at -20°C until time of assay.

Serum samples were used for determination of the following biochemical parameters.

- **Cotinine** using solid-phase competitive chemiluminescent immunoassay with Immulite and Immulite 1000 analyzer manufactured by Euro/DPC Lrd.
- **Superoxide dismutase** (SOD) activity was evaluated by spectrophotometric method [(27)].
- **Lipid peroxidation** marker thiobarbituric acid reactive substances (TBARS) by spectrophotometry at 530 nm[(28)].
- **Serum albumin** was measured by modified bromocresol green colorimetric method [(29)].
- Serum total proteins were measured by colorimetric method
- **Serum copper, iron and zinc** were measured by atomic absorption spectrophotometry. The wavelength for Copper, Iron, and Zinc were 324.7 nm, 248.3 nm, and 213.9 nm, respectively and the Lamp current for the 3 elements was 10 mA.

Urine samples were used for estimation of these parameters.

- **Cotinine** using solid-phase competitive chemiluminescent

immunoassay with Immulite and Immulite 1000 analyzer manufactured by Euro/DPC Lrd.

- **Heavy metals** lead and cadmium were measured by atomic absorption spectrophotometry (Shimadzu-model AA-630-02), with flame emission, using air acetylene flame and hollow cathode lamp. For Cadmium, the wavelength was adjusted to 228.8 nm, and the Lamp current was 6 mA. For Lead, the wavelength was 283.3 nm, and the Lamp current was 6 mA.

Statistical analysis:

Data entry and analysis were done using SPSS (version 13.0) statistical software package. For multiple group comparisons of quantitative data, one-way analysis of variance test (ANOVA) was used. When normal distribution of the data could not be assumed, the non-parametric Mann-Whitney or Kruskal-Wallis tests were used instead. Qualitative categorical variables were compared using chi-square test. Statistical significance was considered at p-value <0.05).

RESULTS

Table (1) describes the patterns of exposure to cigarette smoke, and shows that husbands were smoking 3 to 48 cigarettes per day, for a period of four to twenty years. The mean smoking index was 205.6±113.2 pack/years. They were mostly smoking indoors (90.5%), and with closed windows in 33.3% of the cases. 34.9% were additionally exposed to others smoking at home, with a smoking index 200 pack/years. The total smoking index ranged 36.0 to 772.0 pack/years. The duration of women's exposure to passive smoking ranged three to 15 hours, and the number of cigarettes ranged three to 25 cigarettes. Only 12.7% of the women were aware of the hazards of smoking.

Investigating the relation between exposure to passive smoking and women's obstetric history (Table 2) revealed that those with a history of abortion had a significantly higher mean of smoking index, compared to those with no history of abortion (273.9±78.2 and 178.2±114.1, respectively, (p=0.002). Moreover, women with mucinous consistency of waters had a higher mean of smoking index (229.4±107.9 pack/years), compared to those with watery consistency (169.3±113.6 pack/ years), and the difference was statistically significant.

Table (3) shows women that were categorized according to levels of exposure to passive smoking, statistically significant relations were revealed between the degree of exposure and women's gravidity (p=0.001), number of living children (p=0.02), history of previous abortions (p0.01), and the gestational weeks at labor or pre-term delivery (p=0.03). As evident from table(3), women with the highest grade of exposure were more multigravida (62.5%), mostly had five or more children (54.2%), had more history of previous abortion (50.0%), and had more pre-term labors (70.8%), compared to those with low or moderate exposure.

As regards fetal outcome, Table 4 shows that birth weight was lowest among those with moderate exposure and highest among those with low exposure, but the difference was not statistically significant (p=0.13). Meanwhile, half of the infants born to women with low exposure had their Apgar score at the first minute less than seven (50.0%), compared to 15.8% and 20.8% of those with moderate or high exposure, respectively, and the difference was statistically significant (p=0.034). However, this significant difference disappeared at the fifth minute.

Table (5) illustrates that neither cotinine nor cadmium in urine had any statistically significant relation with pregnancy and labor outcomes. Meanwhile, lead in urine was statistically significantly related to the quality of waters (p=0.01), and Apgar score at first (p=0.01) and fifth (p=0.02) minutes. It is noticed that urine lead levels that were higher with watery quality (89.0±43.9), and Apgar scores less than 7 at the first (94.2±41.0) and fifth (93.6±67.3) minutes.

Women with moderate and high exposures had higher means of urinary cadmium and zinc (Table (6). However, only the relation with zinc reached statistical significance (p=0.02), although the relation with cadmium was of borderline significance (p=0.05). As regard the relation between the levels of maternal exposure to passive smoking and different fetal laboratory tests, table (7) points to no statistical significance.

Table (1): Exposure to passive cigarette smoking among parturient women in the study sample and their related knowledge (n=63)

	Frequency	**Percent**
Husband smoking:		
Cigarettes number:		
Range	3-48	
Mean ± SD	18.0±8.7	
Years:		
Range	4-20	
Mean ± SD	11.3±4.0	
Smoking index (pack/year):		
<200	29	46.0
200+	34	54.0
Range	15-480	
Mean ± SD	205.6±113.2	
Habits while smoking:@		
Inside rooms	57	90.5
Windows are not open	21	33.3
Smoking index for others at home:		
<200	41	65.1
200+	22	34.9
Range	0.0-432.0	
Mean ± SD	144.7±137.1	
Total smoking index at home:		
<200	20	31.7
200	19	30.2
400+	24	38.1
Range	36.0-772.0	
Mean ± SD	350.3±214.4	
Exposure to passive smoking:		
Hours:		
<5	27	42.9
5+	36	57.1
Range	3.0-15.0	
Mean ± SD	5.6±2.3	
Number of cigarettes:		
<10	16	25.4
10+	47	74.6
Range	3.0-25.0	
Mean ± SD	12.2±4.6	
Know the hazards of exposure to smoking	8	12.7

Table (2): Relation between smoking and parturient women obstetric history and current labor

	Total smoking index in pack/year (Mean±SD)	**Mann Whitney Test**	**p-value**
History of abortion:			
No	178.2±114.1		
Yes	273.9±78.2	9.34	0.002*
History of stillbirth:			
No	198.8±112.0		
Yes	241.6±118.8	1.26	0.06
History of preterm labor:			
No	208.7±113.7		
Yes	109.5±14.8	1.58	0.21
History of congenital anomalies:			
No	208.6±111.5		
Yes	15.0±0.0	2.92	0.09
Gestational weeks at labor:			
<37	230.7±107.1		
37+	177.9±115.1	3.49	0.06
Rupture of membranes:			
Spontaneous	188.9±87.1		
Induced	220.8±132.2	1.10	0.29
Quality of waters:			
Watery	169.3±113.6		
Mucinous	229.4±107.9	5.33	0.02*
Baby birth weight (kg):			
<2.5	181.2±126.2		
2.5-	217.1±104.0	H=1.35	0.51
3.5+	21.9.6±115.4		
Apgar score (min 1):			
<7	176.7±96.8		
7-10	217.1±118.2	1.17	0.28
Apgar score (min 5):			
<7	183.0±96.9		
7-10	213.9±118.6	0.50	0.48

Table (3): Relation between obstetric history and current pregnancy and labor characteristics of parturient women in the study sample and the level of exposure to passive smoking

	Smoking exposure index (cigarettes/years)						**X^2 (p-value)**
	Low (<200)		**Moderate (200-<400)**		**High (400+)**		
	No.	**%**	**No.**	**%**	**No.**	**%**	
Gravidity:							
2-4	18	90.0	14	73.7	9	37.5	14.12
5+	2	10.0	5	26.3	15	62.5	0.001*
Parity:							
2-4	18	90.0	16	84.2	17	70.8	--
5+	2	10.0	3	15.8	7	29.2	--
No. of living children:							
1	9	45.0	7	36.8	6	25.0	
2-4	9	45.0	8	42.1	5	20.8	11.31
5+	2	10.0	4	21.1	13	54.2	0.02*
Previous abortion:							
No	18	90.0	15	78.9	12	50.0	9.31
Yes	2	10.0	4	21.1	12	50.0	0.01*
Previous stillbirth:							
No	17	85.0	18	94.7	18	75.0	--
Yes	3	15.0	1	5.3	6	25.0	--
Previous pre-term labor:							
No	18	90.0	19	100.0	24	100.0	--
Yes	2	10.0	0	0.0	0	0.0	--
Previous infant with congenital anomaly							
No	19	95.0	19	100.0	24	100.0	--
Yes	1	5.0	0	0.0	0	0.0	--
Gestational weeks:							
<36	6	30.0	10	52.6	17	70.8	7.29
36+	14	70.0	9	47.4	7	29.2	0.03*
Had antenatal care:							
No	8	40.0	3	15.8	6	25.0	2.98
Yes	12	60.0	16	84.2	18	75.0	0.23
Rupture of membranes:							
Spontaneous	8	40.0	11	57.9	11	45.8	1.30
Induced	12	60.0	8	42.1	13	54.2	0.52
Quality of waters:							
Watery	11	55.0	9	47.4	5	20.8	5.99
Mucinous	9	45.0	10	52.6	19	79.2	0.050
4^{th} stage problems:							
No	16	80.0	19	100.0	24	100.0	--
Yes	4	20.0	0	0.0	0	0.0	--

Table (4): Relation between fetal outcome of parturient women in the study sample and the level of exposure to passive smoking

	Smoking exposure index, (cigarettes/years)						**Test (p-value)**
	Low, (<200)		**Moderate,(200-<400)**		**High,(400+)**		
	No.	%	No.	%	No.	%	
Baby birth weight (kg):							
<2.5	9	45.0	4	21.1	8	33.3	
2.5-	8	40.0	9	47.4	14	58.3	
3.5+	3	15.0	6	31.6	2	8.3	
Range	1.5-3.5		2.0-4.0		2.0-3.5		F=4.07
Mean ± SD	2.4±0.7		2.9±0.7		2.6±0.5		0.13
Apgar score (min 1):							
<7	10	50.0	3	15.8	5	20.8	X^2=6.73
7-10	10	50.0	16	84.2	19	79.2	0.034*
Apgar score (min 5):							
<7	8	40.0	4	21.1	5	20.8	X^2=2.52
7-10	12	60.0	15	78.9	19	79.2	0.28

Table (5): Relation between the levels of cotinine, lead, and cadmium in urine and parturient women obstetric history and current labor

	Urine Cotinine (µg/dl)		**Urine Pb (µg/dl)**		**Cd Urine (µ0g/dl)**	
	Mean±SD	p-value[@]	Mean±SD	p-value[@]	Mean±SD	p-value[@]
History of abortion:						
No	72.3±85.6		74.1±45.3		36.1±20.8	
Yes	81.5±101.8	0.95	75.3±38.7	0.93	38.9±27.1	0.86
History of stillbirth:						
No	67.2±86.3		71.1±42.4		39.1±22.7	
Yes	115.8±101.7	0.051	92.1±45.5	0.31	25.0±18.6	0.09
History of preterm labor:						
No	75.2±90.7		74.7±43.9		36.5±22.9	
Yes	66.6±75.6	0.83	65.0±0.0	0.84	46.6±0.0	0.54
History of congenital anomalies:						
No	75.6±90.3		73.8±43.3		36.6±22.7	
Yes	31.0±0.0	0.93	112.5±0.0	0.46	50.0±0.0	0.46
Gestational weeks at labor:						
<37	66.5±88.5		78.9±43.7		40.5±24.2	
37+	84.2±91.8	0.37	69.5±42.9	0.41	32.8±20.4	0.26
Rupture of membranes:						
Spontaneous	74.7±91.2		67.8±49.2		43.4±26.8	
Induced	75.1±89.9	0.91	80.4±36.7	0.13	30.9±16.1	0.09
Quality of waters:						
Watery	70.1±77.3		89.0±43.9		33.8±20.6	
Mucinous	78.1±98.0	0.93	64.8±40.6	0.01*	38.9±23.9	0.53
Baby birth weight, (kg):						
<2.5	76.3±95.7		72.0±42.0		32.7±24.7	
2.5-	76.7±91.2	0.90	74.9±45.3	0.98	37.8±22.4	0.45
3.5+	67.2±81.3		77.6±43.4		42.0 ±19.4	
Apgar score (min 1):						
<7	66.6±69.5		94.2±41.0		41.2±18.3	
7-10	78.3±97.2	0.96	66.5±42.0	0.01*	35.1±24.1	0.17
Apgar score (min 5):						
<7	79.8±88.1		93.6±44.5		42.3±20.1	
7-10	73.1±91.3	0.65	67.3±41.0	0.02*	34.8±23.3	0.13

Table (6): Relation between laboratory results (Mean±SD) of parturient women in the study sample and the level of exposure to passive smoking

	Smoking exposure index (cigarettes/years)			**Kruskal Wallis Test**	**p-value**
	Low (<200)	**Moderate (200-<400)**	**High (400+)**		
Cotinine in urine (µg/dl):					
Range	13.1-292.0	10.9-292.0	5.0-292.0		
Mean±SD	75.8±83.7	96.6±10703	57.1±77.7	1.58	0.45
Lead in urine (µg/dl):					
Mean±SD	25.0-145.0	24.0-140.0	23.0-140.0		
Median	81.0±42.6	72.1±46.2	70.7±42.6	0.73	0.70
Cadmium in urine (µg/dl):					
Range	7.1-50.0	7.5-70.0	7.1-93.5		
Mean ± SD	26.8±15.5	40.2±22.7	42.5±25.4	5.90	0.05
Serum albumin (mother) (g./dl):					
Range	2.5-5.0	2.1-6.0	2.6-4.0		
Mean ± SD	3.2±0.6	3.5±1.0	3.2±0.3	1.93	0.38
Serum protein (mother)(g./dl):					
Range	4.2-7.7	4.2-7.9	4.2-8.0		
Mean ± SD	5.9±1.1	6.0±1.2	6.±1.2	0.42	0.81
Superoxide Dismutase (mother)(µg/ml):					
Range	1.2-3.6	1.2-3.6	1.2-3.6		
Mean ± SD	2.1±0.8	2.3±0.8	2.5±0.7	1.82	0.40
Lipid peroxide (mother) (mmol/l):					
Range	1.3-4.8	1.8-4.8	1.3-4.8		
Mean ± SD	3.5±1.1	4.1±0.7	4.0±0.9	2.92	0.23
Copper (mother)(µg/dl):					
Range	60.0-150.0	60.0-175.0	65.0-175.0		
Mean ± SD	106.5±29.2	117.8±35.5	114.3±26.9	0.93	0.63
Iron (mother)(µg/dl):					
Range	75.0-155.0	70.0-190.0	70.0-190.0		
Mean ± SD	118.7±24.7	126.9±34.7	118.6±36.5	0.75	0.69
Zinc (mother)(µg/dl):					
Range	75.0-190.0	75.0-200.0	80.0-200.0		
Mean ± SD	117.2±34.1	149.5±44.7	147.0±37.6	7.65	0.02*

(*) Statistically significant at $p<0.05$

Table (7): Relation between laboratory results (Mean±SD) of infants delivered to parturient women in the study sample and the level of exposure to passive smoking

	Smoking exposure index (cigarettes/years)			**Kruskal Wallis Test**	**p-value**
	Low (<200)	**Moderate (200-<400)**	**High (400+)**		
Serum albumin (infant) (g./dl):					
Range	1.5-4.0	2.0-3.2	1.5-4.0		
Mean ± SD	2.7±0.5	2.6±0.4	2.8±0.5	0.69	0.71
Serum protein (infant) (g./dl):					
Range	3.5-6.1	3.5-5.4	3.5-6.1		
Mean ± SD	4.3±0.7	4.4±0.5	4.4±0.7	0.94	0.62
Superoxide Dismutase (infant)(µg/ml):					
Range	0.8-2.7	0.8-3.0	0.9-2.3		
Mean ± SD	1.5±0.7	1.5±0.7	1.3±0.6	2.40	0.30
Lipid peroxide (infant)(mmol/l):					
Range	1.5-6.4	0.6-5.4	0.4-6.4		
Mean ± SD	3.3±0.9	2.7±1.2	3.0±1.2	4.75	0.09
Copper (infant) (µg/dl):					
Range	38.0-100.0	38.0-112.0	43.0-112.0		
Mean ± SD	73.3±20.0	76.8±20.2	71.8±16.4	1.38	0.50
Iron (infant)(µg/dl):					
Range	73.0-175.0	79.0-200.0	72.0-200.0		
Mean ± SD	128.1±33.6	134.6±34.1	128.5±32.8	0.74	0.69
Zinc (infant) (µg/dl):					
Range	85.0±170.0	100.0-180.0	100.0-200.0		
Mean ± SD	135.7±26.9	143.3±29.7	142.1±32.1	0.84	0.66

DISCUSSION

Low birth weight (LBW) constitutes a major concern in different countries, especially in developing countries because of its potential risks in several public health problems. The present study revealed an association between women's gravidity and exposure to passive smoking. Thus, the percentage of grand-multigravida was highest with a high grade of exposure to passive smoking. A similar finding was reported by **Nakamura et al.** [31] who studied the obstetric and perinatal effects of smoking on pregnancy and the infant. Also, it is in agreement with a study in Brazil, which found that active and passive smokers had higher parity. **Nassar et al.**[30] reported that grand multiparity was associated with a low socioeconomic status, and higher incidence of smoking.

Concerning the relation between abortion and exposure to passive smoking, it was found in the present study that women with a history of abortion had a statistically

significantly higher mean of smoking index, compared to those with no history of abortion. Moreover, women with the highest grade of exposure had more history of previous abortion, compared to those with low or moderate exposure. These findings are in congruence with those of **Nielsen et al.**[32] in Denmark, whose study demonstrated a clear dose-response between smoking and the risk of spontaneous abortion.

Also, in agreement with the present study finding, **George et al.**[2], in Sweden, who found that the risk of spontaneous abortion was increased among women exposed to both passive and active smoking and concluded that nonsmoking pregnant women exposed to passive cigarette smoke may be at increased risk of spontaneous abortion. Similarly, Meeker et al. [33] studied maternal exposure to second-hand tobacco smoke and pregnancy outcome. They demonstrated that woman's exposure to second-hand smoke as a child or in utero may be associated with an increased risk of spontaneous abortion in adulthood.

However, in disagreement with the present study, **Wisborg et al.**[34] **in** a prospective study found no association between smoking and first- and second-trimester abortions. Adjustment for alcohol, coffee, maternal age, marital status, occupation, education, pre-pregnancy body mass index and parity did not change the result substantially. Although that study was a prospective one, assessment of exposure depended on self-reported questionnaire, with no measurement of cotinine. This might explain the discrepancy with results of the present study.

The present study also revealed that exposure to passive smoking was associated with a higher risk of pre-term labor. In agreement with **Mohammah- Alizadeh and Mirzaie**[35] who performed a descriptive-analytic study in Iran and demonstrated that women with exposure to cigarette smoke should be considered as a major risk for preterm labor. In a similsr perspective, **Haskins et al.** [36], in USA, studied the effect of cigarette smoking during pre-pregnancy, early pregnancy, and mid pregnancy on preterm birth among Hispanic women. Their results demonstrated that smoking in pre-pregnancy was not associated with preterm birth. However, after adjustment for age, parity, education, and illicit drug use, exposure to smoking in early or mid pregnancy increased the risk of spontaneous preterm birth.

However, in disagreement with the present study results related to risk of pre-term delivery, **Rodrigues and Barros** [37] investigated the risk factors for small-for-gestational-age and preterm in a Portuguese cohort of newborns. They demonstrated that smoking was a risk factor for small-for-gestational-age, but not preterm. No factor could be shown to have a great contribution to preterm birth. The contradiction with the present study finding might be attributed to other risk factors for pre-term delivery that needed to be included in the study.

As regards fetal outcome, the present study findings indicated better Apgar score at the first minute among

infants whose mothers had moderate or high exposure to cigarette smoke. However, this difference disappeared at the fifth minute. The systematic review done by **Grange and Pannier** [38] in France provides support as well as an explanation to that finding. According to these authors, data in the literature is not sufficient to argue in favor of an association between fetal asphyxia during labor and smoking. Nevertheless, the Apgar score does not appear to be modified by moderate maternal smoking. Paradoxically, maternal smoking could have a protective effect on meconial aspiration. However, these findings should be examined with caution because they still need to be confirmed and do not take into consideration other adverse effects of smoking on the fetus. Thus, **Schneider et al.** [39], in Canada, had opposing findings regarding the effects of prenatal cigarette smoke exposure and hypoxemia on cardiorespiratory control in full-term infants. Meanwhile, **Gilman et al.** [40] could not demonstrate any association between maternal smoking during pregnancy and Apgar score.

Concerning heavy metals associated with cigarette smoke, the present study assessed the levels of lead, cadmium, and zinc. In fact, previous studies demonstrated the association of these metals with smoking. Thus, **Szyszko and Czarnowski** [41] determined the levels of cadmium, lead, and zinc in placenta, maternal blood and cord blood of 53 women at delivery from non-smokers [NS], passive smokers [PS] and smokers [S] who were identified by the questionnaire. The results revealed that smoking women had higher cadmium and lead levels than mothers who never smoked, but there was no smoking impact on the zinc levels.

Furthermore, the present study revealed that women with moderate and high exposures to passive smoking had higher means of urinary zinc. The finding is in congruence with that of **Kutlu et al.** [42] who assessed the levels of cadmium, lead, copper, and zinc status in pregnant women exposed to cigarette smoke in Turkey. The results showed that zinc levels were lower in nonsmokers. However, on the contrary, **Razagui and Ghribi** [43] in the United Kingdom, reported that cigarette smoking was associated with lower zinc and higher cadmium and lead concentrations. The discrepancies among various studies might be related to the methods of measurement of heavy metals that need special arrangements to ensure lack of environmental contamination of the samples.

Lastly, the present study findings point to some effects of lead exposure on the quality of the amniotic fluid, as well as on the newborn Apgar score. Higher levels of urine lead levels were associated with significantly lower levels of Apgar scores at the first and fifth minutes. These findings need further investigations for more confirmation, as well as laboratory studies to understand the possible underlying mechanisms.

Normally fetus-placental system is exposed to different metabolites some of them that may be toxic for cell function are destroyed by different defense mechanism systems

that ensure normal intrauterine growth and development of the embryo. Within the unavoidable intermediates produced during metabolism are the reactive oxygen species (ROS), and although they may have beneficial effects to cell function, their level and balance between production and degradation must be controlled, to avoid risk of oxidative damage. Increased levels of ROS like superoxide anion, hydrogen peroxide, peroxynitrite and hydroxyl radical, are involved in pathogenesis of many diseases and ageing [(44)]. ROS are able to block activity or modify several macromolecules such as proteins, lipids and DNA, thereby destroying integrity of cells [(45)]. Human placenta and fetus may be also a target for that toxicity. Placental oxidant/ antioxidants imbalance may release different oxidation products to the circulation, causing damage to endothelial cell membranes related to several pathological conditions during pregnancy e.g. pre-eclampsia, hypertension and pregnancy induced diabetes [(46)]. Efficiency of various ROS scavengers, enzymatic or non-enzymatic present in the placenta and the fetus ensure normal physiology of that system and as a consequence, normal intra-uterine growth.

The role of antioxidants enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in inhibiting lipid peroxidation in placenta and neonatal blood has been already reported. Two of these enzymes are dependent of essential trace elements for its functional activity. Thus, GPx is dependent on selenium (Se-GPx) and SOD dependent on Copper/Zinc (Zn/Cu-SOD). Then, it is conceivable that any decrease in these essential trace elements or the replacement of them with some other toxic heavy metal in the placental enzymes would result in decreased activity of them. This may result in augmented oxidative damage in the fetus-placental system with a potential risk for normal fetal growth[(47)].

CONCLUSION & RECOMMENDATIONS

The study findings indicate that very few women were aware of the hazards of smoking. Exposure to passive smoking was associated with higher history of abortion, more mucinous consistency of waters, and more pre-term labors. Birth weight was not significantly affected. Lead in urine was significantly related to the quality of waters, and Apgar scores. High levels of exposure to passive smoking were associated with higher levels of urinary cadmium and zinc.

In the light of these findings, it is recommended that pregnant women be warned of the detrimental effects of exposure to passive smoking, and encouraged to protect themselves for healthy fetal development. The nurse during antenatal care visits could have an influential role on mothers through health education, or even counseling in the presence of the husband. The effect of lead exposure on the quality of the amniotic fluid deserves further study.

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التأثير البيوكيميائى للتدخين السلبى على الامهات عن طريق قياس مستوى الكوتينين وأثره على وزن الطفل الرضيع والاكسد ة الشدية والعناصر السمية والاساسية النادرة

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ان تعرض الامهات للتدخين السلبى فى فترة ما قبل الولادة يؤثر على الاطفال ، ويؤدى الى مضاعفات فى الحمل تؤدى الى خلل فى المواد المضادة للاكسدة التى لها تغيرات مرضية وفسيولوجية على الرضيع.

اجرى هذا البحث على ٦٣ سيدة حامل واطفالهن الرضع وتم أخذ عينات بول من الام وعينات من الرضع و الامهات ووجد ان هناك علاقة ارتباط ايجابية بين مستوى الكوتينين فى الرضع والامهات ونستنتج أنه لابد من الوقاية والتقليل من التعرض للدخان اثناء الحمل الذى له تأثير يعود على حجم الرضيع عند الولادة