

Effect of Second Hand Smoke Exposure on Serum Estradiol and Progesterone Levels in women having First Trimester Spontaneous Abortions

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ABSTRACT

Background: Spontaneous abortion is a major obstetric complication. Considerable work has been done on its etiology but still in about one fourth of the cases, the cause is unknown. Altered hormonal level especially of progesterone and estrogen is one of the causes of spontaneous abortion. Maternal smoking and maternal second hand tobacco smoke exposure, which is the inhalation of the environmental tobacco smoke by the non-smoker, during pregnancy, might have an association with it.

Aim: To investigate the effect of second hand smoke exposure on serum cotinine, progesterone and estradiol levels and to correlate these in women having spontaneous abortions.

Methods: It was a comparative study. The study comprised of two groups. Group I included non-second hand smoke (SHS) exposed women (serum cotinine level <0.05ng/ml) having first trimester spontaneous abortion, group II comprised of SHS exposed women (serum cotinine level ≥ 0.05ng/ml) having abortion. Blood sample was taken from each subject and analysed for estradiol, progesterone and cotinine. Serum progesterone, serum estrogen and serum cotinine levels were estimated by ELISA. Data was entered into and analysed by using SPSS version 20. Data was explored for normality by using Shapiro-Wilk test. Kruskal-Wallis and Mann-Whitney U were used to compare the differences in groups and Spearman's rho was used to correlate different parameters.

Results: There was no significant difference in serum estradiol and progesterone levels in SHS exposed women having abortion and non-exposed women having abortion ($p>0.05$). There was a significant positive correlation between estradiol and progesterone levels in SHS exposed women having abortion ($\rho=0.63$, $p=0.001$) and in non-exposed women having abortion ($\rho=0.54$, $p=0.002$).

Conclusion: The present study concludes that second hand smoke exposure has negative impact on serum estradiol levels in women having spontaneous abortions. Moreover, serum estradiol and progesterone are positively correlated in women having abortions (SHS exposed and non-exposed women).

Keywords: Estradiol, progesterone, second hand smoke, cotinine, abortion

INTRODUCTION

The tragic event of abortion occurs in about 15-20 percent of all pregnancies¹. In about one fourth of the cases, the cause is unknown. Certain environmental toxins may also contribute to abortions. Maternal smoking, exposure to environmental tobacco smoke and deficient hormone levels especially progesterone and estrogen might be a cause. Tobacco smoke exposure may result in deficiency of hormones leading to spontaneous abortion^{2,3}.

Estrogens and progesterone are indispensable for pregnancy. They balance each other to maintain blood flow at the site of implantation. Estrogens regulate the production of progesterone from placenta. Estradiol induces increased expression of

progesterone in uterus. Many studies have reported significantly lower progesterone and estradiol concentration in women having spontaneous abortions^{4,5,6,7,8}.

The smoke which is present in the atmosphere around us and is inhaled by the non-smokers is termed as "second hand smoke" (SHS) or "environmental tobacco smoke" or "passive smoke". The term "second hand" is preferred as it depicts the involuntary nature of exposure. Second hand smoke contains thousands of chemicals including many reproductive toxins like cadmium, lead, benzene etc. and more than 50 identified carcinogens. It can be said that second hand tobacco smoke exposure from cigarette, biri, cigar, water-pipe, huqqa, sheesha and hubble bubble has similar effects as that of active smoking, however the magnitude might be different depending upon the circumstances and extent of exposure^{9,10}.

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The extent of exposure to the toxins and carcinogens present in the second hand smoke can be measured with the help of nicotine but the problem with nicotine is that it is rapidly metabolized to cotinine in the body. So cotinine, because of greater half-life is said to be the most suitable and reliable biomarker to assess the second hand tobacco smoke exposure. Serum cotinine levels below 0.1ng/ml or even below 0.05 ng/ml depicts non- exposed. In second hand smoke exposed person, the serum cotinine levels are below 15ng /ml. Higher values depict the active exposure^{10,11,12}.

Women do not smoke commonly but are subjected to its harmful effects via second hand smoke. According to a survey conducted by on pregnant women in Pakistan, about half (49.9%) of them reported being exposed to SHS¹³.

Studies performed to see the association between smoking and second hand smoke exposure with abortions show variability in results¹⁴. George et al. reported an increased risk of spontaneous abortions in women who were smokers or exposed to second hand smoke, while Chatenoud et al and Arffin et al. found no association between risk of spontaneous abortion and tobacco smoke exposure^{11,15,16}.

In a prospective study conducted on Chinese women who were planning to conceive, it was found that the levels of estrone conjugate; a major estrogen metabolite in urine, were significantly less during non-conception cycles of SHS exposed women as compared to non-exposed women. No association was found during conceptual cycles. No association of progesterone metabolite concentration in urine to SHS exposure was found¹⁷.

The cigarette smoke may elicit its harmful effects by altering the hormonal profile¹⁸. The direct effect of cigarette smoke alkaloids on human granulosa cells is inhibition of progesterone synthesis¹⁹. However, we do not know if there is any direct association of smoking with progesterone and estrogen levels in women having abortions. In Pakistan, there is a lack of data to emphasize on the magnitude of health harms contributed by second hand smoke exposure²⁰. Studies need to be done to see the impact of second hand smoke exposure on pregnant women. No local study has been conducted so far to check the second hand smoke exposure status of pregnant women by using cotinine as a biomarker. The present study was planned to investigate the effect of second hand smoke exposure as depicted by serum cotinine levels on serum progesterone and estrogen levels in women having spontaneous abortions.

SUBJECTS AND METHODS

It was an observational (cross-sectional) comparative study. This study was conducted in the Department of Physiology and Cell Biology, University of Health Sciences, Lahore in collaboration with Obstetrics and Gynaecology Department, Services hospital, Lahore. The selection criterion was women having spontaneous abortions at 6 to 12 completed weeks of gestation, of 18-35 years of age. The women of age >35 years, having history of induced abortion, second trimester abortion, active smoking, assisted fertilization, progesterone supplementation and any well-established cause of abortion e.g. cervical incompetence, trauma, chronic illness, hypertension, diabetes, autoimmune disorders, or any uterine anomalies were excluded.

Ethical approval was taken from Ethical Review Committee of University of Health Sciences, Lahore and Services Hospital, Lahore. Out of 100 women interviewed 53 women fulfilling the criteria and were willing to participate were selected. Abortion was confirmed by ultrasonography. Written informed consent was taken and history and relevant physical examination was performed. From each subject, 3 ml of venous blood was drawn. Serum was separated and stored at -80 °C in properly labelled eppendorfs. Serum cotinine, estradiol and progesterone were measured in serum quantitatively by immuno-enzymometric assay with an automated EIA analyser CODA, Bio-Rad laboratories, Hercules, CA, USA with the kits (Calbiotech Cotinine Direct ELISA Kit CO096D, Biocheck Estradiol enzyme immunoassay BC-1111 and Biocheck progesterone enzyme immunoassay test kit BC-1113). On the basis of serum cotinine women were divided into two groups. Group I comprised of 30 women, having abortion and serum cotinine levels < 0.05ng/ml and group II comprised of 23 women, having abortion and serum cotinine levels ≥ 0.05ng/ml¹¹.

Statistical analysis: The data was entered and analysed using IBM Statistical Package for Social Sciences (SPSS), version 20. Frequencies and percentages were calculated for qualitative variables. For quantitative variables, mean ±SD (Standard deviation) were calculated for normally distributed variables and median with IQR (interquartile ratio) were calculated for non-normally distributed variables. Student's t-test was applied to observe mean differences of normally distributed variable and Mann Whitney-U was applied for non-normally distributed variables. Spearman's rho correlation was applied to correlate non-normally distributed variables.

RESULTS

Table 1 shows the comparison of age, gestational age, history of previous abortion and gravidity in groups I and II. There was no significant difference between the mean age and gestational age of the two groups. Median serum cotinine level of group II was significantly higher than group I ($p < 0.01$). There was no significant difference between the median

serum progesterone ($p = 0.979$) and estradiol ($p = 0.319$) levels of group I and II (Table 2). Table-3 and figure-1 shows that there was significant negative correlation between serum cotinine and serum estradiol in group II ($p < 0.05$). There was significant positive correlation of estradiol with progesterone in group I and group II ($p < 0.01$). (Table-4, Fig. 2,3).

Table 1: Comparison of age, history of previous abortions, gravidity and gestational age in groups I, II

Parameter		Group I (n=30)	Group II (n=23)	p-value
Age(years) Mean±S.D		27.07±4.42	25.61±4.33	0.075*
Gestational age (days) Mean±S.D		72.77±11.29	70.30±11.32	0.599*
Previous abortions N (%age)	None	16(53.3%)	16(69.6%)	---
	1	12(40%)	3(13%)	---
	2	2(6.7%)	2(8.7%)	---
	≥3	0	2(8.7%)	---
Gravidity N(%age)	Primigravida	4(13.3%)	7(30.4%)	---
	Gravida2	9(30%)	6(26.1%)	---
	Gravida≥3	17(56.7%)	10(43.5%)	---

* $p > 0.05$ is considered statistically non-significant Values of previous abortions and gravidity are expressed as frequency and percentage. Comparison of parameters in groups by student t test

Table 2: Comparison of serum cotinine, progesterone and estradiol in groups I and II

Parameter	Group I (n=30)	Group II (n=23)	p-value
Serum cotinine (ng/ml)	0	0.92(0.71-1.79)	< 0.01*
Serum progesterone(ng/ml)	2.97(1.36-5.27)	3.19(2.00-4.42)	0.979
Serum estradiol(pg/ml)	12.15(4.99-40.84)	6.58(2.46-35.92)	0.319

Values are expressed as Median (IQR) * $p < 0.05$ is considered statistically significant Comparison of parameters in groups by Mann Whitney-U test

Table 3: Correlation of serum cotinine with progesterone and estradiol in group II

Correlation between serum cotinine	Correlation coefficient	p-value
Serum progesterone	-0.14	0.535
Serum estradiol	-0.42	0.049*

* $p < 0.05$ is considered statistically significant Correlation by spearman's rho correlation

Table 4: Correlation between serum estradiol and progesterone in groups I, II

Correlation between serum estradiol and:	Group I		Group II	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Serum progesterone	0.54	0.002*	0.63	0.001*

* $p < 0.05$ is considered statistically significant Correlation by Spearman's correlation

Fig.1: Scatter plot showing correlation ($\rho = -0.42$, $p = 0.049$) between serum cotinine and estradiol in second hand tobacco smoke exposed women having abortion. (Group II)

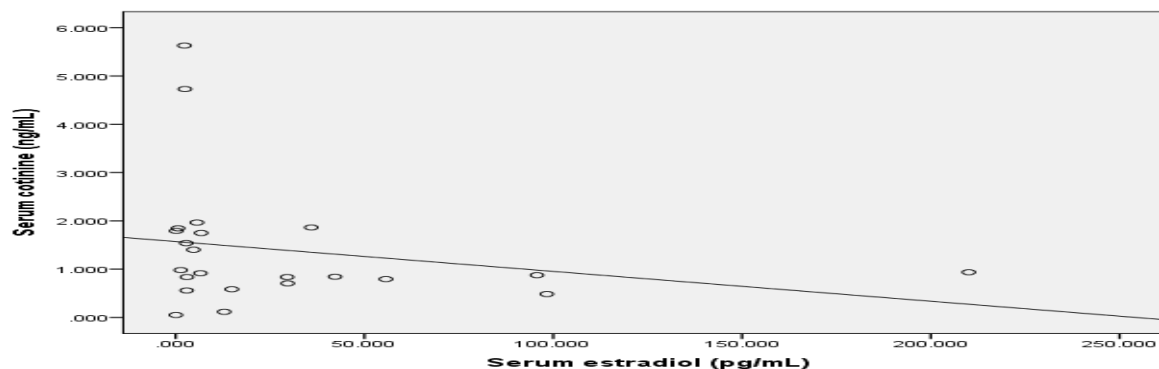


Fig.2: Scatter plot showing correlation ($\rho= 0.63, p=0.001$) between serum estradiol and progesterone in second hand tobacco smoke exposed women having abortion. (Group II)

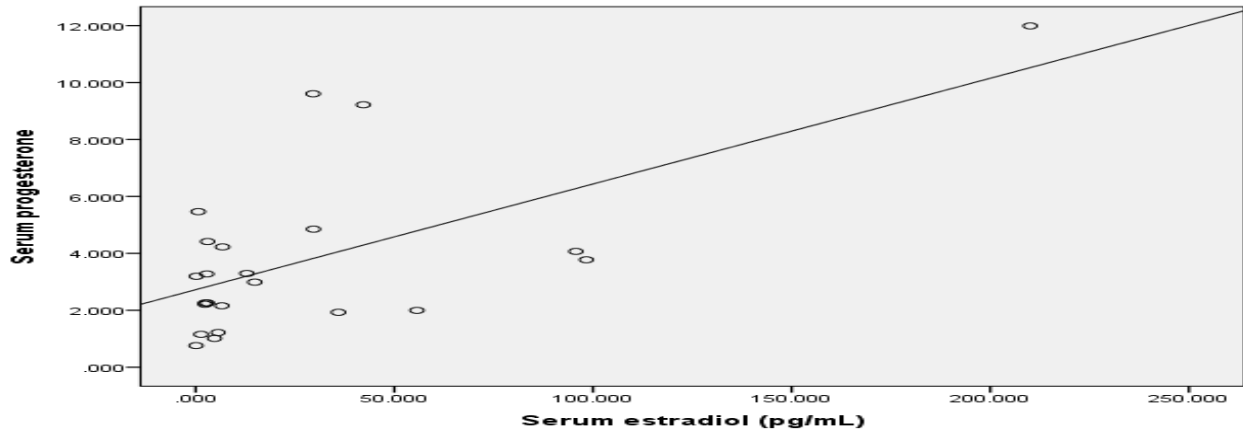
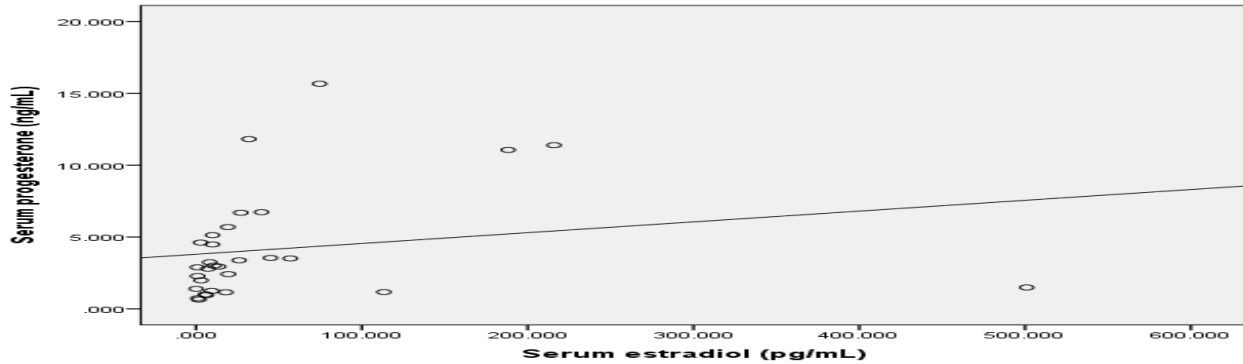


Fig.3: Scatter plot showing correlation ($\rho= 0.54, p=0.002$) between serum estradiol and progesterone in non-exposed women having abortion. (Group I)



DISCUSSION

The present study evaluated the levels of serum progesterone, estradiol and cotinine in SHS exposed women having first trimester spontaneous abortions and non- exposed women having first trimester spontaneous abortions. There was no statistically significant difference between serum estradiol and progesterone levels of SHS-exposed and non-exposed having abortions.

In a meta-analysis of 26 cohort studies, it was found that a low progesterone concentration, less than 3.2 to 6ng/ml depicts non-viable pregnancy⁷. In our study both non-exposed and SHS exposed group had median serum progesterone levels near these values, for Group I it was 2.97(1.36-5.27) and for group II it was 3.19(2.00-4.42)

Aksoy et al found that 80% of the subjects with normal pregnancy had serum estradiol levels above 350pg/ml⁵. In the present study, all the subjects of abortion group with SHS exposure had values below 350pg/ml. 96% of the subjects had value below 100pg/ml. Only one subject in the abortion group that

was not exposed to SHS had serum estradiol value above 350pg/ml.

In a cohort study done by Gu et al. on non-pregnant females of reproductive age, urinary estrogens and 15 estrogen metabolites were measured. Smokers had lower levels; also the ratios of these metabolites were different. Non-smokers had more 16-hydroxylation products. 16-hydroxy-metabolites have more estrogenic activity as compared to 2 hydroxylation products, which do not have potent estrogenic effects. In their study SHS exposure was not estimated²¹. In our study, we only considered estradiol levels. Other estrogens were not measured. Active smoking was also not considered. Although the median serum estradiol level in women having abortion was lower in females who were exposed to SHS as compared to non-exposed but this difference was not significant. However, the present study does find a significant negative correlation between serum estradiol levels and cotinine levels in women exposed to SHS. This means greater the exposure to SHS, lower would be serum estradiol levels. This gives an indirect clue that

SHS exposure has some effect on the hormonal profile. SHS exposure may illicit its effect either by decreasing estradiol synthesis or increasing its degradation to inactive compounds or it may alter its metabolism so that less potent estrogens are formed. These findings might also be due to some chance or some possible unmeasured confounding factors.

The mean cotinine level of SHS exposed women having abortion was 0.92ng/ml. All the subjects had levels in SHS exposed range. Many studies have defined the range of serum cotinine for SHS exposure. Active smoking is depicted by serum cotinine level above 15ng/ml. No subject had serum cotinine levels in the active smoking range which is above 15ng/ mL. The serum cotinine levels reflect the cumulative long term exposure to smoke. It also tells us the magnitude of exposure. Greater the exposure, higher would be serum cotinine.

CONCLUSIONS & RECOMMENDATIONS

It is concluded that serum estradiol and progesterone levels are not different in second hand smoke exposed women having abortions and unexposed women having abortions. Second hand smoke has a negative impact on serum estradiol levels in women having spontaneous abortions. In women having abortions (SHS exposed and non-exposed), serum estradiol levels are positively correlated with serum progesterone levels.

It is recommended that prospective studies in larger sample size be done to see the impact of SHS exposure on hormonal profile during abortion. Further studies on larger scale are recommended to assess the exposure status of pregnant females using a biochemical marker like cotinine. There is also a great need to initiate health education programs of obstetric population to avoid SHS exposure at a national level for the betterment of health.

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