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Effects of Maternal Nicotine Exposure on Expression of Laminin Alpha 5 in Lung Tissue of Newborn

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Abstract: Maternal smoking has been clearly demonstrated to be associated with increased health problems in infants and children. Nicotine is the chemical substance with high level of toxicity. It crosses through the placenta and accumulates in the developing organs of fetus. Previous investigation indicated that maternal nicotine exposures induce decreased fibronectin expression in lung parenchyma. In this study, the effect of maternal nicotine exposure on laminin expression of the newborn mice lungs has been evaluated. Female pregnant Balb/C mice were divided randomly in to four groups as fallow: Experimental group1 (Exp D1); was received 3 mg kg^{-1} nicotine intra peritoneal injection (IP) from gestational day 7 (GD7) to the last day of pregnancy, Experimental group 2 (Exp D14); was received 3 mg kg^{-1} nicotine from GD_7 to postnatal day 14, Groups 3 and 4; as sham control groups (Sha-Con) were received the same volume (3 mg kg^{-1}) of normal saline parallel to experimental groups. At the end of exposure times, all of newborns were anesthetized; their lungs were removed and prepared for immunohistochemical method and real-time polymerase chain reaction. The finding indicated that laminin alpha 5 (Lama5) mRNA expressions in the lung of newborn in the nicotine treated Exp D1 decreased by 0.63 fold but increased in Exp D14 by 1.57 fold comparing to Sh-Con groups. Lama5 immunoreactivity was not similar in different parts of the lungs including alveoli and bronchiole, having a significant increase in the experimental groups in contrast to the Sh-Con groups. However, increase in immunoreactivity observed more in Exp D14. Immunoreactivity intensity in small vessels of all experimental groups was not significantly different. These data also indicate that maternal nicotine exposure may induce abnormal laminin expression which may cause defects in lung function during life time.

Key words: Laminin, lung, nicotine, newborn, immunohistochemistry

INTRODUCTION

Maternal smoking has been associated with pregnancy complications, including intra uterine retardation (IUGR), fetal and neonatal death, spontaneous abortion and premature delivery (Hafstrom *et al*., 2005; Wickstrom, 2007). Nicotine is the causative agent for these effects, because it is a major pharmacological constituent of tobacco that easily crosses the placenta and is concentrated in the fetus to a higher level than the mother (Chen *et al*., 2005). The lung has important role as a gas exchanger in survival of the breathing organism. The lungs development occurs in uterus and is prepared to function at birth time but similar to other mammals, final stages of its development do not complete until birth. Disturbance in developmental stages of lung may affect its maturation and resistance to diseases in future life (Sekhon *et al*., 2004; Wasowicz *et al*., 1998; Wasowicz *et al*., 1996). Investigation in animal models showed that maternal nicotine exposures cause a variety of effects on neonatal lungs including; significant suppression of alveolarization (Maritz, 1988; Maritz and Dennis, 1998), abnormal collagen IV expression and defect in bronchopulmonary development (Jalali *et al*., 2010), decreased expression of fibronectin (Kohbanani *et al*., 2012) and decreased elastin staining of lung parenchyma (Pierce and Nguyen, 2002). Extracellular matrix (ECM) composition is essential for morphogenesis and

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differentiation of virtually all tissues (Gullberg and Ekblom, 1995). Basement membranes are distinctive extracellular matrices having essential roles in tissue organization and development. Components common to all basement membranes include laminin, type IV collagen, entactin/nidogen and sulfated proteoglycans (Kruegel and Miosge, 2010). Among the matrix molecules found, laminins are glycoprotein's that modulate adhesion and signaling through integrin binding; additionally, they adhere to other ECM molecules (Aumailley and Smyth, 1998; Suzuki *et al*., 2005). Each laminin molecule is a composed of three non-identical subunit, called the α, β and γ chains (Bolcato-Bellemin *et al*., 2003). At least 15 isoform types of laminin have been identified which substantially were synthesized and expressed during fetal and adult periods (Lefebvre *et al*., 1999; Miner *et al*., 1997; Nguyen *et al*., 2002). Several study indicated that Alpha 5 chain of laminin is essential for the lung development, both in embryonic and adult lung (Nguyen *et al*., 2005; Nguyen *et al*., 2002), natural development of smooth muscle cell types, basal membrane of blood vessels (Vainionpaa *et al*., 2006) and digestive tract (Bolcato-Bellemin *et al*., 2003). Lama5 is also essential for propagation and polarization of epithelial cells (Fukumoto *et al*., 2006). Deletion of the gene encoding alpha5 chain of laminin during fetal development in mouse lead to death (Miner *et al*., 1998) and imposed abnormality in kidney and digestive tract (Lefebvre *et al*., 1999). In the lungs it would bring about a delay in its evolution along with abnormality in the growth of the surface alveolar cells and disorganization in growth and development of vessels and alveoli (Rahuel *et al*., 2008; Rebustini *et al*., 2007). Because laminins are important BM component essential for morphogenesis of all tissues, this study has evaluated the effect of nicotine on the expression of lama 5 in lung tissue development of the offspring during gestational time and lactation period.

MATERIALS AND METHODS

Nicotine administration and tissue preparation: From January 2011 for about three months female Balbc/c mice were randomly divided into 2 experimental and 2 control groups (n = 6). Sperm positivity in vaginal plaque was designated as day zero of pregnancy. The animals were maintained at the animal house under controlled conditions (12 h light and dark cycle, 21°C and 50% relative humidity) with laboratory chow and water provided *ad libitum*. The experimental group1(Exp D1) received 3 mg kg^{-1} of nicotine (N 3876, sigma.com) daily intra peritoneally (IP) from day 7 of gestation to the last day of pregnancy and experimental group 2 (Exp D14) was received nicotine from day 7 of gestation to two weeks postnatal (lactation period) (Jalali *et al*., 2010). The sham control groups (Sh-Con) were received nicotine solvent (Normal saline) at the same period. Finally, the animals were rapidly sacrificed by cervical dislocation and their lungs were removed in postnatal days one (PD1)and fourteen (PD14), then fixed for 24 h at room temperature in formalin 10% to use for immunohistochemistry (IHC) study.

Immunohistochemistry method: The 5 μm thickness sections were deparaffinized, rehydrated and then washed in PBS (pH 7.4) for 10 min, Antigen retrieval were carried out with Heat-induction by Tries/EDTA buffer, pH 9.0 for 20 min. The slides were washed in Phosphate buffered saline (PBS) plus 0.025% Triton X100 for 5 min and blocked in 10% normal serum (goat, Sigma, USA) with 1% BSA (Sigma, USA) in PBS for 2 h at room temperature. All the sections were incubated with monoclonal anti laminin antibody (Abcam, 75344, USA) diluted 1: 150 in PBS with BSA 0.1% for overnight at 4°C and then washed three times with PBS. For blocking endogenous peroxides activity the slides were incubated in 0.03% H_2O_2 (Merk, Ggermany) dissolved in methanol (Bidestsn, Iran) for 30 min. Next, tissues were incubated for 2 h with secondary antibody (Abcam, 97051, USA) diluted 1:800 in PBS with BSA 0.1% for 2 h. After incubation, the sections were washed extensively with PBS for 3 min and treated with DAB (Sigma, USA) solution (0.03 grDAB in 100 mL PBS and 200 μL H_2O_2/100 mL PBS) for 15 min at room temperature in dark. After being washed in running water, all the sections were counterstained with hematoxylin for 1 min. Finally the sections were dehydrated in increasing graded ethanol, cleared in xylene and mounted in glass slide. Laminin reaction in alveoli and lung parenchyma were graded blind by three separate observers (Table 1), (Jalali *et al*., 2010; Kranenburg *et al*., 2006). Percentile median intensity reactivity was calculated and presented in the form of 50% (25, 75%).

Real time study

RNA extraction: Total RNA was isolated by RNA plus (Cinnagen, Iran) according to the manufacturer's

Table 1: Grade of immunoreactivity intensity reaction to antibody laminin α5

Reaction	Grade
Negative	(-)
Weak	(+)
Moderate	(++)
Strong	(+++)
Very strong	(++++)

(-), no reaction; (+), weak reaction: light brown; (++) moderate reaction: brown; (+++), strong reaction: dark brown and (++++), very strong reaction: very dark brown

instructions briefly, 50-70 mg of lung tissue was homogenized in RNA plus using homogenizer (polytron PT 1200E, Switzerland). The homogenate was centrifuged at 12000 xg for 10 min at 4°C to remove insoluble debris and the supernatant was transferred to a fresh micro centrifuge tube (Eppendorf, Germany). Samples were allowed to sit at room temperature for 5 min and 0.2 mL of chloroform was added per 1 mL of RNA plus ™. The Samples were vortexed (Velp, Italy) for 15 s and allowed to stand for 5 min at room temperature. The mixture was centrifuged at 12000 xg for 15 min at 4°C. The aqueous phase was transferred to a fresh micro centrifuge tube and an equal amount of isopropanol (Merk, Germany) was added. After 30 min incubation at -20°C, the mixture was centrifuged at 12000 xg for 15 min at 4°C. The pellet was washed with 75% ethanol, air-dried and resuspended in 50 μL of diethylpyrocarbonate-treated water. The total RNA was examined by measuring the optical density at 260/280 nm.

cDNA synthesis: First strand cDNA was made using a cDNA synthesis kit (Fermentas, Lithuania) according to the manufacturer's instructions. RNA (3 μL) was mixed with 1 μL of Dnase and incubated for 30 min at 37°C, (Incubator, Memmert, Germany) and then μLl of 100 pmole μL^{-1} Oligo (dT) and 8 μL of H_2o were added to each incubated 10 min at 70°C (Thermal cycler, Bioeer, China). After the above step, 2 μL of 10 mM dNTP Mix 4 μL (fermentas, Lithuania) of 5x reaction buffer, 1 μL of Ribolck (Fermentas, Lithuania) and 1 μL of reverse transcriptase (Fermentas, Lithuania) were added to each sample tube .The tubes were sequentially incubated at 42°C for 60 min and 70°C for 5 min. (Thermal cycler, Bioeer, China) and stored at -20°C (Freezer, Sikat, Iran).

Primers and real-time PCR: The designed primers were as fallows:

- Lama α 5- F, CGTCCCACAGGAATAGGCT, Lama α 5- R, TACCAACGAAGGGCTGCG, GAPDH-F,AACTCCCATTCTTCCACCTTTG, GAPDH-R, CTGTAGCCATATTCATTGTCATACCAG

Real-time PCR was performed using 10 μL SYBR® Real-time PCR Master Mix (Pars tous biotechnology, Iran), 1 μL of each primer (10 pmol μL^{-1}), 1 μL cDNA and 7 μL DEPC-water. Reactions were run using Stratagene Mx-3000P (Stratagene, La Jolla, CA, USA). After a RT-PCR for each target gene, cDNAs from high quality samples for target genes were selected and they serially diluted to obtain six standard solutions to generate the standard curve in the real-time PCR assay. All data were normalized to the house-keeping gene encoding for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the relative mRNA expression ratios were determined using the method that introduced by Pfaffl (2001) and Wong and Medrano (2005).

Statistical analysis: On the basis of staining intensity, sections were graded and Mann-Whitney non-parametric statistical test was used to compare differences between samples. Student t test used for real time PCR. The p-values <0.05 were considered statistically significant.

RESULTS

Our results showed that decrease in lung and body weight of mice offspring born from mothers exposed to nicotine was significant compared to control groups. Therefore nicotine had highest impact on body weight (5.28±0.39) and lung (0.09 ±0.007) in PD14 ($p<0.05$) (Table 2). Analysis of laminin α5 mRNA expression in lung tissue showed that mRNA expression decreased by 0.63 fold in Exp D1 and increased by 1.57 fold in Exp D14 comparing with the Sh Con groups. Statistical analysis indicated that the laminin α5 expression increased significantly from PD1 to PD14 in experimental group ($p<0.05$) (Fig. 1). Thus laminin expression in PD14 was effected in most (1.5 fold) when exposed to nicotine. Immunohistochemical reactivity of lung tissue using rabbit monoclonal antibody against mice was specific for

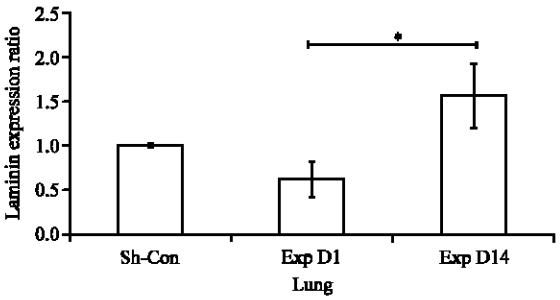

Fig. 1: Relative transcription level of laminin mRNA expression in lung tissue of the experimental groups under treatment with nicotine (3 mg kg^{-1}) on different days (days 1 and 14 of newborn) using Real-time PCR. Sh-Con. (Sham control), Exp D1 (postnatal day one) and Exp D14 (postnatal day fourteen). Values represent the Means±SE (n = 6),* $p<0.05$

Table 2: Effect of nicotine treatment (3 mg kg^{-1}) from the 7th day of gestation to the 14th day postnatal in the new born mice, The body weight and Lung weight index in new born mice at different days are compared to control groups

Groups variable	Control group PD1	Experimental group PD1	Control group PD14	Experimental group PD14
B. wt (g)	1.55±0.05	1.43±0.04 *	5.84±0.33	5.28±0.39 *
L. wt (g)	0.029±.001	0.025±.004*	0.11±0.006	0.09±0.007 *

B. wt: body weight, L. wt: Lung weight, Values is Means±SD, *: $p<0.05$

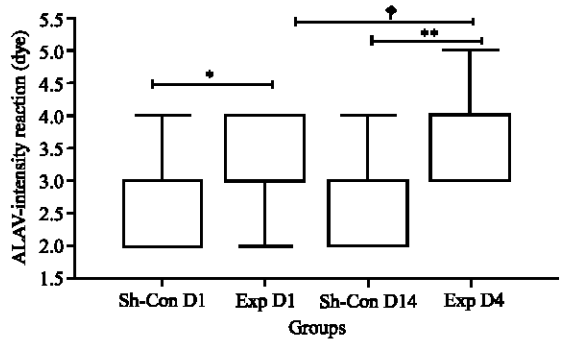

Fig. 2: Effect of maternal nicotine exposure in intensity reaction of laminin α5 in lung alveoli of 1 and 14 days mouse infant, Median is presented in the form of 50% (25, 75%). Sh-Con D1: (Sham control group of day one), Exp D1: (postnatal day one), Sh-Con D14: (Sham control group of day fourteen), Exp D14: (postnatal day fourteen),*p = 0.01, **p = 0.001, †p = 0.01 (n = 17)

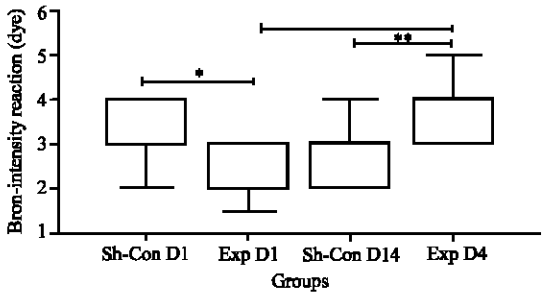

Fig. 3: Effect of maternal nicotine exposure in intensity reaction of laminin α5 in lung bronchiole of 1 and 14 days mouse infants. Median is presented in the form of 50% (25, 75%), Sh-Con D1: (Sham control group of day one), Exp D1: (postnatal day one), Sh-Con D14: (Sham control group of day fourteen), Exp D14: (postnatal day fourteen).*p = 0.002, **p = 0.001, †p = 0.0001 (n = 17)

laminin α5 in alveolar septum and bronchioles, showing positive reactivity in immunohistochemical method. The locations of laminin expression in lung tissue were determined according to the intensity of color darkness. Immunohistochemistry data showed that intensity of immunoreactivity of laminin α5 in infant lung alveoli of Sh-Con D1 group reacted moderate with median of 2(2,3) while at the same position in infants lung born from mothers affected under nicotine Exp D1 was in average reactivity with median of 3(3,4). Statistical analysis showed that this increase was significant (p = 0.01). In addition median of reaction intensity of alveoli in Exp D14 was very intense 4(3-4) compared with Sh Con D14 (p = 0.001). Also expression of laminin α5 between Exp D1

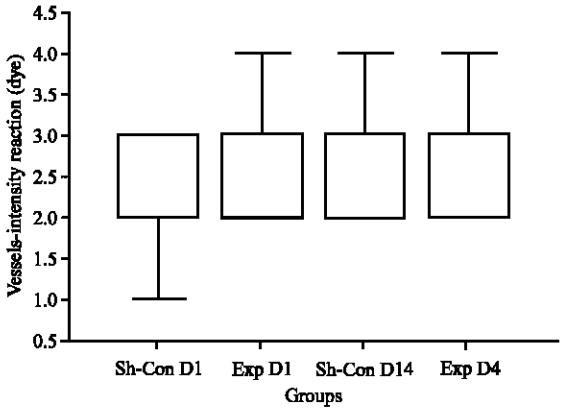

Fig. 4: Effect of maternal nicotine exposure in intensity reaction of laminin α5 in lung vessels of 1 and 14 days mouse infants. Median is presented in the form of 50% (25, 75%). Sh-Con D1: Sham control group of day one, Exp D1: Postnatal day one, Sh-Con D14: Sham control group of day fourteen, Exp D14: Postnatal day fourteen, (n = 17)

and Exp D14 increased significantly (p = 0.01) (Fig. 2-5). Immunoreactivity of laminin α-5 in bronchioles of Exp D14 and Exp D1 showed a decrease reactivity in Exp D1with median 2(2,3) comparing with sh-con 3(2, 4) (p = 0.002). However, intensity reactivity in bronchioles Exp D14 with median 4(3, 4) increased significantly comparing with sh-con 3(2, 3) (p = 0.001). Statistical analysis showed that the expression of laminin α-5 in bronchioles increased significantly between ExpD1 and ExpD14 (p = 0.001) (Fig. 3-5). Increase in immunoreactivity of laminin α-5 in small vessels of Exp D14 and Exp D1 observed with median 3(2,3) and 2(2, 3), respectively comparing with sh-con groups (Fig. 4-5).

DISCUSSION

The results of this study show that nicotine administration during gestation and lactation could change expression of laminin α5 as one of the most important proteins of basement membrane. According to immunohistochemistry (IHC) analysis, maternal nicotine exposure causes laminin reaction intensity increase in the alveolar septum and decrease in bronchiole during gestation period. Therefore, the pattern of laminin expression could be different in various parts of the newborn lungs. Also decrease in expression of laminin would only occur in bronchioles of experimental group 1 while in experimental group 2 increased in both alveoli and bronchioles. Although a little difference in lama5 expression was observed in experimental groups using Real time PCR but it was not significant. Changes found

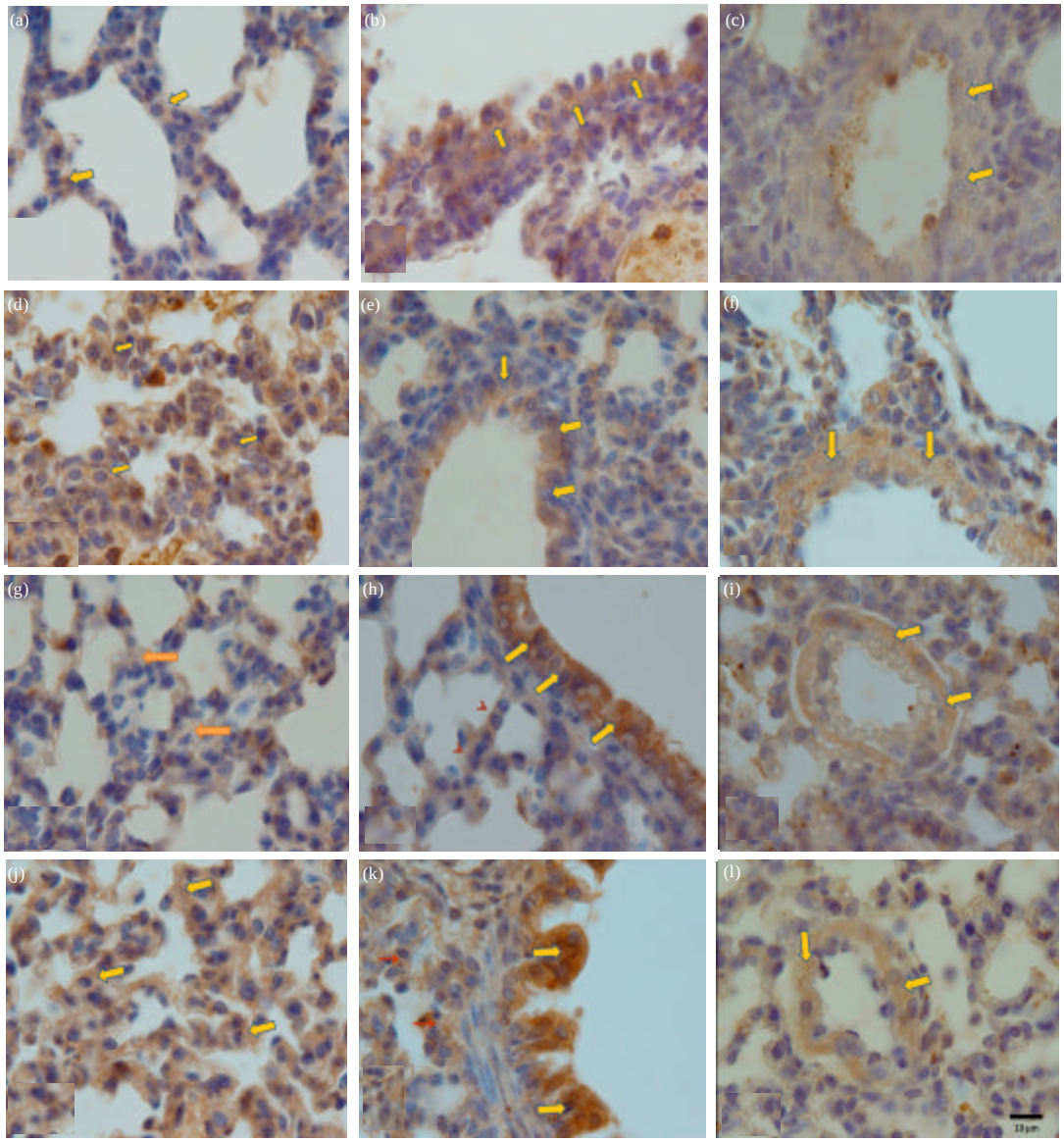

Fig. 5(a-n): Photomicrographs show epithelium of bronchiole, lung alveoli and vessel incubated with laminin α5 antibody (a-n), a, b, c: Alveoli bronchiole and vessel parenchyma in control groups 1, d, e, f: Similar section in experimental groups, g, h, k: Alveol , bronchiole and vessel parenchyma in control groups 2, l, m, n: Similar section in experimental shows increasing reactivity (arrows). Hematoxylin counterstained, (scale bar = 10 μm)

for protein levels followed the same time course as those described for mRNA expression. Data obtained from real time PCR and IHC analyses indicated that lama5 expression increased in experimental groups 2 follow the same developmental pattern. Thus, we concluded that nicotine could have an inhibitory impact on laminin transcription during the gestation period and an stimulatory effect on expression of laminin in lactation period. Schuger *et al.* (1990) indicated that laminin expression increased during late development. In our investigation also laminin expression in alveolus and bronchiole had an increase from day 1 to day 14 which more or less is in conjunction with above studied.

Luck and Nau (1984) showed that nicotine in maternal smoking results in milk concentrations between 1.5 and 3 times the simultaneous maternal plasma concentration. The nicotine in breast milk is rapidly absorbed through the infant's gut and accumulates in some tissues.

Accordingly a reason for increase in lama5 expression in this study might be because of the presence of high nicotine concentration in mother breast milk.

Several studies indicated that nicotine acts on nAChRs, which are ligand-gated ion channels controlling influx of calcium and sodium into cells (Akaike *et al*., 2010). Neuronal nicotinic receptors are present in bronchial epithelium and vascular endothelial cells as well as in the cholinergic nerves innervating the bronchial smooth muscle (Sekhon *et al*., 1999; 2002). Sekhon *et al*. (2002) showed that nicotine administration to pregnant rhesus monkeys caused increase in expression of a7 nicotinic cholinergic receptors within the lungs, which was accompanied by increases in collagen deposition in the airway wall in the lung. The study of Maritz (2009) showed that nicotine increase the production of free radicals parallel with a decrease in the lung antioxidant capacity. Furthermore, suppression of glycolysis and an increase in cAMP results in changes in lung growth (Maritz, 2008). Therefore, we propose that the activation or suppression of intracellular signals by nicotine and change in expression of α7 nicotinic cholinergic receptors could lead to increased or decreased laminin gene transcription. In addition, nicotine exposure during gestation and lactation may result in remarkable low pups birth weight. This finding was consistent with the results of other researchers (Ozokutan *et al*., 2005; Sekhon *et al*., 2004).

Based on results of other researchers, lung laminin alpha5 can be expressed by endothelial cells, smooth muscles of vessels (Bolcato-Bellemin *et al*., 2003) and airways epithelial (Nguyen *et al*., 2005), our results also support these findings. Decrease in lama5 expression observed in this study may also result from malfunctioning of another lung component such as smooth muscles and endothelial vessels.

Several studies showed that laminins affect lung development at multiple stages and in different cellular compartments (Schuger *et al*., 1990; Willem *et al*., 2002). Laminin α2 has been shown to be important for bronchial smooth muscle cell differentiation (Relan *et al*., 1999). Mice lacking laminin γ2 or α3 die 1-3 days after birth from malnutrition (Meng *et al*., 2003). A reduction in laminin α5 expression was reported in breast cancers (Martin *et al*., 1998), Prostate (Calaluce *et al*., 2001), lung (Akashi *et al*., 2001; Manda *et al*., 2000) and colon (Sordat *et al*., 1998). Increases in expression of laminin β2 and alpha1 chains have been reported in airway of asthma patients, allergic airway remodeling and chronic obstructive pulmonary disease (COPD). Therefore, change in laminin expression in embryonic period may cause functional defects especially asthma during either childhood or puberty period. Although above studies highlight the important of laminin in cancer studies but there have not found any record about the effect of nicotine in laminin gene expression.

CONCLUSION

This study has found that maternal nicotine exposure during pregnancy and postnatal produce variable changes in Laminin α5 gene expression at different stages of lung development. This implies that maternal nicotine exposure might change the future development of lung dysfunction.

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REFERENCES

Akaike, A., Y. Takada-Takatori, T. Kume and Y. Izumi, 2010. Mechanisms of neuroprotective effects of nicotine and acetylcholinesterase inhibitors: Role of α4 and α7 receptors in neuroprotection. J. Mol. Neurosci., 40: 211-216.

Akashi, T., E. Ito, Y. Eishi, M. Koike, K. Nakamura and R.E. Burgeson, 2001. Reduced expression of laminin alpha 3 and alpha 5 chains in non-small cell lung cancers. Jpn. J. Cancer Res., 92: 293-301.

Aumailley, M. and N. Smyth, 1998. The role of laminins in basement membrane function. J. Anat., 193: 1-21.

Bolcato-Bellemin, A.L., O. Lefebvre, C. Arnold, L. Sorokin, J.H. Miner, M. Kedinger and P. Simon-Assmann, 2003. Laminin α5 chain is required for intestinal smooth muscle development. Dev. Biol., 260: 376-390.

Calaluce, R., M.W. Kunkel, G.S. Watts, M. Schmelz and J. Hao *et al*., 2001. Laminin-5-mediated gene expression in human prostate carcinoma cells. Mol. Carcinog., 30: 119-129.

Chen, C.M., L.F. Wang and T.F. Yeh, 2005. Effects of maternal nicotine exposure on lung surfactant system in rats. Pediatr. Pulmonol., 39: 97-102.

Fukumoto, S., J.H. Miner, H. Ida, E. Fukumoto and K. Yuasa *et al*., 2006. Laminin α5 is required for dental epithelium growth and polarity and the development of tooth bud and shape. J. Biol. Chem., 281: 5008-5016.

Gullberg, D. and P. Ekblom, 1995. Extracellular matrix and its receptors during development. Int. J. Dev. Biol., 39: 845-854.

Hafstrom, O., J. Milerad, K.L. Sandberg and H.W. Sundell, 2005. Cardiorespiratory effects of nicotine exposure during development. Respir. Physiol. Neurobiol., 149: 325-341.

Jalali, M., M.R. Nikravesh, A.A. Moeen, S. Mohammadi and M.H. Karimfar, 2010. Effects of maternal nicotine exposure on expression of collagen type IV and its roles on pulmonary bronchogenesis and alveolarization in newborn mice. Iran. J. Allergy Asthma Immunol., 9: 169-173.

Kohbanani, M.S., M.R. Nikravesh, M. Jalali, A. Fazel, A. Ebrahimzadeh and M. Sankian, 2012. Nicotine decreased expression of fibronectin in lung parenchyma newborn. Afr. J. Pharm. Pharmacol., 6: 1451-1456.

Kranenburg, A.R., A. Willems-Widyastuti, W.J. Moori, P.J. Sterk, V.K. Alagappan, W.I. de Boer and H.S. Sharma, 2006. Enhanced bronchial expression of extracellular matrix proteins in chronic obstructive pulmonary disease. Am. J. Clin. Pathol., 126: 725-735.

Kruegel, J. and N. Miosge, 2010. Basement membrane components are key players in specialized extracellular matrices. Cell. Mol. Life Sci., 67: 2879-2895.

Lefebvre, O., L. Sorokin, M. Kedinger and P. Simon-Assmann, 1999. Developmental expression and cellular origin of the laminin α2, α4 and α5 chains in the intestine. Dev. Biol., 210: 135-150.

Luck, W. and H. Nau, 1984. Nicotine and cotinine concentrations in serum and milk of nursing smokers. Br. J. Clin. Pharmacol., 18:: 9-15.

Manda, R., T. Kohno, T. Niki, T. Yamada, S. Takenoshita, H. Kuwano and J. Yokota, 2000. Differential expression of the LAMB3 and LAMC2 genes between small cell and non-small cell lung carcinomas. Biochem. Biophys. Res. Commun., 275: 440-445.

Maritz, G.S., 1988. Effect of maternal nicotine exposure on growth *In vivo* of lung tissue of neonatal rats. Biol. Neonate, 53: 163-170.

Maritz, G. and H. Dennis, 1998. Maternal nicotine exposure during gestation and lactation interferes with alveolar development in the neonatal lung. Reprod. Fertil. Dev., 10: 255-261.

Maritz, G.S., 2008. Nicotine and lung development. Birth Defects Res. Part C: Embryo Today: Rev., 84: 45-53.

Maritz, G.S., 2009. Are nicotine replacement therapy, varenicline or bupropion options for pregnant mothers to quit smoking? Effects on the respiratory system of the offspring. Ther. Adv. Respir. Dis., 3: 193-210.

Martin, K.J., C.P. Kwan, K. Nagasaki, X. Zhang and M.J. O'Hare *et al*., 1998. Down-regulation of laminin-5 in breast carcinoma cells. Mol. Med., 4: 602-613.

Meng, X., J.F. Klement, D.A. Leperi, D.E. Birk and T. Sasaki *et al*., 2003. Targeted inactivation of murine laminin α2-chain gene recapitulates human junctional epidermolysis bullosa. J. Invest. Dermatol., 121: 720-731.

Miner, J.H., B.L. Patton, S.I. Lentz, D.J. Gilbert and W.D. Snider *et al*., 1997. The laminin α chains: Expression, developmental transitions and chromosomal locations of α1-5, identification of heterotrimeric laminins 8-11 and cloning of a novel α3 isoform. J. Cell Biol., 137: 685-701.

Miner, J.H., J. Cunningham and J.R. Sanes, 1998. Roles for laminin in embryogenesis: Exencephaly, syndactyly and placentopathy in mice lacking the laminin α5 chain. J. Cell. Biol., 143: 1713-1723.

Nguyen, N.M., J.H. Miner, R.A. Pierce and R.M. Senior, 2002. Laminin α5 is required for lobar septation and visceral pleural basement membrane formation in the developing mouse lung. Dev. Biol., 246: 231-244.

Nguyen, N.M., D.G. Kelley, J.A. Schlueter, M.J. Meyer, R.M. Senior and J.H. Miner, 2005. Epithelial laminin α5 is necessary for distal epithelial cell maturation, VEGF production and alveolization in the developing murine lung. Dev. Biol., 282: 111-125.

Ozokutan, B.H., K.U. Ozkan, I. Sari, F. Inanc, M.E. Guldur and M. Kilinc, 2005. Effects of maternal nicotine exposure during lactation on breast-fed rat pups. Biol. Neonate, 88: 113-117.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic. Acids Res., 29: e45-e45.

Pierce, R.A. and N.M. Nguyen, 2002. Prenatal nicotine exposure and abnormal lung function. Am. J. Respir. Cell Mol. Biol., 26: 10-13.

Rahuel, C., A. Filipe, L. Ritie, W. El-Nemer and N. Patey-Mariaud *et al*., 2008. Genetic inactivation of the laminin α5 chain receptor Lu/BCAM leads to kidney and intestinal abnormalities in the mouse. Am. J. Physiol. Renal Physiol., 294: F393-F406.

Rebustini, I.T., V.N. Patel, J.S. Stewart, A. Layvey, E. Georges-Labouesse, J.H. Miner and M.P. Hoffman, 2007. Laminin α5 is necessary for submandibular gland epithelial morphogenesis and influences FGFR expression through α1 integrin signaling. Dev. Biol., 308: 15-29.

Relan, N.K., Y. Yang, S. Beqaj, J.H. Miner and L. Schuger, 1999. Cell elongation induces laminin α2 chain expression in mouse embryonic mesenchymal cells: Role in visceral myogenesis. J. Cell Biol., 147: 1341-1350.

Schuger, L., S. O'Shea, J. Rheinheimer and J. Varani, 1990. Laminin in lung development: Effects of anti-laminin antibody in murine lung morphogenesis. Dev. Biol., 137: 26-32.

Sekhon, H., Y. Jia, R. Raab, A. Kuryatov, J. Pankow, J. Whitsett, J. Lindstorm and E. Spindel, 1999. Prenatal nicotine increases pulmonary alpha-7 nicotine receptor expression and alters fetal lung development in monkeys. J. Clin. Invest., 103: 637-647.

Sekhon, H., J. Keller, B. Proskocil, E. Martin and E. Spindel, 2002. Maternal nicotine exposure upregulates collagen gene expression in fetal monkey lung. Association with alpha-7 nicotine acetylcholine receptors. Am. J. Respir. Cell. Mol. Biol., 26: 31-41.

Sekhon, H.S., B.J. Proskocil, J.A. Clark and E.R. Spindel, 2004. Prenatal nicotine exposure increases connective tissue expression in foetal monkey pulmonary vessels. Eur. Respir. J., 23: 906-915.

Sordat, I., F.T. Bosman, G. Dorta, P. Rousselle, D. Aberdam, A.L. Blum and B. Sordat, 1998. Differential expression of laminin-5 subunits and integrin receptors in human colorectal neoplasia. J. Pathol., 185: 44-52.

Suzuki, N., F. Yokoyama and M. Nomizu, 2005. Functional sites in the laminin alpha chains. Connect Tissue Res., 46: 142-152.

Vainionpaa, N., Y. Kikkawa, K. Lounatmaa, J.H. Miner, P. Rousselle and I. Virtanen, 2006. Laminin-10 and lutheran blood group glycoproteins in adhesion of human endothelial cells. Am. J. Physiol. Cell Physiol., 290: C764-C775.

Wasowicz, M., S. Yokoyama, K. Kashima and I. Nakayama, 1996. The connective tissue compartment in the terminal region of the developing rat lung. An ultrastructural study. Acta Anat., 156: 268-282.

Wasowicz, M., W. Biczysko, A. Marszaek, S. Yokoyama and I. Nakayama, 1998. Ultrastructural studies on selected elements of the extracellular matrix in the developing rat lung alveolus. Folia. Histochem. Cytobiol., 36: 3-13.

Wickstrom, R., 2007. Effects of nicotine during pregnancy: Human and experimental evidence. Curr. Neuropharmacol., 5: 213-222.

Willem, M., N. Miosge, W. Halfter, N. Smyth and I. Jannetti *et al*., 2002. Specific ablation of the nidogen-binding site in the laminin gamma1 chain interferes with kidney and lung development. Development, 29: 2711-2722.

Wong, M.L. and J.F. Medrano, 2005. Real-time PCR for mRNA quantitation. Biotechniques, 39: 75-85.