Clinical and Biochemical Effects of Environmental Tobacco Smoking On Pregnancy Outcome

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Abstract The effects of environmental tobacco smoke (ETS) are less studied especially on neonates. This study evaluates the clinical and biochemical effects in neonates exposed to ETS during pregnancy. Two hundred pregnant women asked to complete the questioners about their ETS. Ninety from them were enrolled in biochemical assays as two groups according to ETS. The cotinine level determined in saliva and serum of mothers to confirm their tobacco exposure. The routine tracheal suction from the fetus was used to determine the level of neuron specific enolase (NSE), soluble E-cadherin, sApo-1/Fas, nitric oxide (NO) and cotinine. In clinical assessment, the percent of full term babies in non-exposed group (72 %) are higher compared to exposed group (67 %). Apgar score at the first min, admission to intensive care unit (ICU) and morbidity during the first month shows statistical significance increase in exposed compared to non-exposed group ($p = 0.03, 0.05, 0.01$, respectively). The new born weight in exposed group significantly decreased compared to non-exposed group ($2,850 \text{ g} \pm 3.74$

vs $2,967.67 \text{ g} \pm 3.34$; $p = 0.02$). In biochemical assessment, NSE and sE-cadherin significantly increased, while NO significantly decreased ($p = 0.000$) in exposed compared to non-exposed group. There is a positive correlation between level of cotinine and both NSE, sE-cadherin ($r = 0.7, 0.9$; $p = 0.000, 0.006$, respectively). To our knowledge, this is the first study link between prenatal tobacco exposure (PTE) and biochemical parameters measured in tracheal suction. PTE will lead to decrease in birth weight most probably by decreasing NO, sFas, and increasing sE-cadherin. While, increased morbidity of neonates in the exposed group could be attributed to cessation of breast feeding and its complication and increased NSE in the studied markers.

Keywords Neonate · Prenatal tobacco exposure · NSE · sFas · Cotinine · NO · Tracheal suction

Introduction

Passive smoking is the inhalation of smoke from tobacco products used by others. Also, called secondhand smoke (SHS) or environmental tobacco smoke (ETS). SHS has been less well studied and is less consistent in the literature as regard adverse pregnancy outcomes. Moreover, smoking is the most preventable risk for poor pregnancy outcomes. Prenatal tobacco exposure (PTE) defined as any exposure across pregnancy and, or exposure within each trimester. PTE was a significant predictor of increased activity and attention problems in child behavior [1]. Cotinine levels during pregnancy may predict birth weight [2], head circumference [3] and IQ scores [4]. Whereas, reduction in its level associated with improved outcomes. Cotinine concentrations in plasma and urine showed a significant dose-dependent difference among non-smokers, passive and active smokers [5].

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Table 1 Socio-demographic data of all participants

Characteristics	Non-exposed group <i>n</i> = 100 No. (%)	Exposed group <i>n</i> = 100 No. (%)	χ^2 value	<i>p</i> value
Residence				
Rural	67	78	3.03	0.08
Urban	33	22		
Maternal education				
Illiterate	32	47	7.61	0.05*
Basic education	19	9		
Secondary	36	36		
University	13	8		
Maternal occupation				
Employed	13	3	6.79	0.009*
Unemployed ^a	87	97		
Husband's education				
Illiterate	25	24	4.24	0.2
Basic education	16	24		
Secondary	34	37		
University	25	15		
Husband's occupation				
Employee	24	8	10.98	0.02*
Manual	46	58		
Farmer	12	16		
Professional	17	18		
Worker	1	–		

*Significantly difference. As the total number of each group is 100, so the number is the same as percent

^a Unemployed includes irregular temporary jobs

Neuron specific enolase (NSE) is a marker of early cellular brain damage [6]. The cut-off threshold for salivary NSE of 3.7 mg/l was optimum, for differentiation of normal from abnormal individuals with 80 % accuracy [7]. Increased sFas serum concentration in prenatal and early postnatal, possibly indicates gradual decrease of proliferation and apoptosis in early postnatal life [8, 9]. The maternal nicotine exposure during last trimester of pregnancy alters a key component of lung innate immunity in offspring [10]. Specific cell adhesion molecules are important to lung development and airway morphogenesis. E-cadherin protein level was significantly upregulated in congenital lung lesions with abnormal airway branching [11]. Whereas, reduced production of the vasodilator nitric oxide (NO) in fetal vessels in pregnant smokers may lower the blood flow to the fetus and result in lower birth weight, length, and head circumference [12].

Hence, the aim of this study is to determine the cotinine level, NSE, soluble human epithelial cadherin (sE-cadherin),

Table 2 The effects of environmental tobacco smoking on current labor of both groups

Items	Non-exposed group <i>n</i> = 100 No. (%)	Exposed group <i>n</i> = 100 No. (%)	χ^2 value	<i>p</i> value
Membrane rupture				
Spontaneous	83	73	2.91	0.08
Induced	17	27		
Time of membrane rupture				
At first stage	45	41	0.33	0.5
At second stage	55	59		
Amniotic fluid status				
Clear	99	98	0.33	0.5
Stain with meconium	1	2		
Mode of delivery				
Normal vaginal delivery	85	97	8.79	0.000*
C.S.	15	3		
Causes of C.S.	Total No: 15	Total No: 3		
Failure of progress	7 46.7	2 66.7	0.5	N.A
Fetal distress	7 46.7	1 37.3		
Leakage of amniotic fluid.	1 6.7	0 0		

*Significantly difference. As the total number of each group is 100, so the number is the same as percent except in causes of Caesarian section (C.S)

sApo-I/Fas and NO in tracheal suction of exposed and non-exposed neonate to PTE just after delivery as it could reflect the internal environment of the fetus. Also, to explain some possible mechanisms of adverse effects of PTE.

Subjects and Methods

This study carried out among primiparous women in Maternal Health Center of Assiut University Hospital in Assiut city. It is prospective, cross sectional, hospital based study. Women with mal position baby, or ante-partum hemorrhage were excluded. At the start of the work, 200 pregnant women with a singleton pregnancy and gestational age more than 28 weeks were asked to complete the questionnaires (maternal sheet) by the help of well-trained nurse, also, another sheet after delivery to determine the morbidity of the fetus (neonatal assessment sheet). From those, only 90 women with their infants were submitted to biochemical analysis. The women were divided into two groups according to exposure to ETS each group includes 45 women. Apgar score at 1 and 5 min and assessment observation sheet used to determine the fetal condition after birth and 1 month later. Before labor, two samples

from the mother (urine by catheter and saliva) obtained to confirm exposure or non-exposure to ETS and one sample from newborn as routine tracheal suction to measure specific biochemical markers. Suctions contained meconium were excluded. Ethical approval for the study was obtained from the Institutional Ethics Committee and Review Board of the Faculty of Medicine, Assiut University. Informed and written consent was obtained from all participants.

Biochemical Assays

Tracheal suction obtained from all new born just after delivery for determination of specific biochemical markers. NSE (EC 4.2.1.11) determined by two monoclonal antibodies using EIA kit (Sweden). Quantitative sandwich enzyme immunoassay technique used for determination of sE-cadherin and sAPO-1/Fas by commercial kits (USA). Serum level of NO measured by colorimetric method as total nitrite concentration [13]. Cotinine measured by chemical method according to Peach et al. [14]. Briefly, 0.5 ml of sample pipetted into small test tube with 4 M acetate buffer (0.2 ml), pH 4.7. Then, the mixture reacted by the sequential addition at 15 s intervals of freshly prepared 10 % aqueous KCN (0.1 ml), 10 % aqueous chloramine-T (0.1 ml), and 0.5 ml of 1 % barbituric acid in acetone and water (1: 1 v/v). A positive result was indicated by the appearance of an orange color within 20 min. The samples were measured at 506 nm 20 min after reaction and compared with the reading given by an aqueous solution of 10 µg/ml cotinine.

Statistical Analysis

Data were statistically analyzed using SPSS version 16 for Windows and expressed as mean $\pm$ SE. Statistical analysis performed using Mann–Whitney U test, Chi square and Pearson's test. A p value of ≤ 0.05 was considered statistically significant.

Results

Clinical Assessment

In this study, no significant differences between maternal age in exposed and non-exposed groups (22.56 ± 3.9 vs 22.83 ± 3.5 , respectively) was observed. There is increased percent of full term babies in non-exposed group (72 %) compared to exposed group (67 %). The new born weight in exposed group significantly decreased ($2,850 \pm 3.74$ vs $2,967.67 \pm 3.34$ g; $p = 0.02$) compared to non-exposed group. Demographic data of all mothers shows significant differences in maternal's education and occupation

Fig. 1 Apgar score at the first min after labor, showed significant differences between *exposed* and *non-exposed* groups to ETS by Chi square, ($X^2 = 6.96$, $p = 0.03$). While majority of new born in both groups had normal Apgar score after 5 min ($X^2 = 0.803$, $p = 0.3$)

Table 3 The effect of PTE on newborn in both groups

Items	Non-exposed group		Exposed group		X^2 value	p value
	No	%	No	%		
Vital status	$n = 100$		$n = 100$		2.202	0.1
Live	98	98	100	100		
Still birth	2	2	0	0		
Admission to NICU	$n = 98$		$n = 100$			
Yes	7	7.1	16	16	3.78	0.05*
No	91	92.9	84	84		
Apgar score at 1st min	$n = 98$		$n = 100$			
Normal	77	78.6	63	63	6.96	0.03*
Mild distress	19	19.4	36	36		
Severe distress	2	2	1	1		
Apgar score after 5 min	$n = 98$		$n = 100$			
Normal	94	95.9	93	93	0.803	0.3
Mild distress	4	4.1	7	7		
Newborn examination	$n = 98$		$n = 100$			
Normal	97	99	98	98	0.318	0.5
Abnormal	1	1	2	2		
Causes of admission to NICU	$n = 7$		$n = 16$			
LBW	3	42.8	7	43.8	2.38	0.3
Fetal distress	2	28.6	8	50		
LBW and fetal distress	2	28.6	1	6.2		

NICU neonatal intensive care unit, LBW Low birth weight

*Significantly difference

(Table 1). One of interesting results in this study, is the increased rate of cesarean section in non-exposed group than exposed group ($p = 0.000$; Table 2). Apgar score at the first min after labor shows significant differences between two groups ($p = 0.03$; Fig. 1), but after 5 min Apgar score became insignificant (Table 3). Also, there is significant

Fig. 2 There is significant differences between *exposed* and *non-exposed* groups to ETS as regard *admission to ICU* by Chi square ($X^2 = 3.78$, $p = 0.05$). But the cause of admission as low birth weight, fetal distress or both did not differ significantly ($X^2 = 2.38$, $p = 0.3$)

differences between both groups as regard admission to intensive care unit (ICU) ($p = 0.05$), but the cause of admission as low birth weight, fetal distress or both did not differ significantly (Fig. 2). In follow up after 1 month, there was significant increase in morbidity in exposed group ($X^2 = 15.16$, $p = 0.01$). As regard the cause of illness, only diarrhea that show significant increase ($p = 0.05$) in exposed group (Fig. 3). The effects of PTE on fetus (Table 4) show significant decreased in breast-feeding continuation in exposed compared to non-exposed group ($p = 0.008$; Table 5).

Biochemical Assessment

There is significant increase in cotinine level in maternal urine, saliva and neonatal suction of exposed group compared to non-exposed ($p = 0.000$; Table 4). A correlation between level of cotinine in mother and neonates ($r = 0.5$; $p = 0.000$) was detected. Also, a significant increase of NSE and sE-cadherin ($p = 0.000$, 0.001 , respectively) but decrease of NO ($p = 0.000$) in exposed compared to non-exposed group was detected. However, no significant difference in sFas level detected (Table 4). There is a negative

Fig. 3 The main morbidity of infants were *jaundice*, *chest infection*, *diarrhea* and *ear inflammation* with statistically significant differences between *exposed* and *non-exposed* groups to ETS ($X^2 = 15.16$, $p = 0.01$)

Table 4 The effects of PTE on new born within one month after labor

Items	Non-exposed group A		Exposed group	X^2 value	p value
	No.	%	No.		
Vital status	$n = 98$		$n = 100$	2.30	0.1
Alive	95	96.9	92		
Dead	3	3.1	8		
Newborn morbidity	$n = 95$		$n = 92$	15.16	0.01*
Jaundice	22	22.3	24		
Ear inflammation	3	3.2	6		
Chest infection	14	14.9	16		
Diarrhea	1	1.1	11		
Jaundice and ear inflammation	1	1.1	2		
Jaundice and chest infection	1	1.1	1		
None	53	56.4	32		
Breast feeding					
Continue	75		62	9.71	0.008*
Need supplementation	19		22		
Stopped	1		8		

*Significantly difference

correlation between birth weight and sFas and NSE ($r = -0.9$, -0.3 ; $p = 0.01$, 0.03), but, a positive correlation between level of cotinine in neonatal suction and NSE, sE-cadherin ($r = 0.7$, 0.9 ; $p = 0.006$, 0.000 , respectively). By regression analysis, sE-cadherin is a constant predictor for prognosis in neonate when level of cotinine is a dependent variable ($F = 26.74$; $p = 0.004$).

Discussion

ETS represents a potentially large public health problem as 22–30 % of non-smoking pregnant women are exposed to it.

Table 5 The effects of environmental tobacco smoking on mother after labor in both groups

Items	Non-exposed group <i>n</i> = 100		Exposed group <i>n</i> = 100		X^2 value	<i>p</i> value
	No.	%	No.	%		
Puerperium						
Normal	95	95	90	90	1.80	0.1
Complicated	5	5	10	10		
Type or complication						
Bleeding	2	40	6	60	1.50	0.4
Infection	3	60	3	30		
Others			1	10		

ETS-exposed women have increased risks of infants with lower birth weight, congenital anomalies, longer lengths, and trends towards smaller head circumferences [15].

Cotinine level in children's urine statistically differentiated children from exposed homes compared with non-exposed homes [16]. Most of the children considered non-exposed if cotinine levels below 1 ng/ml in their urine [17]. In this study, the presence of ETS exposure confirmed by questionnaires and cotinine level in urine and saliva of pregnant women. The new born weight in exposed group significantly decreased ($2,850 \pm 3.74$ vs $2,967.67 \pm 3.34$; $p = 0.02$) while the cotinine level (17.28 ± 0.81 , 1.81 ± 0.43) significantly increased compared to non-exposed group, the mean weight decrease is ~ 117 g corresponding to ~ 15 ng/ml increase in the cotinine level. This in agreement with El-Mohandes et al. [2] as reported that the urinary cotinine was the best predictor of infant irritability ($r = 0.7$) and salivary cotinine level of smoking mothers (20 to ≥ 100 ng/ml) was associated with significant reduction in birth weight ranged from 88 to 205 g, respectively [2]. In this study, there is significant increased level of NSE in exposed group to PTE and a positive correlation between level of cotinine and NSE ($r = 0.7$; $p = 0.006$) that may suggest some neuronal affection most probably in the form of irritability as El-Mohandes et al. [2] reported (Table 6).

Small for gestational age pregnancies had lower sFas levels than control subjects in the second trimester, but not in the first trimester [18]. This in agreement with this study as there is a decrease in the level of sFas in exposed group to ETS compared to non-exposed but not reach a significant level ($1,560 \pm 276$ vs $2,078 \pm 148$ pg/ml, $p = 0.1$). This could be explained as intrauterine fetal development is characterized by increased rates of proliferation and apoptosis, while both these processes may be attenuated in post-natal period. A higher apoptosis rate in the first trimester of pregnancy, possibly affecting maternal immuno-tolerance,

Table 6 Biochemical markers in tracheal suction of neonates after delivery

	ETS	Min–Max	Mean $\pm$ SE	<i>p</i>
Markers in maternal samples				
Cotinine in saliva (ng/ml)	Exposed	3.14–24.51	14.28 ± 0.70	0.000*
	Non-exposed	0.9–1.24	1.63 ± 0.45	
Cotinine in urine (ng/ml)	Exposed	2.57–20.92	11.09 ± 0.27	0.000
	Non-exposed	0.80–1.50	1.06 ± 0.07	
Markers in neonatal suction				
Cotinine (ng/ml)	Exposed	3.66–32.56	17.28 ± 0.81	0.000*
	Non-exposed	0.30–2.16	1.81 ± 0.43	
NSE (μ l)	Exposed	3.70–8.94	5.65 ± 0.19	0.000*
	Non-exposed	0.47–1.40	0.77 ± 0.11	
E-cadherin (ng/ml)	Exposed	2.40–4.40	$3.12 \pm .25$	0.001*
	Non-exposed	0.80–1.50	1.07 ± 0.22	
sAPO-1/Fas (pg/ml)	Exposed	803–2445	1560 ± 276	0.1
	Non-exposed	1248–2411	2078 ± 148	
NO (μ mol/ml)	Exposed	0.424–1.854	0.79 ± 0.04	0.01*
	Non-exposed	0.96–1.03	0.99 ± 0.02	

ETS environmental tobacco smoking, Min–Max Minimum–Maximum, NSE neuron specific enolase, NO nitric oxide

*Significantly difference

followed by a down-regulation during the post-natal period [19]. There is an acceleration of Fas-FasL-mediated apoptosis during delivery and a respective decrease postpartum in both normal and IUGR pregnancies [20].

Maternal smoking reduced endothelial nitric oxide synthase (eNOS) activity in the fetal vascular bed lead to reduction of vasodilator capacity and retard fetal growth. So that, smoking cessation early in pregnancy may prevent these effects in newborns [12]. This in agreement with this study, as there is significant decrease in the level of the vasodilator, NO in exposed neonates. Reduced production of NO in fetal vessels may lower the blood flow to the fetus and result in lower birth weight. To our knowledge, this is the first time to use tracheal suction in the biochemical assay that can reflects the true internal environment of the fetus. However, this need further studies to measure hormones and other biochemical markers in neonatal tracheal suction that will help in screening and early detection of some diseases. One of interesting results, in this study, is the increased rate of cesarean section in non-exposed group than exposed group.

In conclusion, PTE decreased the birth weight most probably by decreasing NO. The decrease in sFas level

may indicate decrease proliferation and consequently birth weight. While, increased morbidity of neonates in the exposed group could be attributed to increased NSE and sE-cadherin in the studied markers. Also, cessation of breast feeding and its sequel. So that, neonates should be more protected from ETS exposure in prenatal and post-natal periods.

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