Cord serum cotinine as a biomarker of fetal exposure to environmental tobacco smoke

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ABSTRACT

الأهداف: عمل مقارنة بين تقرير الأمهات لتعرضهم للتدخين السلبي من جهة ونتائج الفحص المختبري لمادة الكوتينين في دم الحبل السري لأطفالهن حديثي الولادة من جهة أخرى.

الطريقة: أُجريت هذه الدراسة المقطعية في مستشفى العلوية التعليمي، بغداد، العراق وذلك خلال الفترة من أغسطس إلى نوفمبر 2008م، وشملت عينة الدراسة 88 أماً مع طفلها الوليد. لقد تم أخذ المعلومات من الأمهات عن طريق توزيع أوراق الاستبيان الخاصة، فيما تم تحليل الكوتينين في الحبل السري من خلال المقايسة المناعية المرتبطة بالإنزيمات (إليزا).

النتائج: أظهرت نتائج الدراسة بأن معدل التعرض لمادة النيكوتين والذي اعتمد على تقرير الأمهات قد كان 4.267±2.557 نانوغرام/مل، بينما كان معدل الكوتينين في الفحص المختبري لدم الحبل السري 8.10±1.925 نانوغرام/مل. وقد كان هناك علاقة خطية واضحة من الناحية الإحصائية بين معدل النيكوتين في تقرير الأمهات والفحص المختبري لمادة الكوتينين في مصل الحبل السري ($p<0.0001$, $r=0.8043$). لقد تم اللجوء إلى المعادلة ($y=1.1912\ x - 5.3814$) من أجل تقدير مدى تعرض الأمهات الحوامل الغير مدخنات للتبغ وذلك من خلال جمع البيانات من الاستبيان الذي أُعطي لهؤلاء الأمهات.

خاتمة: أثبتت الدراسة الحالية أهمية المعلومات التي تعطيها الأم الحامل عن التعرض للتدخين السلبي، حيث أن ذلك قد يُستعمل كبديل للفحص المختبري للكوتينين في الحبل السري وخصوصاً عند عدم توفره.

Objective: To compare the results obtained from pregnant mothers' self-reports of smoke exposure with those obtained from laboratory tests of fetal cord blood.

Methods: This cross-sectional study was conducted in Al-Elwyea Maternity Teaching Hospital, Baghdad, Iraq. Eighty-eight mothers and their newly born babies were included in the study through convenient sample techniques from August to November 2008. Information was obtained from the mother, and cord serum cotinine was analyzed using the enzyme linked immunosorbent assay method.

Results: The calculated mean nicotine level according to mothers' self-report was 4.267±2.557 ng/ml, while the mean cotinine level was 8.10±1.925 ng/ml. There was a linear relationship between nicotine level and laboratory levels of cotinine, and the correlation was strong ($r=0.8043$, $p<0.0001$). The equation $y = 1.1912\ x - 5.3814$ could be used to estimate approximate exposure to environmental tobacco smoke among nonsmoker pregnant women using self-reported information given by the nonsmoker pregnant mother.

Conclusion: Information on second hand smoke exposure provided by pregnant women is helpful, and can be used when cotinine level estimation is not available.

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The prevalence of tobacco use is increasing in many middle-income countries, although it is decreasing in many high-income countries, which leads to devastating health and economic burdens particularly for vulnerable populations such as women. In addition to the health risks that maternal tobacco smoke exposure in pregnancy has on women, there is substantial fetal morbidity and mortality.[1-3] Cotinine is specific for exposure to tobacco, and it is preferred as a marker over nicotine itself, partly because of the half-life of cotinine in the body of around 18 hours.[4,5] A recent study[6] found the mean half-life of cotinine in pregnant women was 8.8 hours (95% confidence interval [CI] 5.5-12) compared to 16.6 hours for the same women 3 months postpartum (95% CI 14.8-19). The use of an objective measure of a biomarker for accurate assessment of fetal

exposure could be of major importance for investigating the effect of pre- and postnatal environmental tobacco smoke (ETS) exposure.[7] Studies of the effects of ETS exposure tend to rely heavily on maternal self-report. With the establishment of cotinine as a biomarker of ETS exposure along with the determination of levels that discriminate exposed and truly non-exposed pregnant women, it is possible to examine the association between self-reported ETS exposure and that indicated by serum cotinine levels.[8] Self-reported exposure to ETS can provide a good estimate of exposure, if the information is gathered by a well-trained interviewer in a structured way, which can be an alternative to cotinine estimation in pregnant women, especially when laboratory estimation is not available.[9] The daily exposure of nicotine (DEN) is the term used to explain the quantity of daily exposure to nicotine in milligrams in nonsmoking mothers with passive exposure. The DEN was calculated as: nicotine daily intake (NDI) of the active smoker in the environment of the mother multiplied by the hours when exposure was reported to occur (as a fraction of 24 hours) according to the brand, accordingly the equation will be as follows: DEN = NDI × hours spent with the smoker/24 hours. Finally, the equation for all people that the mother is exposed to will be as follows:[10]

$$DEN = \Sigma \text{ smokers } ([\text{mg nicotine/cigarettes} \times \text{number of cigarettes smoked/day}] \times \text{hours spent with the smoker/24hours})$$

This study was carried out to investigate the relationship between maternal self-reported exposure to nicotine using a questionnaire, and the laboratory test of nicotine Biomarker (cotinine) in the cord blood of their newly born babies.

Methods. This cross-sectional study was conducted in Al-Elwyea Maternity Teaching Hospital, Baghdad, Iraq on 88 mothers and their newly born babies from August to November 2008, using a convenient sampling technique of pregnant women attending the hospital for delivery. Inclusion criteria included single pregnancy mother, age range from 14-45 years, non-smoker and exposed to ETS from her husband or relatives at the same home or from work, college, and so forth, and a sufficient amount (>1 ml) of cord blood. Exclusion criteria included all mothers exposed to other types of smoke (such as Argil, cigar, or pipe, due to the limitations in calculating the exact amount of nicotine the mother is exposed to in these types of smoke), women who stayed in the hospital for more than 8 hours,[6] mothers with hypertension or diabetes, and mothers who were unstable in one home during the pregnancy period. Information was usually taken directly from the mother via the questionnaire form, which includes some demographic characteristics and socioeconomic status. Measurement of exposure to ETS was taken from the mother before going to the delivery room, nonsmoking mothers were asked whether they were regularly exposed to ETS, where and by whom (husband or other member in the family or/and at work),[11] also the average number of cigarettes, the brand of cigarette smoked by those people, and the average hours of exposure were calculated as milligrams DEN. For each nonsmoking mother, the DEN will be obtained from the number of cigarettes smoked per day multiplied by nicotine content (in milligrams) of each cigarette multiplied by the hours during which exposure was reported to occur (as a fraction of 24 hours),[12] accordingly, for more than one exposure, the different exposures will be added.[11] Umbilical cord blood samples were taken from the baby of each mother included in the study in the delivery room, and then left for 30 minute before being centrifuged at 4000 cycle/minute for 5 minutes. The separated serum was stored at -20^{0}C until analysis. The cord serum cotinine level was analyzed using the enzyme linked immunosorbent assay (ELISA) technique using Cotinine Serum ELISA (DRG Diagnostic, Frauenbergstr, Germany). A limit of 14 ng/ml was used in this study for cord serum cotinine to distinguish active smokers from nonsmokers,[13] between 1-14ng/ml was considered an exposed nonsmoker, and below one ng/ml considered a nonexposed nonsmoker.[14] The use of the currently accepted cut-off point of 14 ng/ml overestimates the number of nonsmokers in comparison with the proposed new overall cut-off point of 3 ng/ml, or the race/ethnic-specific cut-off points of 1-6 ng/ml.[15] Two mothers were excluded from the study because cord blood samples from their babies were higher than 14 ng/ml.

The study design and the questionnaire were reviewed and agreed upon by the local committee of the College of Medicine, Al-Nahrain University, Baghdad, Iraq. Ethical Approval was obtained from the Center for Research and Educational Methods, Ministry of Health, and from Al-Elwyea Gynecological & Obstetric Teaching Hospital to perform the research.

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 11. Descriptive measures included mean and standard deviation, and test of significance was carried out using a correlation test. A p-value ≤0.05 was considered significant.

Results. **Table 1** shows that the highest proportion of studied mothers were in the 20-24 years age group, from

urban areas, had a primary school education, and had male newborns. **Figure 1** shows that the highest number of mothers reported exposure to nicotine, according to the questionnaire, between levels of 1.00-2.99 ng/ml, and the lowest number of mothers at levels of 8.00-13.00 ng/ml, while the highest number of cord blood samples shows laboratory cotinine levels between 6.00-6.99 ng/ml. **Table 2** compares the nicotine level calculated from the questionnaire (4.267±2.557 ng/ml) with laboratory calculated cotinine levels (8.10±1.925 ng/ml). **Figure 2** describes the linear relationship between final nicotine level using the questionnaire and the laboratory level of cotinine taken from babies cord blood, and shows a strong significant correlation (r=0.8043, p=0.0001).

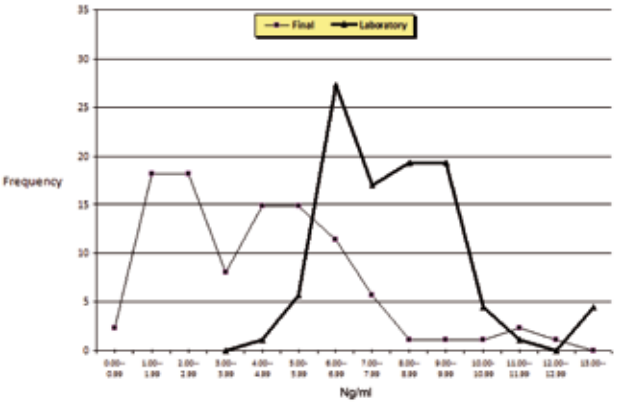

Figure 1 - Distribution of nicotine level from the questionnaire and cotinine level from babies cord blood.

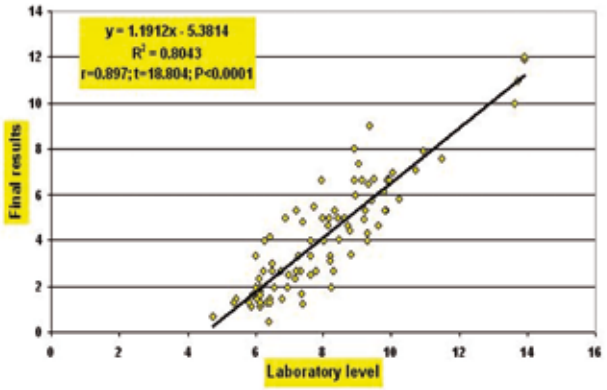

Figure 2 - Linear relationship between nicotine estimation in the questionnaire and laboratory cotinine level from babies cord blood.

Discussion. It was found that 81.8% of the parents were from urban areas, while only 18.2% were from rural areas. Rauh et al[16] and Goel et al[17] found that exposure to ETS was high among low-income, urban parents, both because of the uneven distribution of outdoor pollution sources and the higher smoking rates in those populations. In the present study, it was obvious that the percentage of parents exposed to ETS was higher among those with primary school education than secondary and college, and this result is consistent with previous findings of a positive association of low parental education with children exposure at home.[18] Soliman et al[19] found that exposure declined in higher education and income groups, and showed a negative relation between maternal education and the prevalence of home ETS as the mother's education increased. Soliman et al[19] also concluded that underreporting might have been greatest among higher socioeconomic groups in which a lower prevalence of smoking and greater education made smoking and ETS less socially acceptable. Goel et al[17] found that women who reported exposure to ETS had a lower socioeconomic status than non-exposed women. Pichini et al[20] studied cases of cord serum and maternal urine samples, and found no differences in social class (based on occupation).

Table 1 - Socio-demographic characteristics of the study sample of 88 mothers with newborns.

Variables	Number	(%)
Mothers' age (years)		
<20	21	(23.9)
20-24	30	(34.1)
25-29	20	(22.7)
30-34	9	(10.2)
35+	8	(9.1)
Mean±SD = 24.32±6.01		
Residency		
Urban	72	(81.8)
Rural	16	(18.2)
Education of mother		
Illiterate	5	(5.7)
Primary school	38	(43.2)
Intermediate	27	(30.7)
Secondary	16	(18.2)
College	2	(2.3)
Mothers' occupation		
Housewives	82	(93.2)
Employed\self employed	6	(6.8)
Gender of newborn		
Male	50	(56.8)
Female	38	(43.2)
Order in the family		
First	36	(40.8)
Second	25	(28.4)
Third and more	27	(30.8)

Table 2 - Comparison between nicotine level as calculated from the questionnaire form, and cotinine level as calculated from laboratory level.

Variables (N=88)	Nicotine calculated from the questionnaire form	Cotinine calculated from laboratory result
Number	88	88
Mean	4.267	8.10
Standard error of mean	0.273	0.205
Median	4.000	7.955
Standard deviation	2.557	1.925

The present study shows that babies who were first in order present the highest proportion (40.9%), and the percentage starts to decrease with increase in order in the family. Pichini et al[20] reported that babies first in order represent 48%, and those second in order represents 37%, and those third in order and more represent 15%. This indicates that we have to start to increase the awareness of mothers, especially those newly married, on the risk of ETS as they were at more risk probably because of their lack of knowledge and little interest in health problems.

It is clear from these results that there was a relationship between cotinine level in the laboratory and nicotine level from the questionnaire. The laboratory cotinine level was higher than the questionnaire, and this may be explained by the difficulties in recognizing exposure behavior in the questionnaire, or possibly difficulty in recalling exposure during gestation. In addition, in our society pregnant women may fear telling the truth about their exposure because of fear of risks of tobacco smoke for the fetus, or due to social pressure. All these factors will lead to underreporting of the real exposure in the questionnaires compared with laboratory cotinine level. With biomarkers of smoking exposure and mean laboratory cotinine level from babies cord blood, it seems that laboratory cotinine level was approximately 2 times the nicotine level recorded by the questionnaire, and this may give us an initial idea on the relationship between those 2 readings. Pichini et al,[20] Tappin,[21] and Roumans[22] reported that nicotine levels from the questionnaire were less than the laboratory cotinine level due to maternal unawareness and underestimation of hours of ETS exposure, as well as an unwillingness to declare smoking exposure during pregnancy.

The present study shows a linear relationship between laboratory cotinine level from the cord blood of babies and the questionnaire nicotine level. This relationship was strong with a correlation r square of 0.8043 and 0.897 and significant (p<0.0001), in agreement with other studies.[9,23] Accordingly, this relation can be used to predict exposure to ETS among pregnant women through the use of a self-report questionnaire to predict exposure level without depending on laboratory cotinine estimation (ELISA test for cotinine is expensive and the kit is not available in our country) with limitation to use (short half life) and considerable intersubject variability in uptake, metabolism, and elimination. According to the result of this study the equation y=1.1912, and x=5.3814 (were y is nicotine level from the questionnaire and x is laboratory cotinine level from babies cord blood) can be used to estimate approximate exposure to ETS among non smokers using information taken from the person. Using (y) and then apply the equation to find (x), for example: X=Y+5.3814/1.1912. Pichini et al[20] also found a linear relationship between cord serum cotinine level and nicotine questionnaire levels of ETS, however, the relationship was inaccurate in high doses of active smoking.

According to the present results, we can conclude that cotinine is a sensitive measure of ETS exposure, but if not available, then self-reported exposure to ETS can be helpful to predict exposure status. However, the number of mothers who participated in this study was only 88 due to difficulties in obtaining the cotinine ELISA kit in Iraq, and future studies with a larger sample size are needed to confirm our results.

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