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Original Research Article

Self-Reported Exposure to ETS (Environmental Tobacco Smoke), Urinary Cotinine and Oxidative Stress Parameters in Pregnant Women – the Pilot Study

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Abstract: Background: Exposure to ETS (Environmental Tobacco Smoke) is one of the most toxic environmental exposures. Objective: To investigate the impact of ETS on physiological, biochemical, psychological indicators, on the urine antioxidant capacity (AC) and oxidative damage to lipids in a pilot sample of healthy pregnant women. Methods: The exposure to ETS was investigated by a validated questionnaire, urine cotinine and the marker of oxidative damage to lipids - 8-isoprostane concentrations using an ELISA kit. Urine AC was determined by the spectrophotometric TEAC method. From the sample of pregnant women (n=319, average age 30.84 ± 5.09 years) in 80 the levels of cotinine and oxidative stress markers were analyzed. Results: From our sample, 5 % individuals (7.4 % objectified by cotinine) were current smokers and 25 % reported passive smoking in the household (18.8 % objectified by cotinine). The Kappa was 0.78 for smokers and 0.22 for ETS exposed non-smokers. Smokers as well as non-smokers had significantly higher ($p<0.05$) urine AC than ETS exposed non-smokers. Non-smokers had significantly lower levels of 8-isoprostane than smokers ($p<0.01$) and ETS-exposed non-smokers ($p<0.05$). Correlations between urine levels of cotinine and AC were positive in ETS exposed non-smokers. Conclusion: The harmful effect of active and passive smoking on oxidative stress parameters has been indicated.

Keywords: Environmental Tobacco Smoke (ETS); pregnant women; questionnaire; urinary cotinine; oxidative stress parameters

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1. Introduction

Exposure to environmental risk factors has a negative impact on health, especially in vulnerable population groups, which include the children, mothers and pregnant women. Exposure to tobacco smoke is one of the most toxic environmental exposures. Globally, more than a third of all people are regularly exposed to the harmful effects of smoke. This exposure is responsible for about 600,000 deaths per year, and about 1% of the global burden of diseases worldwide [1]. Around the world, 40% of children, 33% of male non-smokers, and 35% of female non-smokers were exposed to ETS in 2004 [2]. According to the Global Adult Tobacco Survey (GATS) (2008-2010), which investigated the prevalence of smoking and passive smoking among women aged 15-49 years in 14 low- and middle-income countries, the prevalence was 0.4% in Egypt, 30.8% in Russia, 17.8% in Mexico and 72.3% in Vietnam. In Poland 26.9% of women smoke, 45.4% are exposed to ETS at home and 24.3% at work. Slovakia and the Czech Republic did not take part in this survey [3]. According to the WHO, the prevalence of daily adult tobacco smokers in Slovakia in 2016 was 29%, 24% of women and 34% of men [4].

Diseases arising from smoking are referred to as „smoking-related diseases“. These include tumors (lips, throat, esophagus, colon, kidney, bladder, liver, lung), non-cancerous respiratory system diseases, cardiovascular diseases and many other diseases affecting a wide variety of organ systems that increase the morbidity, mortality, shorter life expectancy, and worse quality of life [5-10]

Smoking, however, also affects non-smokers in households and public places, where smoking is allowed [11-13]. Environmental tobacco smoke (ETS) exposure, defined as smoke emitted from the burning end of a cigarette or cigar or exhaled by a smoker, represents a well-established and significant health risk [11, 12]. Recent studies demonstrate that ETS is composed not only of second-hand smoke (SHS) but also of third-hand smoke (THS). Third-hand smoke is a complex phenomenon resulting from residual tobacco smoke pollutants that adhere to the clothing and hair of smokers and to surfaces, furnishings, and dust in indoor environments. Exposure can even take place long after smoking has ceased, through the close contact with smokers and in indoor environments in which tobacco is regularly smoked [14, 15].

There have been many studies pointing to the harmful effects of passive smoking on exposed groups of adults, children, pregnant women and their fetuses [13, 16-20].

The most serious complications of ETS in pregnancy include spontaneous abortion, preterm birth fetal developmental anomalies, ectopic pregnancy, preterm labor, intrauterine growth retardation of the fetus (IUGR), fetal death, sudden infant death syndrome (SIDS) [11, 12, 21-24]. Newborns exposed to cigarette smoke during pregnancy are more affected by neurological disorders with long-term deterioration in behavioral, emotional and cognitive functions at a later age [25-27].

Tobacco smoke contains toxic, carcinogenic and mutagenic chemicals as well as free radicals and reactive oxygen species with the potential of oxidative damage to biomolecules. The increased production of reactive oxygen species is related to the depletion of antioxidants and the formation of oxidative stress in the organism [28, 29]. As a result, lipid oxidation, cell membrane damage, DNA strand breaks and the inactivation of some enzymes may occur [30]. Exposure of pregnant women to tobacco smoke causes oxidative stress not only in pregnant women but also in their fetuses [31, 32]. Nicotine and its major metabolite cotinine (the most common biomarker for exposure to cigarette smoke assessed in hair, serum or urine) have high lipid solubility; therefore, they pass rapidly through the placenta into the fetal circulation, with higher levels of cotinine recorded in the fetus than in the mother's plasma [33-37].

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The aim of this international and interdisciplinary project is to assess the degree of ETS exposure and its impact on physiological, biochemical and psychological indicators and on the urine antioxidant capacity and oxidative damage to lipids in a pilot sample of healthy pregnant women. The specific aim is to determine the extent to which self-reported smoking and exposure to ETS are in agreement with the levels of nicotine metabolite (urinary cotinine). The benefit of the study will be the development of the basis for primary preventive interventions in clinical and preventive practice.

2. Materials and Methods

Researchers from the Comenius University's Obstetrics and Gynecology (OB/Gyn) Department and Institute of Hygiene in Bratislava, Slovakia distributed surveys to pregnant women in the 36th − 41st week of pregnancy being seen for the follow-up at the OB/Gyn Department of the Faculty Hospital and Clinic. This survey is the continuation and re-analysis of the previous survey that was designed to evaluate environmental, behavioral, and psychosocial factors in the lives of women [20]. The results of the study have shown that ETC exposure is an independent risk factor associated with the worse physical health of non-smoking mothers in the reproductive age and the worse mental health in the smaller sample of pregnant women [20]. In the present study, we have enlarged the sample of the pregnant women and objectified the self-reported smoking and ETS exposure by the levels of urinary cotinine. The study was approved by the Ethical Committee of the Faculty of Medicine, Comenius University Bratislava, Slovakia and by the Institutional Review Board of New York University School of Medicine, New York, U.S.A (IRB number: 09-0331).

In the present study 319 (average age 30.84 ± 5.09) healthy pregnant women without any medical treatment were included and in 80 of them (average age 30.24 ±4.92 years) the levels of cotinine and oxidative stress markers in urine specimens were analyzed from March to June 2018. Exposure to tobacco smoke as well as the analysis of the lifestyle and demographic determinants of passive smoking were assessed by the validated Questionnaire for mothers used in the previous study [20]. Based on the obtained data we have evaluated the environmental, behavioral and psychosocial factors in the mother's life. For the verification and objectification of women´s exposure to tobacco smoke, the levels of urinary cotinine were evaluated [38].

Urine specimens were taken at the routine control into plastic containers that were subsequently frozen at −20 °C. In the urine samples, levels of cotinine and oxidative stress marker (8-isoprostanes) were analyzed within 3 months of sampling.

2.1 Sample

In the sample of healthy pregnant women (n=319) 79.9 % were younger than 35 years old, most of Slovak nationality (94.3 %), 78.2 % were married or in a relationship, 50.5 % graduated from the university, 60.6 % were employed, 57.4 % had children under 18 years of age in their household (Table 1). In the sample of healthy pregnant women in whom we analyzed the levels of cotinine and oxidative stress markers in urine specimens (n=80), 81.20 % were younger than 35 years old, most of Slovak nationality (93.8 %), 78.8 % were married or in a relationship, 68.80 % graduated from the college, 66.3 % of mothers employed, 31.3 % had children under 18 years of age in their household (Table 2).

2.2 Questionnaire

The validated "Questionnaire for Mothers" administered by a trained person, contained questions on environmental, behavioral and psychosocial factors in the life of pregnant women. Besides questions on personal (age, nationality, marital status, education, employment, children), behavioural (smoking, lifestyle, nutrition), housing (residence) and economic characteristics (household income), it also included questions on mothers' smoking and ETS exposure in the

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household (smoking spouse or other members of the family, number of cigarettes and number of years of smoking). In the case of a former smoker, there was a question for how many years she/he has not smoked. Former smokers were considered non-smokers.

Table 1. Characteristics of a sample of pregnant women (n=319)

Indicator*	**N**	**%**
Age group		
≤ 35	255	79.9
> 35	64	20.1
Nationality		
Slovak	299	94.3
other	18	5.7
Marital status		
married/in a relationship	248	78.2
single/divorced	69	21.8
Number of children under 18		
no	100	42.6
1	107	45.5
2	24	10.2
≥ 3	4	1.7
Mother´s education		
secondary school or lower	42	13.2
high school graduate	116	36.4
university degree	161	50.5
Employment status of the mother		
employed	191	60.6
unemployed	124	39.4
Father´s education		
secondary school or lower	63	20.0
high school graduate	118	37.5
university degree	134	42.5
Employment status of the father		
employed	307	98.4
unemployed	5	1.6
Household income		
≤ 700 €	62	20.1
> 700 €	246	79.9
Residence		
urban-metropolitan area	229	72.2
rural-non-metropolitan area	88	27.8
Physical activity		
regular	129	41.1
irregular	185	58.9

Healthy lifestyle		
yes	207	65.9
no/not sure	107	34.1
Number of daily meals		
≤ 4	192	60.4
> 4	126	39.6
Smoking status (self-reported)		
non-smoker	187	58.6
ex-smoker	103	32.3
current smoker	29	9.1
Exposure to tobacco smoke (self-reported)[a]		
not exposed	156	62.2
exposed	95	37.8

[a] If somebody living in the household is smoking. * There are some data missing in each variable category

Table 2. Characteristics of a sample of pregnant women with urinary cotinine and oxidative stress parameters (n=80)

Indicator*	N	%
Age		
≤ 35	65	81.2
> 35	15	18.8
Nationality		
Slovak	75	93.8
other	5	6.2
Marital status		
married/in a relationship	63	78.8
single	17	21.3
Number of children under 18		
no	55	68.7
1	20	25.0
2	5	6.3
≥ 3	0	0.0
Mother´s education		
secondary school or lower	6	7.5
high school graduate	19	23.7
college graduate and higher	55	68.8
Employment status of the mother		
employed	53	66.3
unemployed	27	33.7
Father´s education		
secondary school or lower	10	12.6
high school graduate	21	26.6

college graduate and higher	48	60.8
Employment status of the father		
employed	78	98.7
unemployed	1	1.3
Household income		
≤ 700 €	5	6.5
> 700 €	72	93.5
Residence		
urban-metropolitan area	57	72.2
rural-non-metropolitan area	22	27.8
Physical activity		
regular	40	50.6
irregular	39	49.4
Healthy lifestyle		
yes	54	67.5
no/not sure	26	32.5
Number of daily meals		
≤ 4	43	54.5
> 4	36	45.5
Smoking status (self-reported)		
non-smoker	59	73.8
ex-smoker	17	21.2
smoker	4	5.0
Smoking status (cotinine objectified)		
no	74	92.6
yes	6	7.4
Exposure to tobacco smoke (self-reported)[a]		
not exposed	56	70.0
exposed	20	25.0
Exposure to tobacco smoke (cotinine objectified) [a]		
not exposed	59	73.8
exposed	15	18.8

[a] If somebody living in the household is smoking. * There are some data missing in each variable category

2.3 Chemical analyses

2.3.1 Cotinine

The level of cotinine was measured in urine samples using a competitive ELISA kit (MyBioSource, San Diego, CA, USA) according to the manufacturer´s instructions. Obtained results were expressed in mg/mol of creatinine. Pregnant women were assigned into three experimental groups based on the urine cotinine levels: 58 women with cotinine levels above 2 mg/mol creatinine were included in the smoker group (S), 15 women with cotinine levels between 0.06 - 2 mg/mol

creatinine into the ETS group (environmental tobacco smoke) and 7 women with cotinine levels below 0.06 mg/mol creatinine were included into the non-smoker group (NS).

2.3.2 Antioxidant capacity of urine (TEAC)

Trolox equivalent antioxidant capacity (TEAC) decolorization assay (Re et al. 1999) is a decolorization method applicable for both the lipophilic and hydrophilic antioxidants. A cation radical 2,2′-azino-bis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS.+) is produced by the oxidation of ABTS with potassium persulfate (K2S2O8). Added antioxidants reduce the cation radical in a dose- and time-response manner. Decolorization of the cation radical is related to the standard trolox (synthetic, water-soluble form of vitamin E). Results are expressed in mmol of trolox/L/mol of creatinine.

2.3.3 8-isoprostane

Isoprostane (8-iso prostaglandin F2α) levels in urine were determined by the commercial competitive ELISA kit (Cayman Chemical, USA) following manufacturer´s instructions. Results are expressed in ng/mL/mmol of creatinine.

2.3.4 Creatinine

Urine creatinine was determined in the certified laboratory (Medirex, a.s., Bratislava, Slovakia)

2.4. Statistical analysis

To evaluate the results, we used the methods of descriptive and analytical statistics (categorical data analysis) to identify mutual associations between factors assessed in the questionnaire and self-reported ETS exposure. Kappa statistics, sensitivity, specificity and correlations were used to determine the extent to which self-reported smoking and exposure to ETS are in agreement with the degree of ETS exposure determined by the levels of urinary cotinine (i.e. to determine the accuracy of self-reported smoking status). Kappa is the percentage of cases in which the two measures are in agreement after accounting for chance agreement [39]. It does not take into account which measure is considered the gold standard. Sensitivity is the percentage of true positive (the percentage of respondents who reported being smokers or ETS exposed non-smokers among those classified as smokers or ETS exposed non-smokers based on cotinine concentrations). Specificity is the percentage of true negatives (the percentage of respondents who reported being non-smokers among those classified as non-smokers based on cotinine concentrations). The predictive value positive (PVP) and predictive value negative (PVN) are the complements of the percent false positive and percent false negative, respectively [38, 40, 41]. Statistical package SPSS, version 24 (International Business Machines Corp.; New Orchard Road; Armonk, New York, USA) was used for the data analysis.

To evaluate the results of chemical analysis the statistical package SPSS ver. 18 (SPSS Inc., Chicago, IL, USA) was used. The results are expressed as mean ± standard deviation (SD) for normally distributed data, or median (lower quartile – upper quartile) for data not normally distributed. The Student´s unpaired t-test or non-parametric Mann-Whitney test were used for the comparison between groups of continuous parameters as appropriate. To quantify the association between two variables, Pearson or Spearman correlations were used.

The significance level was set at $p<0.05$.

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3. Results

In the sample of 319 healthy pregnant women, there were 58.6% (187) self-reported non-smokers, 32.3 % (103) ex-smokers and 9.1% (29) current smokers smoking from one to 15 cigarettes a day. The average number of cigarettes was 6.66± 4.16 per day; median 5 (lower quartile 3 – upper quartile 10); the average duration of smoking was 8.90 ± 5.46 years; median 10 (4– 12). Current smokers were excluded from the analysis. ETS exposure (somebody living in the household is smoking) reported 37.8 % (95) non-smoking respondents. The average number of cigarettes smoked by the partner/person living in the same household was 13.12 ± 8.12 per day; median 11.5 (7– 20). The average duration of smoking was 12.81 ± 6.38 years (Table 1). In the analysis of the lifestyle and demographic determinants of passive smoking in the household significant negative relationships between ETS and the level of mother's and father's education ($p<0.001$) and household income ($p<0.05$) were found. ETS exposed non-smoking pregnant women live mostly in the urban/metropolitan area, have reportedly worse healthy lifestyle ($p<0.05$) and indicated lower physical activity ($p=0.057$) (Table 3).

Table 3. The relation between demographic factors and mother's exposure to tobacco smoke (self-reported)

Indicator*	ETS- (N = 156)		ETS+ (N = 95)		p-value
	N	%	N	%	
Age group					
≤ 35	117	75.0	78	82.1	n.s.
> 35	39	25.0	17	17.9	
Nationality					
Slovak	148	94.9	91	95.8	n.s.
other	8	5.1	4	4.3	
Marital status					
married/in a relationship	127	81.9	72	76.6	n.s.
single/divorced	28	18.1	22	23.4	
Number of children					
any	50	40.0	31	40.8	
1-2	73	58.4	43	56.6	n.s.
≥ 3	2	1.6	2	2.6	
Mother´s education					
secondary school or lower	12	7.7	21	22.1	
high school graduate	48	30.8	44	46.3	<0.001
university degree	96	61.5	30	31.6	
Employment status of the mother					
employed	100	65.8	56	58.9	n.s.
unemployed	52	34.2	39	41.1	
Father´s education					
secondary school or lower	19	12.3	32	33.7	<0.001

high school graduate	58	37.7	42	44.2	
university degree	77	50.0	21	22.1	
Employment status of the father					
employed	149	98.0	93	98.9	n.s.
unemployed	3	2.0	1	1.1	
Household income					
≤ 700 €	23	15.3	24	26.1	<0.05
> 700 €	127	84.7	68	73.9	
Residence					
urban-metropolitan area	114	73.1	56	60.2	<0.05
rural-non-metropolitan area	42	26.9	37	39.8	
Physical activity					
regular	70	44.9	30	32.6	0.057
irregular	86	55.1	62	67.4	
Healthy lifestyle					
yes	112	72.3	54	57.4	<0.05
no/not sure	43	27.7	40	42.6	
Number of daily meals					
≤ 4	97	62.2	60	63.8	n.s.
> 4	59	37.8	34	36.2	

ETS+ exposed to tobacco smoke (self-reported); ETS- not exposed to tobacco smoke (self-reported); $p<0.05$ is considered as statistically significant * There are some data missing in each variable category

In the sample of healthy pregnant women in whom we analyzed the levels of cotinine and oxidative stress markers in urine specimens (n=80), there were 5 % (4) self-reported smokers, 73.8 % (59) non-smokers and 21.2 % (17) ex-smokers. The average number of cigarettes was 5.67± 4.04 per day; median 5 (2–5), the average duration of smoking was 14.50 ± 7.78 years; median 14.50 (9.0–14.5). ETS exposure (somebody living in the household is smoking) reported 25 % (20) non-smoking respondents. The presence of ETS exposure objectified by cotinine was confirmed in 18.8 % (15) respondents. The average number of cigarettes smoked by the partner/person living in the same household was 10.39 ± 6.50 per day; median 10 (5–16.3). The average duration of smoking was 13.43 ± 5.90 years (Table 2). There were 5 % (4) self-reported current smokers and 7.4 % (6) current smokers objectified by the level of cotinine in the urine sample and 25 % (20) self-reported ETS exposed non-smokers and 18.8 % (15) ETS exposed non-smokers confirmed by the level of cotinine in the urine sample. The sensitivity for self-reported smoking status was 66.7 %, specificity 100 %, positive predictive value 100 % and negative predictive value 95.8 %. Kappa was 0.78 indicating the substantial agreement [42] or excellent agreement [39]. The sensitivity for self-reported ETS exposure was 46.7 %, specificity 78 %, positive predictive value 35 %, negative predictive value 85.2 %. Kappa was 0.22 indicating the fair agreement [42] or the poor agreement [39]. The agreement for self-reported ETS exposure was better for women from the younger age group (≤ 35yrs) and with lower education reaching to moderate or fair to good agreement (Kappa=0.44) [39, 42].

Table 4. Measures of agreement to determine the accuracy of self-reported smoking status and exposure to ETS in the sample of pregnant women (n=80)

	Smoking status			
	Non-smoker vs. current smoker	ETS- vs. ETS+		
Measures of agreement	**Total**	**Total**	**Younger age group (≤35yrs)**	**Lower education**
Kappa	0.78	0.22	0.30	0.45
Spearman correlation	0.80	0.22	0.29	0.44
Sensitivity	66.7%	46.7%	54.6%	66.7%
Specificity	100.0%	78.0%	79.6%	80.0%
Positive predictive value	100.0%	35.0%	37.5%	57.1%
Negative predictive value	95.8%	85.2%	88.6%	85.7%
Diagnostic accuracy	96.2%	71.6%	75.0%	76.2%

ETS+ exposed to tobacco smoke; ETS- not exposed to tobacco smoke

The median value of cotinine in ETS exposed pregnant women was 0.22 (0.129-0.338) and in currently smoking pregnant women 253.19 (181.82-498.31) mg/mol creatinine. The urine antioxidant capacity (TEAC) mean value in ETS exposed pregnant women was 0.91±0.28 and 1.3±0.43 mmol trolox/L/mol creatinine in current smokers; median values of isoprostanes 258.41 (112.26-411.88) in ETS exposed and 293.74 (250.17-377.51) ng/mL/mmol creatinine in currently smoking pregnant women (Table 5).

Pregnant women in the ETS+ group had significantly reduced urine antioxidant capacity (TEAC) compared to both the non-smoker (ETS-) and the smoker groups (Tables 5 and 6). There was no significant difference in urine antioxidant capacity between the non-smokers and the smokers. The marker of oxidative damage to lipids - 8-isoprostanes were significantly increased in the ETS+ and the smoker group compared to the non-smoker group. 8-isoprostane levels were the highest in the smoker group; however, there was no significant difference between ETS+ and smoker groups.

Significant positive correlation between urine cotinine levels and urine antioxidant capacity (TEAC) in the ETS exposed group was found (Table 7). The same correlation was negative in the non-smoker group; however, this correlation was marginally significant.

Table 5. Cotinine levels, TEAC and levels of 8-isoprostanes in the analyzed groups

	Cotinine	**TEAC**	**8-isoprostanes**
Smoking status	mg/mol creatinine	mmol trolox/L/mol creatinine	ng/mL/mmol creatinine
ETS-	0.00±0.00	1.2±0.4	143.6(73.91-197.54)
ETS+	0.22(0.129-0.338)	0.91±0.28	238.41(112.26-411.88)
current smoker	253.19(181.82-498.31)	1.3±0.43	293.74(250.17-377.51)

ETS+ exposed to tobacco smoke; ETS- not exposed to tobacco smoke (cotinine objectified). Results are expressed as the mean ± SD or the median (lower quartile – upper quartile).

Table 6. Statistical significance (p-value) of TEAC and levels of 8-isoprostanes between the analyzed groups

Smoking status	**TEAC**	**8-isoprostanes**
ETS+ vs. ETS-	0.0105*	0.0487*
ETS+ vs. current smoker	0.0199*	0.4702
current smoker vs. ETS-	0.7374	0.0055*

ETS+ exposed to tobacco smoke; ETS- not exposed to tobacco smoke (cotinine objectified); * significant at $p<0.05$

Table 7. Correlations between cotinine levels in urine and oxidative stress parameters in the analyzed groups

Antioxidant parameters	**Rho**	**p-value**
ETS-		
TEAC	-0.2036	0.0642
isoprostanes	0.0676	0.3097
ETS+		
TEAC	0.7607	0.0007*
8-isoprostanes	-0.2179	0.2171
Current smoker		
TEAC	-0.0857	0.4014
8-isoprostanes	-0.5429	0.1208

ETS+ exposed to tobacco smoke; ETS- not exposed to tobacco smoke (cotinine objectified); Rho - Spearman's rank correlation coefficient; * significant at $p<0.05$

4. Discussion

The results of our previous studies show that ETS is one of the most important health hazards influencing the physical and mental health of the exposed non-smoking partners [20]. The study published by the members of our research team on a nationally representative data from the year 2000 to 2004 Medical Expenditure Panel Survey in the USA [18] showed a relationship between living with smokers and worsened maternal physical and mental health in non-smoking mothers with children. The risk was discernible with the presence of a single adult smoker in a household and increased with the number of smokers [18]. The limitation of our previous studies was the fact that the smoking status was ascertained via self-reporting. Since there is a considerable public awareness about the effects of cigarette smoking and ETS exposure on humans, participants might be motivated

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to under-report their smoking status although there is evidence in some studies to show that self-report is an accurate way to measure smoking behaviors [38, 43, 44].

The problem might be the ETS exposure of pregnant respondents and the motivation to under-report [45, 46, 47] or over-report their exposure (we have not found a study on pregnant women over-reporting their ETS exposure).

The strength of our present study is the determination of the accuracy of self-reported smoking and ETS exposure status by urinary cotinine and investigation of the impact of ETS besides physiological, biochemical, and psychological indicators on the urine antioxidant capacity (AC) and oxidative damage to lipids. Active smoking of pregnant women or ETS exposure results in several problems such as intrauterine growth retardation, an increased risk of spontaneous abortion, reduction of pulmonary function in healthy neonates or a higher risk of sudden infant death syndrome [22]. One of the mechanisms explaining these effects is the presence of the smoke-induced oxidative stress leading to the oxidative damage to molecules and to the inflammatory response [48]. The cigarette smoke contains a large number of free radicals as well as metals such as copper, mercury and zinc [49], which may catalyze the production of the very reactive hydroxyl radical by the Fenton reaction [50]. Smoking may increase oxidative stress not only through the generation of free radicals but also through the depletion of the antioxidant systems protecting the organism against deleterious effects of oxygen radicals.

In our study we have examined the effect of the ETS exposure and the active smoking on the oxidative damage to lipids and on the antioxidant capacity of urine in pregnant women. In the past decades, numerous studies have shown that 8-isoprostanes are extremely accurate markers of lipid peroxidation [51, 52, 53, 54]. 8-isoprostanes are compounds produced by the non-enzymatic oxidation of arachidonic acid. We have found that pregnant women exposed to ETS had significantly higher oxidative damage to lipids and significantly lower urine antioxidant capacity than non-smokers. These results indicate that ETS-exposed pregnant women are under increased oxidative stress which is in accord with other studies [55, 56, 57] Smoking pregnant women had 8-isoprostanes level similar to the ETS group and antioxidant capacity similar to the non-smokers. In the smoker group compared to the ETS and the non-smoker groups women are exposed to the higher load of oxidants, which may stimulate compensatory mechanisms leading to the increased antioxidant capacity. Results of other studies on oxidative stress of smoking pregnant women are ambiguous. Similar results were reported also in plasma and saliva by other studies [28, 32, 58]. In contrast, Fayol et al (2005) have detected higher plasma antioxidant activity in ETS exposed pregnant women than in controls [59].

In addition, we have observed a strong, significant, positive correlation between the urine antioxidant capacity and the urine cotinine levels only in the ETS+ group. ETS exposed pregnant women might be sensitive to tobacco smoke and able to correspondingly stimulate their antioxidant system. However, in the smoker group this correlation was non-significantly negative which might be the consequence of the higher use of antioxidant compounds by the fetus in order to counteract the increased oxidative burden in active smokers.

ETS exposure or active smoking of pregnant women can have negative effects on their fetuses. There are several reports providing evidence of increased oxidative damage to lipids, DNA and proteins in the blood of such neonates (Kurt et al, 2016) and the correlations between oxidative stress parameters of pregnant women and their neonates [60]. Increased oxidative damage to important biomolecules in fetus caused by cigarette smoke has been implicated in the etiopathogenesis of over

100 disorders [59]. Increased consumption of dietary antioxidants might be a potential therapeutic means against increased oxidative stress in ETS exposed pregnant women and actively smoking pregnant women.

The validity of self-reported smoking in population surveys remains an important question [44]. In our study self-report seems to be in the best agreement with the self-reported smoking status (78 % agreement). Sensitivity was 66.7% and specificity 100 %. There are studies with higher sensitivities, but using larger samples [38, 44, 61]. Self-reported non-smokers who seem to be smokers based on biochemical measurements are generally considered "deceivers" of their true smoking status [43]. In a summary of studies in which questionnaire responses regarding smoking status were compared with cotinine measurements, the estimated misclassification rates (proportion of self-reported non-smokers with increased cotinine levels indicative of active smoking) ranged from 0.9% to 9.8% [43, 44, 62]. Misclassification rates reported among pregnant women may be as different as 3% in a population-based survey and 26.2% in a smoking cessation trial [43, 46, 63]. In our study the misclassification rate for pregnant smokers was 3.9 %.

The agreement for ETS exposed pregnant non-smokers is much lower (22 % agreement, 46.7 % sensitivity and 78 % specificity). The misclassification rate for under-reported ETS exposure was 10.81 %, but for over reported ETS exposure 17.57 %. The older and more educated respondents seem to overestimate their ETS exposure (Table 4). The pregnancy itself may also play a role in overestimation of ETS exposure. The analysis of the lifestyle and demographic determinants on a larger sample of 319 pregnant non-smokers revealed negative relationships between ETS and the level of mother's and father's education ($p<0.001$) and household income ($p<0.05$) similar to the other studies [18, 20].

The main conclusion of several studies on large population samples is that the validity of self-reported smoking is consistently high in population-based studies and therefore the extended use of cotinine measurements for validation purposes may not be justified [38, 43, 44]. Nevertheless, further research may focus on assessing the optimal cut off point for validating smoking status among specific groups, such as pregnant women. These studies will also improve our understanding of the effects of gender, social conditions, and pregnancy status on the metabolism of nicotine and on smoking behaviors that may affect nicotine intake.

Findings of several studies suggest that most pregnant women disclose their smoking and ETS exposure as well. Universal urinary cotinine screening of pregnant women could aid in appropriately counseling women about second-hand exposure, as well as monitoring women at high risk for adverse pregnancy outcomes [64, 65]. In contrast, there is a substantial within-person fluctuation in pregnancy smoking, as women try repeatedly to quit or cut down. In this case, cotinine measures may be of limited use for validation of amount smoked, as they are informative only about a recent exposure, vary with individual smoking topography and are dependent on the time elapsed since the last cigarette smoked [61]. The results of the study by Xiao, 2018 on rural pregnant women indicated that, regardless of trimester, more than 15% of pregnant women with actual exposure to ETS might not perceive themselves as passive smokers in prenatal care, especially in the first trimester [66]. The third trimester of pregnancy was the proper period to follow our respondents.

The limitation of our study is the small sample size, the cross-sectional design, and self-reported ETS exposure in the larger sample. In our study, we did not use the medical outcomes short form-12 (SF-12) to quantify the mental and physical health of mothers because pregnancy itself could

influence mental and physical health as well. Especially, physical health is very much influenced by pregnancy and its analysis by SF-12 could be biased [20, 66]. The strength of our study is the separation of current smokers, ETS exposed and ETS not exposed non-smokers and the investigation of the harmful effects of active and passive smoking on detected oxidative stress parameters.

5. Conclusions

Data from our study show that maternal cigarette smoking and ETS exposure during pregnancy may compromise the balance between reactive oxygen species (ROS) and antioxidant defense and can cause potent oxidative stress with all negative consequences in pregnancy.

Combining the maternal self-report of smoking with the level of urine cotinine concentration could improve the precision of the exposure to tobacco smoke. Urine testing for cotinine may be useful in reducing the nondisclosure surrounding prenatal tobacco use. This screening could be a valuable tool for counseling to help pregnant women in tobacco smoking cessation. The presented results might be used in clinical practice and in campaigns for smoke-free environments and in the promotion of community-based smoke-free programs. Furthermore, they represent an important argument for intervention in families. A complete smoking ban at home should be considered to avoid potential adverse effects on pregnancy outcomes due to ETS.

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