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The effect of cigarette or sheesha smoking on first-trimester markers of Down syndrome

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Objective To investigate the influence of cigarette or sheesha smoking on first-trimester markers of Down syndrome.

Design A prospective observational study.

Setting Primary care centres and antenatal clinics of Maternity and Children Hospital, King Abdulaziz University Hospital and New Jeddah Clinic Hospital, Jeddah, Saudi Arabia.

Population Women with a singleton pregnancy who were either nonsmokers (n = 1736) or cigarette smokers (n = 420) or sheesha smokers (n = 181).

Methods Fetal nuchal translucency thickness (fetal NT), maternal serum free beta-human chorionic gonadotrophin (free β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) were measured at 11 weeks 0 days to 13 weeks 6 days of gestation in all women. Women were grouped according to smoking status, confirmed by maternal serum cotinine measurements, and analyte levels between groups were compared.

Main outcome measures Fetal NT, maternal serum free β-hCG, PAPP-A and cotinine measurements.

Results Compared with nonsmoking women, fetal NT was significantly increased and free β-hCG and PAPP-A levels were significantly decreased in both cigarette and sheesha smokers. There were significant relationships between all three markers and the number of sheeshas consumed per day.

Conclusions Cigarette and sheesha smoking significantly affect first-trimester markers of Down syndrome (fetal NT, free β-hCG and PAPP-A). Correction for this effect in women who smoke might improve the effectiveness of first-trimester screening for Down syndrome in these women. The underlying mechanism(s) relating smoking to the changes in first-trimester markers require further studies.

Keywords Cigarette, fetal NT, first-trimester, free β-hCG, PAPP-A, sheesha, smoking.

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Introduction

A combination of fetal nuchal translucency thickness (fetal NT) and maternal serum levels of free beta-human chorionic gonadotrophin (free β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) has been shown to be an effective approach to screening for Down syndrome, and other fetal trisomies, in the first trimester of pregnancy.[1–9] While the effect of cigarette smoking on second-trimester markers of Down syndrome has been reported extensively, increasing maternal serum levels of α-fetoprotein and decreasing unconjugated estriol and both total and free β-hCG.[10–13] However, there are few studies on the effect of cigarette smoking on first-trimester markers, and no reported studies on the effects of sheesha smoking. Cigarette smoking decreases maternal serum PAPP-A levels by 15–20%,[14,15] while the reported effect on free β-hCG has been inconsistent with some authors observing decreased levels of free β-hCG[14–18] and others reporting no effect.[19] Similarly, while there is a single report that cigarette smoking is associated with an increase in fetal NT,[14] the majority of authors have observed no effect.[16,17,19] Sheesha smoking (also known as water-pipe smoking, hubble-bubble, argila and hookah) has old traditions and is widely used in Saudi Arabia and the Middle East.[20] The effect of sheesha smoking on first-trimester screening markers for Down syndrome has not been studied before.

The main objectives of the present study were to prospectively examine the influence of cigarette or sheesha smoking

on the first-trimester markers of Down syndrome (fetal NT, maternal serum free β-hCG and PAPPA) in Saudi singleton pregnant women.

Methods

Over a period of 40 months (2001–04), 2494 healthy Saudi women with a normal singleton pregnancy were prospectively recruited from women attending antenatal care clinics at King Abdulaziz University Hospital (KAUH), Maternity and Children Hospital (MCH) and New Jeddah Clinic Hospital (NJCH) and 12 associated primary health care centres in Jeddah. All women were in the first trimester of pregnancy (from 11 weeks 0 days to 13 weeks 6 days of gestation) at recruitment and all completed a medical examination. Women with significant medical disorders (e.g. hepatic, renal endocrine disorders and diabetes) were excluded, and all women had a normal pregnancy outcome. All women gave written informed consent to participate. The study protocol was in agreement with KAUH ethical standards and was approved by the Local Ethics Committee.

All women were examined by ultrasound scan to assess viability, gestation (crown rump length) and fetal NT, using standardised techniques following previous established criteria.[21] A blood sample was obtained from each woman, which was immediately transferred to the Clinical Endocrine and Metabolic Research Unit at King Fahd Medical Research Centre where sera were separated by centrifugation and stored at –130°C until analysis. All assays were performed within 48 hours of sample collection. Free β-hCG and PAPP-A were assayed using commercially available kits (DELFIA dual-labelled time-resolved fluorescent assay system, Perkin Elmer, Wallac, Turku, Finland). The within and between assay variations for free β-hCG were both <3.1% and for PAPP-A were <1.4 and <2.6%, respectively. The analytical sensitivities of free β-hCG and PAPP-A were 0.2 ng/ml and 5 mU/l, respectively.

At recruitment, women were asked about their smoking habit and were classified as smokers if they smoked one or more cigarettes or sheeshas per day. Smoking was analysed in three categories (cigarette smokers, sheesha smokers and nonsmokers) and also in ordered categories according to the number of cigarettes (≤5, >5–≤10, >10–≤20 and >20) or sheeshas (1, 2 and ≥3) consumed per day. Women who smoked both cigarettes and sheesha ($n = 157$) were excluded from study, leaving 2337 women for study. To validate information on smoking status, cotinine was measured using a commercial enzyme immunosorbent assay (Cozart Biosciences Ltd, Abingdon, UK) in the collected sera as per manufacturer's instructions. Serum cotinine levels were expressed as ng/ml. A cutoff of 15 ng/ml of cotinine was used to differentiate smokers (cigarettes or sheeshas) from nonsmokers.[22]

All marker results were expressed as multiple of the gestation-specific medians (MoMs) in our population. The median MoM equations were established by least squares regression of the logarithmic-transformed marker values against gestational age (in decimal weeks). Median marker values = $10^{(A + B \times \text{gestation age})}$ where A and B are intercept and slope, and gestation age is in decimal weeks. For maternal body weight adjustment of the MoM values for weight, each was divided by the expected weight-specific MoM: this was obtained by regression analysis using an inverse model.[23] The Gaussian distribution parameters of $\log_{10}$ MoM were estimated for the women studied. The mean was estimated from the $\log_{10}$ median value. To avoid the undue influence of occasional outliers, the SD was calculated from the 10th to 90th centile range divided by 2.563. Similarly, the relationship between fetal NT and gestational age (days) was regressed and applied to a log-transformation of fetal NT. The generated equation was used to compute the mean of log fetal NT for a given gestational age, equivalent to the median log fetal NT since fetal NT was log-normally distributed. Comparisons between nonsmokers and smokers were undertaken using the Wilcoxon rank sum test. All statistical analysis was carried out using SPSS statistical package (version 11.0 for Windows) (SPSS Inc., Microsoft Corp., Chicago, IL, USA).

Results

A total of 2337 healthy Saudi women with a normal singleton pregnancy at 11 weeks 0 days to 13 weeks 6 days of gestation were studied. Basic demographic and pregnancy outcome data are detailed in Table 1. Serum cotinine levels were significantly higher in smoking women than in nonsmokers (Table 1), with the highest levels in sheesha smokers. The population parameters of distribution (median MoM, mean $\log_{10}$ MoM and $\log_{10}$ SD) for fetal NT were: 1.02, 0.0142 and 0.1321 for nonsmokers; 1.18, 0.0496 and 0.1674 for cigarette smokers and 1.20, 0.0561 and 0.1720 for sheesha smokers, respectively. The same parameters for free β-hCG were: 1.00, 0.0372 and 0.2943 for nonsmokers; 0.87, 0.0390 and 0.3027 for cigarette smokers and 0.84, 0.0425 and 0.3236 for sheesha smokers, respectively. The same parameters for PAPP-A were: 1.01, –0.0301 and 0.2943 for nonsmokers; 0.82, –0.0892 and 0.2965 for cigarette smokers and 0.78, –0.0934 and 0.3125 for sheesha smokers, respectively.

Table 2 summarises fetal NT, free β-hCG and PAPP-A levels among nonsmokers, cigarette and sheesha smokers. Compared with nonsmoking women, cigarette-smoking women were associated with a significant decrease in levels of free β-hCG (13%; $P < 0.001$) and PAPP-A (18.8%; $P < 0.001$) and with a significant increase in fetal NT (15.7%; $P < 0.001$). Similarly, sheesha-smoking women were associated with significantly decreased levels of free β-hCG (16.0%; $P < 0.05$) and PAPP-A (22.8%; $P < 0.001$) and with

Table 1. Maternal age, body weight, gravida, duration of pregnancy at screening, week of delivery, fetal birthweight and fetal gender of Saudi nonsmoking and smoking pregnant women studied during the first trimester (11–13^{+6} weeks)

Clinical and anthropometric variables	Smoking status		
	Nonsmokers	Cigarette smokers	Sheesha smokers
Number	1736	420	181
Age (years)	29.5 ± 4.2	27.1 ± 3.6*	31.2 ± 4.8*,**
Body weight (Kg)	67.8 ± 14.6	63.4 ± 13.4*	64.4 ± 12.9*
Gravida	3.8 ± 2.4	3.4 ± 2.6	3.6 ± 2.1
Duration of pregnancy at screening (days)	85 ± 6	84 ± 6	85 ± 6
Week of delivery	39.6 ± 1.4	39.4 ± 2.6	39.2 ± 2.2
Fetal birthweight (g)	3336 ± 292	3120 ± 316*	3075 ± 285*,**
Fetal gender (M/F)	920/816	227/193	92/89
Serum cotinine (ng/ml)	4.9 ± 3.6	295 ± 166*	385 ± 177*,**

Values are presented as means ± SD.
*Significantly different from nonsmokers ($P < 0.05$).
**Significantly different from cigarette smokers ($P < 0.001$).

significantly increased fetal NT (17.6%; $P < 0.001$). Stratifying women according to the number of cigarettes smoked per day showed a positive trend for an increase in the median MoM values of fetal NT with increasing number of cigarettes smoked per day and a trend for a decrease in that of free β-hCG and PAPP-A. However, such relationships did not reach statistical significance. Similarly, when sheesha smoking pregnant women were stratified according to the number of sheeshas consumed per day, a significant tendency ($P < 0.05$) for an increase in the median MoM values of fetal NT with increasing number of sheeshas consumed per day and a significant tendency of a decrease in the values of free β-hCG ($P < 0.05$) and PAPP-A ($P < 0.05$) with increasing number of sheeshas consumed per day were evident.

Discussion

In the present study, we have shown that both cigarette and sheesha smoking significantly affect measurements of fetal NT, maternal free β-hCG and PAPP-A in the first trimester of pregnancy. This observation suggests that correcting these

Table 2. Median (MoM) values for fetal NT, free β-hCG and PAPP-A according to smoking status and number of cigarettes or sheeshas consumed per day during pregnancy in Saudi women studied

Status and cigarettes/ sheesha per day	Number	Median MoM		
		Fetal NT	Free β-hCG	PAPP-A
Cigarettes smokers				
≤5	106	1.09	0.981	0.912
>5–≤10	161	1.15	0.853	0.806
>10–≤20	115	1.20	0.830	0.784
>20	38	1.23	0.826	0.772
Cigarettes smokers (all)	420	1.18	0.87	0.82
Sheesha smokers				
1	96	1.15	0.898	0.865
2	48	1.19	0.835	0.801
≥3	37	1.22	0.816	0.702
Sheesha smokers (all)	181	1.20	0.84	0.78
Nonsmokers (all)	1736	1.02	1.00	1.01
Statistical significance*		$P < 0.001$	$P < 0.001$	$P < 0.001$

*Statistical significance was based on Wilcoxon rank sum test (two-tail).

markers for either cigarette or sheesha smoking may improve first-trimester screening performances in women who smoke.

In the present study, the percentage of cigarette or sheesha smokers among the population studied were 11.8 and 5.1%, respectively, similar to rates previously reported for cigarette smoking among Saudi women[24] but lower than that reported in another study (being 0.9% in Saudi women)[25] or in lactating Saudi women (being 2.0%).[26] The smoking status of women in our study was confirmed by serum cotinine levels, confirming the previous reports on cigarette smokers and nonsmokers.[27,28] Higher serum cotinine levels were evident in sheesha smokers compared with cigarette smokers: this is due to the larger amount of tobacco consumed during sheesha smoking compared with cigarette smoking.[20]

Our observation that smoking, either cigarette or sheesha, is associated with a significant increase in fetal NT and is consistent with that previously described in Finnish pregnant women who were cigarette smokers[14] but contrast with the observations of others.[16–19] Such differences could be related to smoking habits among the studied pregnant women and/or genetic, nutritional or other unknown contributing factors. However, although the results of the present study are significant, it may have no clinical relevance on the performance and measurement of fetal NT screening due to the small difference observed among the studied groups.[14] In addition, it is possible that the increased fetal NT among smokers is related to impaired and/or delayed development of the fetal lymphatic system, which can be attributed to smoking.[29] Similar conclusions can be made on sheesha smokers; however, further studies are needed in this regard. The median MoM values of maternal serum free β-hCG and PAPP-A were significantly decreased in cigarette or sheesha smokers compared with that of nonsmokers. The results on cigarette smokers are consistent with previous studies.[14–19] However, to our knowledge, the results on sheesha smokers are considered to be the first reported in the literature. The reasons why smoking affects the maternal biochemical markers are still poorly understood. Three possibilities can be offered. First, smoking has been shown to damage the placental barrier and to disturb transportation across the placenta, which may influence the extent of maternal-fetal handling of the markers studied;[30] second, the placental syncytiotrophoblast undergoes apoptosis, and this process is inhibited by smoking, which in turn might modify the maternal-fetal exchange process;[31] and third, the number of placental areas in which the syncytiotrophoblastic necrosis was significantly higher among smokers compared with nonsmokers,[32] which may affect the availability of these markers between the maternal-fetal processes. Whether these changes may have an impact on the screening process in the first trimester is still controversial. Stratifying the MoM values of fetal NT together with that of maternal serum free β-hCG and PAPP-A in smoking pregnant women according to the number of cigarettes consumed per day showed no significant relationship between the number of cigarettes consumed per day and the changes in the marker median MoM values. The latter is consistent with the results of Spencer *et al.*,[17] but contrast with that of Yigiter *et al.*[19] However, stratifying the MoM values for fetal NT, maternal serum free β-hCG and PAPP-A according to the number of sheeshas consumed per day showed significant relationship between the two variables ($P < 0.05$). This difference in the relationship between the first-trimester markers studied and the daily consumption of cigarettes compared with sheeshas requires further studies.

Conclusions

In conclusion, the impact of smoking on the first-trimester markers (namely: fetal NT, maternal serum free β-hCG and PAPP-A) with MoM values may be related to the number of cigarettes or sheeshas consumed per day, which is more evident for the latter. The effect of smoking may start after a certain level of consumption has been achieved by the pregnant women and seems to be evident on the first-trimester screening markers. Currently, the use of the combined first-trimester screening method (namely combination of fetal NT, maternal serum free β-hCG and PAPP-A) is considered to be an effective method for early pregnancy screening for Down syndrome. According to the results of the present study, the changes in the three first-trimester markers due to cigarette or sheesha smoking may have an impact on the performance of the screening programme during the first trimester. The changes in fetal NT and the biochemical markers in smoking pregnant women will put them at a higher risk for Down syndrome than nonsmokers. Thus, correction for these parameters for smokers might improve the accuracy of risk assessment calculation for a given pregnant woman undergoing prenatal screening accordingly. Further studies are needed on affected pregnancies with Down syndrome to establish the underlying mechanism(s) of smoking on these variables accordingly.

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