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Effect of Prenatal Cigarette Smoke Exposure on Hematological Characteristics in Adult Rat Offspring

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Abstract

Maternal cigarette smoking during pregnancy is associated with many severe developmental abnormalities on the fetus. The present study investigates the hematological parameters of the blood of adult rat offspring exposed prenatally to Cigarette Smoke (CS). Pregnant albino rats were exposed to CS 3 times/day (each 15 minutes) or room air (controls) from gestational day 0 to parturition. Hematological parameters were measured for 3 month–old adult offspring. Prenatal CS exposure significantly increases total leukocyte counts, monocytes ($P < 0.05$), and lymphocytes ($P < 0.01$); and significantly decreases mean corpuscular volume of red blood cells ($P < 0.05$) in adult rat offspring as compared with controls. However, prenatal CS exposure had no significant effect on other hematological parameters, including red blood cells count, granulocytes, platelets, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin, red cell distribution width, prothrombin time, mean platelet volume and platelet distribution width in adult rat offspring. These findings may indicate that cigarette smoking during pregnancy is associated with a significant increase in total leukocyte counts, lymphocytes and monocytes, and significant decreases mean corpuscular volume of red blood cells of adult rat offspring.

Keywords: Pregnant rats; prenatal cigarette smoke exposure; hematological parameters; adult rat offspring.

1. Introduction

Maternal cigarette smoking during pregnancy is a known risk factor for many severe pre- and postnatal developmental complications in the human fetus and infant. Prenatal CS exposure is the main risk factor in postnatal morbidity and mortality, intrauterine growth retardation, preterm delivery and sudden infant death syndrome (Andres and Day, 2000; Dempsey and Benowitz, 2001; Duncan et al., 2009). In addition, cigarette smoking during pregnancy can induce several prenatal complications, including spontaneous abortion, low birth weight, and increase in neonatal nucleated red blood cells (Blake et al., 2000; Voigt et al., 2006; Dollberg et al., 2000; Giovanni et al., 2013). CS is known to be a complex mixture of large number of highly toxic substances can lead to damage to neonates through active or passive smoking. Many of these metabolites, such as nicotine, cotinine, polycyclic aromatic hydrocarbons, metals and carbon monoxide can readily cross the placental barrier to the developing embryo and cause both prenatal and postnatal health consequences (Lambers and Clark 1996; Pichini et al., 2000). Some of these compounds were identified in the fetus blood and urine of newborns of smoking mothers (Pichini et al., 2000; Mamsen et al., 2010; Berlin et al., 2010; Giovanni et al., 2013), and in fetal blood of rodents (Carmines et al., 2003). These compounds can cause vasoconstriction to the uterine placental blood vessels (Donnenfeld et al., 1993; Albuquerque et al., 2004), resulting in serious reduction in oxygen supply and malnutrition to the developing fetus (Ganapathy et al., 1999). There also gestational exposure to CS stimulates the release of catecholamines from adrenal glands and sympathetic autonomic nerves which consequently limits the placental blood flow, causing intrauterine reduction in oxygen supply and under nutrition to the fetus (Ernest et al., 2001).

Several studies demonstrated that CS exposure or nicotine during gestational period is associated with significant permanent postnatal neurodevelopmental abnormalities in the activity of the central and peripheral nervous systems in adult offspring (Chen et al., 1998; Eugenin et al., 2008; Duncan et al., 2009; Bublitz and Stroud, 2012). This will result in severe deficits of many regulatory functions, including endocrinal (Fowler et al., 2009; Shields et al., 2009), cardiovascular (Blake et al., 2000; Xiao et al., 2008; Lawrence et al., 2008), respiratory (Gilliland et al., 2000; Singh et al., 2003; Pendleburg et al., 2008), reproductive (Jensen et al., 2005; Fowler et al., 2009; Mamsen et al., 2010),

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autonomic neural control (Khan et al., 1994; Franco et al., 2000; Parslow et al., 2004; Schneider et al., 2008) and neurobehavioral outcomes (Pauly and Slotkin, 2008; Ernst et al., 2001). It is known that large majority of neurons in the cerebral cortex and other brain regions are generated during gestational period.

In addition, human studies have demonstrated significant immunomodulatory effects of maternal cigarette smoking during pregnancy on the immune system parameters by increasing in lymphocyte counts (Giovanni et al., 2013) and serum immunoglobulin levels (Cederqvist et al., 1984) in newborns of smoking mothers compared to those born to non-smoking mothers. In addition, other studies have described the immunosuppressive properties of maternal CS exposure by decreasing total leukocyte counts, lymphocytes and modulating the number and function of alveolar macrophages in neonates of smoking mothers (Mercelina-Roumans et al., 1996; Sopori and Kozak, 1998). Animal models have shown that gestational nicotine exposure down-modulates the immune function of adult offspring by suppressing lymphocyte responsiveness (Basta et al., 2000).

The aim of the present study is to investigate the effect of CS exposure during gestational period on hematological parameters of adult rat offspring.

2. Materials and Method

2.1. Animals

Two groups (each of 5 females) of young adult albino rats (10 weeks of age and weighing 150 - 160 g body weight) were used for mating. The first group was CS exposure, and the second group was controls. The animals were housed individually in plastic cages, and allowed to acclimatize for one week. The rats were provided with standard laboratory chow and water ad libitum except during CS exposure. Animal room was maintained at 20 – 25°C. Care and handling of the animals were performed in accordance with the international guidelines for the use of laboratory animals. During the mating period, the female rats were placed with fertile males overnight, and the next morning female rats were examined to have mated by the presence of sperms in the vaginal smears (considered gestational day 0). At the end of mating period, the males were removed, and the pregnant females were divided into two groups of 5 animals each: experimental CS exposure group and control group.

2.2. Cigarette Smoke Exposure

The pregnant females of CS exposure group were daily exposed to CS via whole-body inhalation (3 times/day, each 15 minutes) from gestational day 0 to parturition. CS was generated from the burning of filtered cigarettes using a smoking device (designed in Neurobiology laboratory, Mutah University) which pumps CS in the form of puffs (each 35 ml) at regular intervals (one puff/min, and puff duration of 2 seconds). There were no deaths or any other abnormal signs in the pregnant females associated with CS exposure. The control group was subjected to the same experimental conditions of the CS exposure group, but without exposure to CS. Food consumption and body weight gain of pregnant females of CS exposure group were similar to that of pregnant females of the control group. Newborn pups were kept with their mothers until weaning. After weaning, male and female offspring of CS exposure group and control group were separated and kept in the animal house. The hematological parameters of the blood were measured in 3 month–old adult offspring (150 – 170 g body weight) of both groups.

2.3. Hematological Investigation

Blood samples (5 ml) were collected by cardiac puncture under light ether anesthesia from adult rat offspring of CS exposure group (males = 15 and females = 11) and control group (males = 13 and females = 10). Immediately after blood collection, complete blood cell counts and hematological analysis were performed according to laboratory routine using automated hematology analyzer (Mythic) in Al-Karak hospital, Ministry of health. In addition, differential cell counts of leukocytes were performed manually.

2.4. Statistical Analysis

Statistical evaluations were performed by Student's t-test for independent samples. The level of significance of all tests was set at $P < 0.05$, and the results were expressed as means ± S.E..

3. Results

3.1. Cellular Components of the Blood of Adult Rat Offspring after Prenatal CS Exposure

The cellular components of the blood of adult rat offspring after prenatal CS exposure and controls are shown in Table 1. Prenatal CS exposure significantly increases the total leukocyte counts and monocytes ($P < 0.05$), and lymphocytes ($P < 0.01$) in male and female adult rat offspring as compared with controls. However, prenatal CS exposure had no significant effect ($P > 0.1$) on red blood cells count, granulocytes and platelets in male and female adult rat offspring as compared with controls.

3.2. Hematological Parameters of the Blood of Adult Rat Offspring after Prenatal CS Exposure

The hematological parameters of adult rat offspring after prenatal CS exposure and controls are shown in Table 2. Prenatal CS exposure significantly ($P < 0.05$) decreases the mean corpuscular volume of red blood cells in male and female adult rat offspring as compared with controls. However, prenatal CS exposure had no significant effect ($P > 0.1$) on other hematological factors including, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution

width, prothrombin time, mean platelet volume and platelet distribution width in male and female adult rat offspring as compared with controls.

Table 1. Cellular components of the blood of adult rat offspring after prenatal CS exposure and controls.

Cellular component	Males		Females	
	CS exposure (n = 15)	Controls (n = 13)	CS exposure (n = 11)	Controls (n = 10)
RBCs ($X10^6$/ml)	8.31 ± 0.1	8.13 ± 0.11	7.71 ± 0.11	7.53 ± 0.33
Total Leukocytes ($X10^3$/ml)	9.92 ± 0.60*	6.81 ± 0.56	9.13 ± 0.64*	6.71 ± 0.60
Lymphocytes ($X10^3$/ml)	6.13 ± 0.43**	3.54 ± 0.40	4.78 ± 0.44**	3.60 ± 0.49
Monocytes ($X10^3$/ml)	1.44 ± 0.19*	0.97 ± 0.17	1.59 ± 0.33*	0.92 ± 0.13
Granulocytes ($X10^3$/ml)	2.34 ± 0.30	2.27 ± 0.21	1.32 ± 0.18	1.78 ± 0.27
Platelets ($X10^3$/ml)	611.50 ± 34.57	670.60 ± 24.12	657.18 ± 20.84	599.09 ± 15.14

Significantly different from controls (*P < 0.05, **P < 0.01).

Table 2. Hematological parameters of the blood of adult rat offspring after prenatal CS exposure and controls.

Hematological parameters	Males		Females	
	CS exposure (n = 15)	Controls (n = 13)	CS exposure (n = 11)	Controls (n = 10)
Hemoglobin (g/dl)	15.67 ± 0.25	15.50 ± 0.19	15.24 ± 0.26	14.77 ± 0.39
Hematocrit (%)	44.28 ± 0.43	44.54 ± 0.65	42.50 ± 0.56	42.65 ± 1.10
MCV (fL)	53.15 ± 0.52*	55.74 ± 1.15	54.37 ± 0.42*	56.81 ± 1.06
MCH (pg)	19.07 ± 0.43	19.1 ± 0.21	19.75 ± 0.23	19.68 ± 0.42
MCHC (g/dL)	34.80 ± 0.37	34.91 ± 0.64	35.64 ± 0.38	34.65 ± 0.38
RDW (%)	14.18 ± 0.27	15.16 ± 0.48	13.02 ± 0.53	13.6 ± 0.86
PT (%)	0.12 ± 0.01	0.16 ± 0.02	0.13 ± 0.01	0.13 ± 0.01
MPV (fL)	2.15 ± 0.13	2.59 ± 0.22	2.63 ± 0.34	2.98 ± 0.34
PDW (%)	15.75 ± 0.29	16.13 ± 0.30	16.48 ± 0.36	16.47 ± 0.30

Significantly different from controls (*P < 0.05). MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, PT: prothrombin time, MPV: mean platelet volume, PDW: platelet distribution width.

4. Discussion

The present study investigates the hematological parameters of the blood of adult rat offspring exposed prenatally to maternal cigarette smoking. It reveals that prenatal CS exposure caused a significant increase in the total leukocyte counts, lymphocytes and monocytes in both male and female during late adult life. These findings are in agreement with a recent study which showed significant increase in lymphocyte counts in newborns of smoking mothers than those born of non-smoking mothers without significant change in the percentages of lymphocyte subpopulations (Giovanni et al., 2013). Therefore, it seems that maternal cigarette smoking during pregnancy has a long term stimulatory effect on the immune response in adult rat offspring. However, Schmid et al. (2007) showed that total leukocyte counts and lymphocytes were reduced in neonates of smoking mothers compared with those of non-smoking mothers. The decrease in total leukocyte counts might be due to the suppressive effect of nicotine on myeloid dendritic tissue of the bone marrow (Vassallo and Chen, 2004).

In addition, chronic fetal hypoxia and/or ischemia induced by carbon monoxide and nicotine of the CS can cause reduction of the uteroplacental blood flow during gestational period (Donnenfeld et al., 1993; Albuquerque et al, 2004) which may affect the development of hematopoietic tissues in bone marrow. This is supported by the findings of Dollbreg et al., 2000 who showed that infants born to women exposed to passive smoking during pregnancy have increased the number of circulating nucleated red blood cells. This is due to the increase in levels of erythropoietin hormone levels as Varvarigou et al. (1994) found in cord blood of infants born to smoking mothers. Since, it is known that this hormone has a stimulatory effect on bone marrow tissue after severe reduction in oxygen supply to body cells.

The present results show that prenatal CS exposure during pregnancy had no significant effect on red blood cell and platelet counts in male and female adult rat offspring. This is in agreement with the findings of Giovanni et al. (2013) who showed that red blood cell and platelet counts were similar in newborns of smoking mothers and non-smoking mothers. In addition, the present findings demonstrate that prenatal CS exposure caused a significant decrease in the mean corpuscular volume of red blood cells in male and female of adult rat offspring. However, other hematological factors, including, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, prothrombin time, mean platelet volume and platelet distribution width, were not significantly affected after prenatal CS exposure of adult rat offspring..

5. Conclusion

The present study suggests that maternal cigarette smoking during pregnancy is associated with a significant increase in the total leukocyte counts, lymphocytes and monocytes, and significantly decreases the mean corpuscular volume of red blood cells of adult rat offspring. However, other hematological parameters were not significantly affected after prenatal CS exposure in adult rat offspring.

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References

Albuquerque CA, Smith KR, Johnson C, Chao R and Harding R. 2004. Influence of maternal tobacco smoking during pregnancy on uterine, umbilical and fetal cerebral artery blood flow. *Early Hum Dev*, **80**: 31-42.

Andres RL and Day MC. 2000. Perinatal complications associated with maternal tobacco use. *Semin Neonatal*, **5**: 231-241.

Basta P V, Basham K B, Ross W P, Brust M E and Navarro H A. 2000. Gestational nicotine exposure alone or in combination with ethanol down-modulates offspring immune function. *Int J Immunopharmacol*, **22**: 159-169.

Berlin I, Heilbronner C, Georgieu S, Meier C, Spreux-Varoquaux O. 2010. Newborns cord blood plasma cotinine concentrations are similar to that of their delivering smoker mothers. *Drug Alcohol Depend*, **107 (2**): 250-252.

Blake KV, Gurrin LC, Evans SF, Beilin LJ, Landau LI, Stanely FJ and Newnham JP. 2000. Maternal cigarette smoking during pregnancy, low birth weight and subsequent blood pressure in early childhood. *Early Hum Dev*, **57**: 137-147.

Bublitz MH, and Stroud LR. 2012. Maternal smoking during pregnancy and offspring brain structure and function: review and agenda for future research. *Nicotine Tob Res*, **14(4):** 388-397.

Carmines EL, Gaworski CL, Faqi AS and Rajendran N. 2003. *In utero* exposure to 1 R4F reference cigarette smoke: Evaluation of developmental toxicity. *Toxicol Sci*, **75**: 134-147.

Cederqvist LL, Eddey G, Abdel-Latif N and Litwin SD. 1984. The effect of smoking during pregnancy on cord blood and maternal serum immunoglobulin levels. *Am J Obstet Gynecol*, **148**: 1123-1126.

Chen WJ, Parnell SE and West JR. 1998. Neonatal alcohol and nicotine exposure limits brain growth and depletes cerebellar Purkinje cells. *Alcohol*, **15**: 33-41.

Dempsey DA and Benowitz NL. 2001. Risks and benefits of nicotine to aid smoking cessation in pregnancy. *Drug Safety*, **24:** 277-322.

Dollberg S, Fainaru O, Mimouni F, Shenhav M, Lessing J and Kupfermine M. 2000. Effect of passive smoking in pregnancy on neonatal nucleated red blood cells. *Pediatrics*, **106 (3)**: 34-39.

Donnenfeld A, Pulkkinen A, Palomaki GE, Knight GJ and Haddow JE. 1993. Simultaneous fetal and maternal cotinine levels in pregnant women smokers. *Amer J Obstet Gynecol*, **167**: 781-782.

Duncan JR, M, Myers M, Fifer WP, Yang M, Kinney HC, and Stark RI. 2009. Prenatal nicotine-exposure alters fetal autonomic activity and medullary neurotransmitter receptors: implications for sudden infant death syndrome. *J Appl Physiol*, **107(5)**: 1579-1590

Ernst M, Moolchan ET, Robinson ML. 2001. Behavioral and neural consequences of prenatal exposure to nicotine. *J Am Acad Child Adolesc Psychiatry*, **40**: 630–641.

Eugenin J, Otarola M, Bravo E, Coddou C, Cerpa V, Parada M, Llona E and Bernhardi R. 2008. Prenatal to early postnatal nicotine exposure impairs central chemoreception and modifies breathing pattern in mouse neonates: A probable link to sudden infant death syndrome. *J Neurosc*, **28(51)**: 13907-13917.

Fowler PA, Bhattacharya S, Gromoll J, Monteiro A and Oshaughnessy PJ. 2009. Maternal smoking and developmental changes in luteinizing hormone (LH) and the LH receptor in the fetal testis. *J Clin Endocrinol Metab*, **94**: 4688-4695.

Franco P, Chabanski S, Szliwowski H, Dramaix M and Kahn A. 2000. Influence of maternal smoking on autonomic nervous system in healthy infants. *Pediatr Res*, **47(2)**: 215-220.

Ganapathy V, Prasad PD, Ganapathy ME and Leibach FH. 1999. Drugs of abuse and placental transport. *Adv Drug Deliv Rev*, **38**: 99-110.

Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Vora H, Rappaport EB, Avol E and Peters JM. 2000. Maternal smoking during pregnancy, environmental tobacco smoke exposure and childhood lung function. *Thorax*, **55**: 271-276.

Giovanni A, Gernot E, Andrea L, Christian S, Christian K, Peter H, Rudolf T, David B and Martina P. 2013. Maternal cigarette smoking and its effect on neonatal lymphocyte subpopulations and replication. *BMC Pediatr*, **13:** 57.

Jensen MS, Mabeck LM, Toft G, Thulstrup AM and Bonde JP. 2005. Lower sperm counts following prenatal tobacco exposure. *Hum Reprod*, **20**: 2559-2566.

Khan A, Groswasser J, Sottiaux M, Kelmanson J, Rebuffat E, Franco P, Dramaix M and Wayenberg JL. 1994. Prenatal exposure to cigarettes in infants with obstructive sleep apneas. *Pediatrics*, **93**: 778-783.

Lambers DS and Clark KE. 1996. The maternal and fetal physiologic effects of nicotine. *Semin Perinatol*, **20**: 115-126.

Lawrence J, Xiao D, Xue Q, Rejali M, Yang S and Zhang L. 2008. Prenatal nicotine exposure increases heart susceptibility to ischemia/perfusion injury in adult offspring. *J Pharmacol Exp Ther*, **324**: 331-341.

Mamsen LS, Lutterodt MC, Andersen EW, Skouby SO, Sorensen KP, Andersen CY and Byskov AG. 2010. Cigarette smoking during early pregnancy reduces the number of embryonic germ and somatic cells. *Hum Reprod*, **25(11)**: 2755-2761.

Mercelina-Roumans PE, Breukers R B, Ubachs J M and van Wersch J W. 1996. Hematological variables in cord blood of neonates of smoking and nonsmoking mothers. *J Clin Epidemiol*, **49**: 449-454.

Parslow PM, Cranage SM, Adamson TM, Harding R and Horne RS. 2004. Arousal and ventilator responses to hypoxia in sleeping infants: effects of maternal smoking. *Respir Physiol Neurobiol*, **140**: 77-87.

Pauly JR and Slotkin TA. 2008. Maternal tobacco smoking, nicotine replacement and neurobehavioral development. *Acta Pediatrica*, **97**: 1331-1337.

Pendlebury JD, Wilson RJ, Bano S, Lumb KJ, Schneider JM and Hasan SU. 2008. Respiratory control in neonatal rats exposed to prenatal cigarette smoke. *Am J Respir Crit Care Med*, **177**: 1255-1261.

Pichini S, Basagana XB, Pacifici R, Garcia O, Puig C, Vall O, Harris J, Zuccaro P, Segura J and Sunyer J. 2000. Cord serum cotinine as a biomarker of fetal exposure to

cigarette smoke at the end of pregnancy. *Environ Health Perspect*, **108**: 1079-1083.

Schmid JMP, Kuehni CE, Strippoli MP, Roiha HL, Pavlovic R, Latzin P, Gallati S, Kraemer R, Dahinden C and Frey U. 2007. Maternal tobacco smoking and decreased leukocytes, including dendritic cells, in neonates. *Pediatr Res*, **61**: 462-466.

Schneider J, Mitchell I, Singhal N, Kirk V and Hasan SU. 2008. Prenatal cigarette smoke exposure attenuates recovery from hypoxemic challenge in preterm infants. *Am J Respir Crit Care Med*, **178**: 520-526.

Shields B, Hill A, Bilous M, Knight B, Hattersley AT, Bilous RW and Vaidya B. 2009. Cigarette smoking during pregnancy is associated with alterations in maternal and fetal thyroid function. *J Clin Endocrinol Metab*, **94(2)**: 570-574.

Singh SP, Barrett EG, Karla B, Boroujerdi SR, Langley RL, Kurup V, Tesfaigzi Y and Sopori ML. 2003. Prenatal cigarette smoke decreases lung cAMP and increases airway hyperresponsivness. *Am J Respir Crit Care Med*, **168**: 342-347.

Sopori, ML and Kozak, W. 1998. Immunomodulatory effects of cigarette smoke. *J. Neuroimmunol*, **83**: 148-156.

Varvarigou A, Beratis NG, Makri M and Vagenakis AG. 1994. Increased levels and positive correlation between erythropoietin and hemoglobin concentrations in newborn children and mothers who are smokers. *J Pediatrics*, **124**: 480-482

Vassallo R and Chen L. 2004. Selective suppression of dendritic cell functions by cigarette smoke extract. *Chest*, **125**: 107S.

Voigt M, Hermanussen M, Witter-Backofen U, Fusch C and Hesse V. 2006. Sex-specific differences in birth weight due to maternal smoking during pregnancy. *Eu J Pediatrics*, **165**: 757-761.

Xiao D, Xu Z, Huang X, Longo LD, Yang S and Zhang L. 2008. Prenatal Gender-related nicotine exposure increases blood pressure response to angiotensin II in adult offspring. *Hypertension*, **51**: 1239-1247.