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The effects of smoking on the placentas of smoker mothers in terms of MPO, MMP-9 and FGF

Placenta and smoke

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Abstract

Aim: Smoking during pregnancy remains a common habit. Pregnant smokers harm both themselves and their fetus. Therefore, in this study, we aimed to investigate oxidative stress and angiogenic effects of smoking on pregnant smokers by analyzing FGF, MMP-9, and MPO levels.

Material and Methods: The placentas of 68 pregnant women which referred to the pathology laboratory between the years 2010 and 2011 were included in the study. Of the total, 28 women were smokers (S) and 40 women were non-smokers (NS). The FGF, MMP-9, and MPO immunostaining of placental tissues were examined by manual microarray study. The number of cigarettes smoked, age, systemic disease rate, mean abortus number, blood pressure, hemoglobin and hematocrit, Apgar, and pathological parameters were also evaluated.

Results: Statistically significant positive relationships were found between S and NS groups in terms of MMP-9 staining (grade 1) (p=0.039). There was no MMP-9 staining in S group. Systemic diseases were more frequent in S than NS group (p=0.049). There was no statistically significant relationship between other parameters.

Discussion: The effects of smoking on the fetus have been demonstrated with MMP-9. More comprehensive studies are needed to reveal the relationship between fetus and smoking.

Keywords

Smoking, FGF, MMP-9, MPO, Placenta

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Introduction

Smoking during pregnancy remains a common habit. Smoking accounts for a significant proportion of fetal morbidity and mortality through both the fetal and placental effects. Smoking causes placental abruption ranging from 1.2% to 4.0 [1]. Previous studies have reported that smoking during pregnancy is significantly associated with increased risks of intrauterine growth retardation (IUGR), stillbirth, early weaning, low birth weight, miscarriage, and sudden infant death syndrome [2, 3].

Normal pregnancy is associated with increases in uterine and umbilical blood flows [2]. These increases in blood flows are attributed to both placental angiogenesis and vasodilation. These are directly correlated with fetal growth, survival, and neonatal birth weight [3,4]. The basic fibroblast growth factors (bFGF or FGF-2) are expressed by the trophoblast and endothelial cells in the uterine and placentas. Researchers thought that FGF-2 plays a key role in regulating placental angiogenesis and vasodilatation [5, 6]. There are many functions of FGF in many cellular processes including proliferation, differentiation, migration, and cell survival during all stages of prenatal and postnatal life [7]. This place is important for the bidirectional mother-fetus exchanges of nutrients and respiratory gases essential for fetal growth/survival during pregnancy [8].

Smoking is known to activate oxidative stress and healthy pregnancy, like other physiological states, is characterized by a stable redox balance between ROS and antioxidants. In vitro and in vivo studies have demonstrated that nicotine induces the excessive generation of reactive oxygen species (ROS). It leads to the development of embryonic and fetal oxidative stress during pregnancy. ROS brings about changes in most types of lipid peroxidation, protein oxidation, and DNA damage. The embryo has a relatively weak antioxidant defense. It is susceptible to severe damage caused by smoking [9, 10, 11]. A study on children between the ages of 4-17 found that passive smoking increases oxidant MPO levels and does not affect antioxidants. It is declared that this situation can lead to the development of various diseases [12]. Myeloperoxidase (MPO) has been demonstrated to accumulate on the surface of neutrophils in pregnant women and contributes to the metabolic activity and oxidant production of neutrophils during pregnancy. However, the specific role of MPO during pregnancy remains unclear [13].

MMPs include collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs. Matrix metalloproteinases (MMPs) are a family of proteases that degrade the extracellular matrix (ECM) and connective tissue proteins [14]. Cigarette smoke condensate increases the expression of metalloproteinase-9 [13]. Increased activity of MMP-2 and MMP-9 has been found to be associated with a detrimental effect on the brain after neonatal hypoxia in rats [15].

The aim of this study was to evaluate the possible harmful effects of smoking based on some clinical parameters and immunohistochemical analysis with FGF, MMP-9, and MPO in placental tissue.

Material and Methods

This retrospective study used formalin-fixed tumor samples including placental tissues taken from 68 patients who were diagnosed between 2010 and 2011 in the Department of Pathology, Medical Faculty. The samples were grouped as smokers (S) and non-smokers (NS) analyzed immunohistochemically.

We examined many placental tissues on a single slide by manual microarray method for each placental sample. The slides were stained with anti-FGF, anti-MMP-9, and anti-MPO. The sections were examined and scored using light microscopy (Olympus BX50). The study protocol was reviewed and approved by the Ethics Committee of xxx University with the approval number 2016/28.

1.1. Immunohistochemistry

The expression of FGF, MMP-9, and MPO was respectively analyzed by staining with anti-bFGF, anti-MMP-9, and anti-MPO in 68 placental villous tissues. For this application, samples (0.5x0.5 in diameter) were taken from the maternal surface of each placenta. All tissues were fixed in formalin, embedded in paraffin, and cut into 5-µm-thick sections, which were collected on slides coated with poly-L-lysine. After the paraffin was removed, the sections were rehydrated. Immunostaining was performed using the streptavidin-biotin-peroxidase method. The samples were microwaved for 10 minutes to fix the antigens and then were washed with PBS (phosphate-buffered saline). They were then incubated with anti-bFGF (1-24), [Polyclonal] Conc. 0.1 ml (1:100), code: F3393, Sigma Aldrich, anti-MMP-9 [15W2] Conc. 1 ml (1:40-80), code: MMP9-439, Leica/Novocastra, anti-MPO (Dako Corp, Carpinteria, CA) for 1 hour. At this time, the slides were ready to be assessed with the IHC technique with streptavidin-biotin.

1.2. Evaluation of Immunohistochemical Staining

The intensity and localization of the staining reaction in cytotrophoblasts, syncytiotrophoblasts, chorionic villous stromal cells, villous vascular endothelial cells, vascular smooth muscle cells, and extravillous trophoblasts were evaluated by two investigators blind to the purpose of the study. Immunoreactivity to antibodies was scored using a semi-quantitative scale for the intensity of staining (as a score of 0-3). The scores were evaluated as follows: score 0 negative, no staining; 1+ weak positive; 2+ moderately positive; 3+ strongly positive (Figure1 (a,b,c)).

1.3. Statistical analysis

The analysis was performed using SPSS software (the Statistical Package for the Social Sciences, Version 19.0, SPSS Inc, Chicago, Illinois, USA). Descriptive statistics, percentage, and the median (minimum, maximum) values were expressed using either the Chi-Square test or the Kruskal-Wallis test.

Results

Of the total, 28 women were smokers (S) and 40 women were non-smokers (NS). The group of smokers averaged 8 (2 to 20) cigarettes per day. The age range of the pregnant smokers was 27.48 in NS group and 28.46 in S group. Gravida, parity, the average weight of the pregnant women and were similar in S group according to NS group. Blood pressure was higher in S group (114.64/71.43) than NS group. But it was not statistically significant. Systemic diseases were observed more frequently in S than NS group (p=0.049) (Table 1). MMP-9 staining (Grade 1) density was statistically significant in NS group when

compared to S group (p=0.039). There was no MMP-9 staining in S group. There was a range of 15% staining density of MMP-9 (Grade 1) (Table 1) (Figure 1). FGF staining was not statistically significant in NS group compared to S group (p=0.791) (Figure 2). MPO staining was also not statistically significant in NS group compared to S group (P=0,640) (Table-1) (Figure 3). We did not find a statistically significant relationship between other parameters (Tables 1,2).

Table 1. Relationship between groups and both stain and clinical parameters

			Groups		p-values
			NS (n=40)	S (n=28)	
FGF (grade)	0	n- (%)	21- (52.5)	15- (53.6)	0.791
	1	n- (%)	16- (40.0)	12- (42.9)	
	2	n- (%)	3- (7.5)	1- (3.6)	
MMP-9 (grade)	0	n- (%)	34- (85.0)	28-(100.0)	0.039
	1	n- (%)	6- (15.0)	0- (.0)	
MPO (grade)	0	n- (%)	27- (67.5)	18- (64.3)	0.640
	1	n- (%)	12- (30.0)	10- (35.7)	
	2	n- (%)	1- (2.5)	0- (.0)	
Abortus (mean number)	0	n- (%)	30- (75.0)	18- (64.3)	0.326
	1	n- (%)	8- (20.0)	6- (21.4)	
	2	n- (%)	0- (.0)	2- (7.1)	
	3	n- (%)	1- (2.5)	2- (7.1)	
	4	n- (%)	1- (2.5)	0- (.0)	
Systemic disease (no:1, yes:2)	1	n- (%)	5- (12.5)	9- (32.1)	0.049
	2	n- (%)	35- (87.5)	19- (67.9)	
Caesarean section (no:1, yes:2)	1	n- (%)	38- (95.0)	28-(100.0)	0.230
	2	n- (%)	2- (5.0)	0- (.0)	

Table 2. Identifier values in the numeric properties of both group

	Groups	N	Mean	SD	P
Age	NS-S	40- 28	27.48- 28.46	7.380- 6.362	0.557
Gravida	NS-S	40- 28	2.53- 2.82	1.485- 1.701	0.448
Parity	NS-S	40- 28	2.08- 2.04	1.248- 1.261	0.899
Child number	NS-S	40- 28	2.10- 2.07	1.236- 1.086	0.922
Weight	NS-S	40- 28	76.35- 77.29	11.356- 16.126	0.793
Length	NS-S	40- 28	1.6229- 1.6210	.05068- .06660	0.897
BMI	NS-S	40- 28	29.0562- 29.2254	4.18753- 5.30904	0.884
Weight (during pregnancy)	NS-S	40- 28	12.95- 12.71	5.074- 5.032	0.851
Systolic	NS-S	40- 28	108.63- 114.64	11.602- 15.982	0.076
Diastolic	NS-S	40- 28	67.63- 71.43	8.770- 11.455	0.126
Hemoglobin	NS-S	40- 28	11.308- 11.458	1.6967- 1.2982	0.696
Hematocrit	NS-S	40- 28	34,579-35.160	4.3694- 3,3398	0.555
Pregnancy week	NS-S	40- 28	37.595- 38.814	4.1647- 1.3848	0.141
Baby weight	NS-S	40- 28	3308.21- 3186,00	606.321- 622.802	0.421
Percentile weight	NS-S	40- 28	48.46- 45.98	26.514- 29.427	0.717
APGAR 1	NS-S	40- 28	7.85- 7.89	1.312- .685	0.537
APGAR 5	NS-S	40- 28	9.70- 9.89	1.588- .315	0.676
Placenta weight	NS-S	40- 28	616.14- 580.75	170.778- 148.710	0.379

Discussion

Vasoconstriction is one of the most harmful effects of the compounds in cigarette smoke. Vasoconstriction leads to a reduction in nutrient and oxygen flow to the fetus [16]. Habek et al. confirmed that smoking more than 20 cigarettes per day in pregnant women who smoked was associated with a high risk for pregnancy by causing maternal anemia and fetal hypoxia [17]. Anblagan et al. reported that fetuses exposed to maternal smoking are smaller in size and have smaller organs (brain, liver, lung). Besides, they noted that smokers' placental volumes are smaller than non-smokers in pregnant women [18]. In this study, it was observed that S groups are less than (range of 6%) NS groups. Vielwerth et al. observed that both prenatal and postnatal growth patterns were affected by maternal heavy smoking. The growth rate of weight in the third trimester was lower in fetuses whose mothers smoked heavily during pregnancy. Weight and length are reduced in the fetus of pregnant smokers [2]. In this study, newborn body weight and lengths of pregnant smokers were slightly lower. But, the differences were not statistically significant.

Reciprocal embryo transfer experiments demonstrated that embryonic MMP9 is a major contributor to normal implantation, but maternal MMP9 also plays a role in embryonic trophoblast development [19]. Plaks et al. investigated the role of embryonic and maternal MMP9 in embryo implantation and placentation [20]. In this study, there was no MMP-9 staining in S group. But there was mild staining in NS group (range of 15%).

It has been reported that maternal smoking causes strong oxidative stress in placental tissue. Myeloperoxidase (MPO) is evidence of the inflammation and endothelial dysfunction associated with oxidative stress in the circulation, vasculature, and placenta. The placenta, which normally has a rich vasculature, plays an important role in the development of IUGR. The most common cause of IUGR is placental ischemia. Ischemia results from regressed uteroplacental perfusion. Insufficient uteroplacental perfusion leading to abnormal angiogenesis may result in the pathophysiology of IUGR [3, 4, 9]. In this study, S group had more MPO staining than NS groups. Although the difference was not statistically different, it could be related to smoking-induced stress.

Previous studies have demonstrated that the production of angiogenic factors such as basic fibroblast growth factor (b-FGF), by villous placental cells [5, 6]. Many of these angiogenic factors are produced by trophoblasts [21]. Active and passive maternal smoking has a harmful effect in every trimester of human pregnancy. Smoking is associated from early in pregnancy with a decrease in vascularisation and thickening of the trophoblastic basement membrane, and an increase in the collagen content of the villous mesenchyme in placenta [22]. Angiogenesis may be regulated by oxygen status, and production of angiogenic growth factors. Vascular endothelial cells, pericytes, and trophoblasts include receptors and antagonists of the angiogenic growth factors. The resulting changes in fetal vasculature are associated with altered patterns of villous growth. Abnormal vasculogenesis, angiogenesis, and pseudo-vasculogenesis are correlated with the impaired placental and fetal development seen in complicated pregnancies such as IUGR. The results from

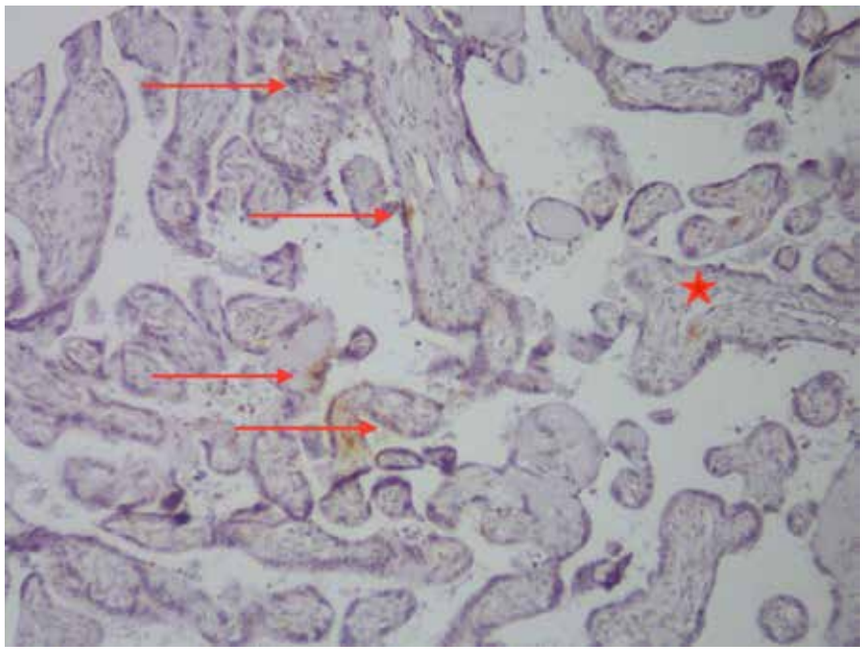

Figure 1. Weak staining of the cytotrophoblast (arrow) and endothelial cells(asteriks) (MMP-9x200)- (1+ weak positive)

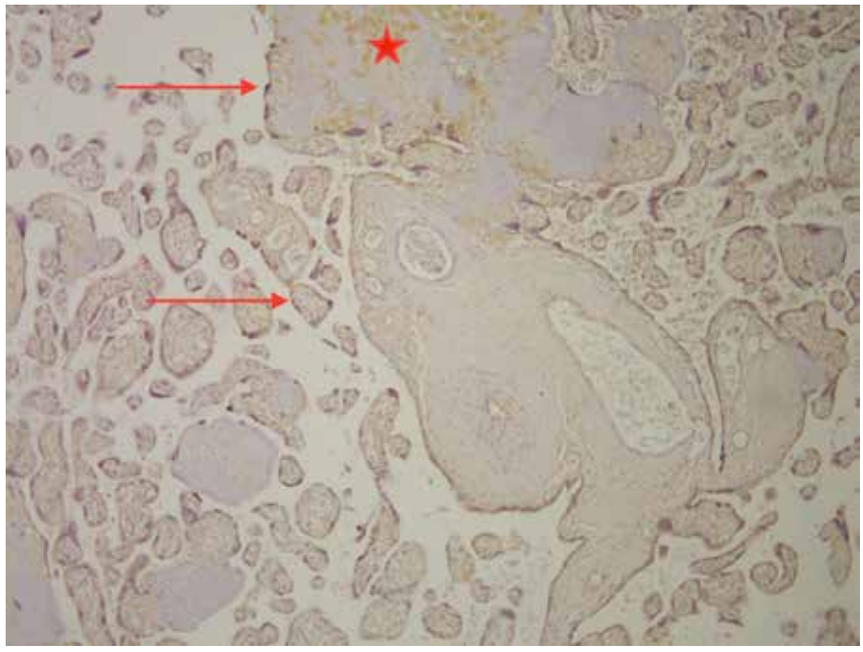

Figure 2. Weak staining of the cytotrophoblast (arrow) and stromal cells(asteriks) (FGFX100)- (1+ weak positive)

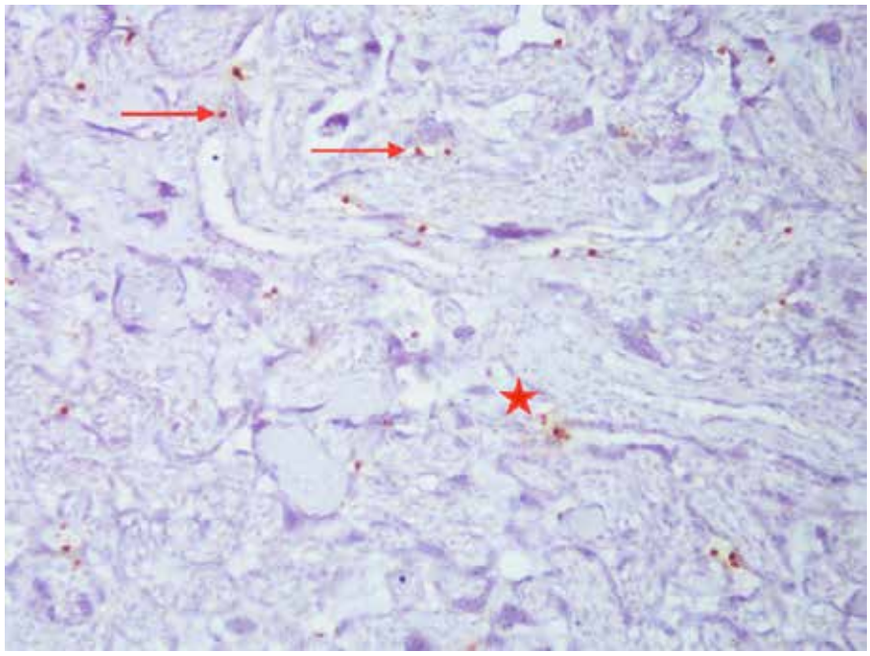

Figure 3. Weak staining of the endothelial cells (arrow) and cytotrophoblasts (asteriks) (myeloperoksidasex200) (1+ weak positive)

many studies support the literature which reports that a change in placental development accompanying deteriorated angiogenesis occurs in IUGR [4, 23, 24]. Chełchowska et al. showed that the birth weight of the smoking mothers' infants was lower by about 400 g ($p< 0.01$) and their birth body length by 1.5 cm ($p< 0.05$), and negatively correlated with the number of cigarettes smoked per day ($r = -0.55$; $p< 0.005$) [21]. Barut et al. reported that the interaction of maternal, placental, and fetal factors with increased oxidative stress, and angiogenesis may lead to pathologies such as placental chorangiosis [9]. In this study, FGF staining was not statistically significant in NS group compared to S group ($p=0.791$).

These results might be attributable to the small number of cases and the small number of smoking cigarettes per day.

Conclusion

In conclusion, systemic diseases were more common in pregnant smokers. In addition, the effects of smoking on the fetus have been demonstrated with the MMP-9 in this study. More comprehensive studies are needed to reveal the relationship between the fetus and smoking.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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