

Estimation of Oxygen Diffusing Conductance in Different Dissection Portion of the Placental Cotyledon of Passive Smoking Mothers

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ABSTRACT

A study was undertaken to estimate the amount oxygen that can diffuse across the placenta of the passive smoking mothers in an Indo-Asian population. A total of 24 human term placentas, 11 from mothers exposed to tobacco smoke and 13 from those not exposed to tobacco smoke were collected for estimating weight, surface area and volume term placentas. Five placentas from each of the groups were used for estimating the various parameters for calculating the oxygen-diffusing conductance. A random sampling technique at 7 levels of tissue preparation was adopted. Cotyledons were cut into blocks and each block was further cut into upper (maternal), middle (foeto-maternal) and lower (foetal) components. Semithin sections, 1 μm thick, stained using a modified toluidine was viewed and the images were analysed. A Merz grid was used to estimate the vascular volumes, exchange surface areas and tissue diffusion distances for calculation the oxygen-diffusing conductance of the placenta. These were combined with physicochemical quantities (oxygen-haemoglobin reaction rates and tissues oxygen diffusion coefficients) obtained from literature in order to estimate the partial conductance of the six tissue compartments of the oxygen pathway. The present study demonstrates that there is no significant difference in the total oxygen-diffusing conductance in the placenta of passive smokers and those not exposed to tobacco smoke. However, the significant increase in the foetal capillaries and foetal erythrocytes conductance suggests that an adaptive mechanism in the foetal part of the placenta may be occurring to ensure adequate oxygen to the foetus

Keywords: Indo-Asian population, oxygen-diffusing conductance, passive smoking mothers, placental cotyledons

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INTRODUCTION

The placenta, a vital interface component between mother and fetus is important for nutrients and gas exchange, waste disposal, immunity and organ maturation (1). Placenta provides a large surface area for nutrients exchange to occur between the mother and fetus (2). Placental membranes are important for fetomaternal nutrients exchange since the nutrients must pass a myriad of membranes (syncytiotrophoblast, cytotrophoblast, stroma, vascular endothelium), which present barriers between the mother and fetus blood circulation (3). The passage of nutrients across these barriers are dependent on physical and chemical properties (4).

There are many modes of nutrient and gas transport, which include diffusion, pinocytosis, and active or facilitated transport (5). The transport of oxygen is a diffusion-limited process since it is dependent on the oxygen pressure gradient between mother and fetus blood (6). Physiologists express the ability of an organ to act in oxygen diffusion exchange as oxygen conductance. This denotes the volume flux of oxygen per minute over a given partial pressure gradient (7). Transplacental oxygen flow can be measured using a morphometric model formulated by Mayhew et al in 1984 (8). This model measures the total resistance in six tissue compartments of placenta, which reflect the microstructure of the diffusion pathway (8, 9). The estimation of total resistance is the reciprocal to the total oxygen conductance (D_p) (9). This morphometric model has been successfully used in many placenta studies (7-11).

The adverse effect of passive smoking leading to pregnancy complication and mortality is well documented (12-14). Cigarette smoke contains more than 3000 toxic substances that result in damage of the placenta particularly in the membrane compartments (15). Strong evidence indicates that smoking cause profound changes to membrane thickness, vessel size and number of capillaries that is important in determining placental oxygen diffusive conductance (9, 16). However most

studies have been conducted in the placenta of active or passive smoking mothers in developed countries (12, 13, 17).

There is, however, a paucity of information on amount of oxygen can diffuse across the placenta of the passive smoking mothers in an Indo-Asian population. The present study was initiated to fill the gap that may exist in this area of research of the placenta. Furthermore, it will be interesting to measure the oxygen conductance in different dissection of placental cotyledon of passive smoking mothers as the cotyledons with its villous components are important for nutrients and oxygen transfers between the fetus bloods in the capillaries (6).

SUBJECTS AND METHODS

A total of 24 fresh placentas (10-15 minutes afterbirth) were collected from the Kuala Lumpur Maternity Hospital with approval from the hospital ethics committee in accordance with the Helsinki declaration. The study design was observational based and stratified sampling method was used to select the sample randomly for histological study. Informed consent was obtained from all the admitted mothers and a questionnaire was distributed before the study. The passive smoking is defined as mothers who are exposed to 5-14 cigarettes per day from their husband or close associates. The placenta collected comprised of 13 non-exposed and 11 exposed to tobacco smoke.

The rate of exposure was assessed subjectively (from volunteered cigarette exposure) and objectively (determining levels of urine cotinine, a major metabolite of nicotine). All the infants whose placenta was taken for the study were live-born, full term and no detectable congenital malformations. The exclusion criteria include pregnant women with no diabetes, hypertension and

pregnancy complication that would lead to fetal and placental growth disorder. The collection and measurement of placental parameters were done according to established method. The umbilical cord was clamped at the placental insertion immediately after delivery and the cot was cut 5-20 mm from the insertion point before the membranes were trimmed away and the blood clots removed.

Urine cotinine level was determined using a HPLC method to estimate maternal smoking pattern(18). The urine sample was collected from the pregnant women immediately prior to delivery (0.25-1hour before childbirth). The cotinine was extracted from urine using quinolone as internal standard and run in a HPLC system with a mobile phase flow rate of 1.5 ml/min and absorbance at 254 nm. The method to measure oxygen diffusion was adapted from a well-established protocol (8, 9). The capacity for diffusion is expressed as a diffusive conductance, D_p , in $\text{mlO}_2/\text{min}/\text{kPa}$ (9).

The cotyledons were portioned into upper, middle and lower section using a point grid system. Systematic random sampling procedures were adopted in all levels of samplings such as blocks, sections field of view and individual measurement. The conductance for each tissue layers was carried out by stereology method and physicochemical variables. For stereological studies, the light microscope and Merz grid were used. Results were assessed statistically by analyses of variance (ANOVA) and Student's t-test. The form of t-test used was an independent samples two-tailed t test. Significance was set at the $p < 0.05$ levels Statistical analyses were executed by using SPSS for Windows (Version 9.0, SPSS Inc. Chicago, Illinois, USA).

RESULTS

The mean age of all participating mothers were 28.6 ± 4.76 years and the gestational age between 37-42 weeks (Table 1). For the group of smoking exposed mothers, the mean height was 1.57 m, the mean weight on admission was 62.6 kg and the mean body mass index (BMI) was 25.5 kg/m^2 respectively. No significant differences were observed in non-exposed groups I in comparison with the exposed groups for all the variables mentioned (Table 1).

Urine cotinine determination by HPLC method shows that the mean cotinine concentration was not significantly different between exposed (17.10 ± 15.65) and non-exposed (10.11 ± 7.50) groups (Table 1). The gross surface differences of normal and passive smokers placenta showed some interesting observations. The entire passive smokers placenta showed the presence of blood clot (abruption) on the cotyledons surface while three of the smokers showed calcification and multiple infarction on the surface. There is no difference in the placenta size between and passive and normal mothers (Table 1). The apparent increase in placental surface area, weight and specific weight in the exposed group was not statistically significant. However, we did observe a significantly higher placental volume ($p < 0.05$) in the exposed group (mean 491.2, CI: 446.7-535.6) compared to non-exposed group (mean 411.5, CI: 363.6-459.3) (Table 1).

The structural variables for oxygen conductance determination are shown in Table 2. Maternal-intervillous spaces and foetal-intervillous spaces of the three region of cotyledon (upper, middle and lower) showed no significant difference between exposed and non-exposed groups. The exchange surface area of maternal erythrocytes in the upper region of the cotyledon was significantly higher ($p < 0.05$) in the exposed group. However, exchange surface area in the middle and lower regions of the cotyledon were not significantly different. A significant difference ($p < 0.05$) was observed in the surface area of maternal aspect of the trophoblast in the lower region of

the cotyledon for the exposed group. The exchange surface area of foetal aspect of trophoblast, luminal aspect of foetal capillaries and foetal erythrocytes showed no significant difference between exposed and non-exposed group in all the regions of the cotyledon. Moreover, we found no significant difference in the harmonic mean thickness of maternal plasma, trophoblast and stroma for all the regions of the cotyledon in both exposed and non-exposed groups. Interestingly, we found that the harmonic mean thickness of foetal plasma in the upper region of the cotyledon was significantly higher ($p < 0.05$) in the exposed group.

Partial diffusing conductance of foetal plasma in all three regions of the cotyledon was not significantly different between exposed and non-exposed groups. However, when the foetal erythrocytes were taken into consideration, the partial diffusing conductance in the lower region of the cotyledon was significantly ($p < 0.05$) higher in the exposed groups. The total diffusive conductance of the placentae in all the regions of the cotyledons was significantly different between exposed and non-exposed groups. Furthermore, the specific diffusive conductance of the placental oxygen pathway in all three regions was not significantly different. The mean conductance in both exposed and non-exposed group was in the range of 10-15 ml/min/kPa/kg. In exposed groups, the regression equation for the mean total conductance against birth weight took the allometric form $D_p = 24.56W^{5.77}$ with no significant difference.

The partial relative resistance of maternal erythrocytes, maternal plasma, trophoblast and stromal layers in all three regions of the cotyledon were not significantly different between exposed and non-exposed groups. The main contributors to total oxygen diffusive resistance were the trophoblast (46-48 %), maternal plasma (15-23 %) and stroma (16-19 %). In addition, foetal plasma accounted for 7-11 % and foetal erythrocytes about 4 % of the total oxygen diffusive resistance. The number of villi was significantly higher ($p < 0.05$) in the lower region of the

cotyledon in the exposed group. In addition, the total number of villi was found to be higher in the exposed group compared to non-exposed group.

DISCUSSION

Gross pathological changes, such as placental abruption, infarction and calcifications have been observed on the placenta of those mothers who were exposed to tobacco smoke. This raises the possibility of foetal hypoxemia occurring in those women exposed to tobacco smoke during pregnancy (19). The non-significant alteration in the total oxygen conductance between exposed and non-exposed group is related to non-decrease birth weight in both group tested (9). The insignificant mean partial conductance of maternal erythrocytes and plasma could be attributed to the effects of carbon monoxide in the tobacco smoke. Carbon monoxide has a far higher affinity for hemoglobin than oxygen and thus reduces oxygen capacity of the maternal blood (functional anemia). Other studies have shown that the levels of carboxyhaemoglobin are greater in mothers exposed to tobacco smoke (20-22) and this factor is not accounted for in the morphometric model. Therefore, mean conductances of maternal erythrocytes and plasma, probably underestimated for exposed group.

Our findings further indicate that the upper portion of cotyledon comprised a proportionally lower volume of villous structure but a proportionally higher volume of maternal blood space, which are vice versa in lower portion of cotyledon. The present study also showed a significant increase in partial conductance of maternal plasma in upper region of cotyledon in the placenta of passive smokers. This suggests that there are greater surface areas of erythrocytes plasma membrane on this part of cotyledon. This finding varies with others, where it is in an

agreement with (9) but in contrast with (23). Moreover, ours may be in advantage than the others since we have partitioned the cotyledons into three parts namely upper, middle and lower section. Our findings further indicate that the upper portion of cotyledon comprised a proportionally lower volume of villous structure but a proportionally higher volume of maternal blood space, which are vice versa in lower portion of cotyledon.

Trophoblast conductance was not altered in passive smoking group and this result could be contributed to the rate of exposure to tobacco smoke that is in the range of 15-30 ng/ml. There is no significant increase in the harmonic mean distance across the villous stroma indicating that other factors might affect the conductance across the layers. The augmented value of foetal erythrocytes conductance and number of foetal capillaries in exposed group indicate that post-placental compensation may have taken place to improve diffusion in passive smokers placenta. The post-placental compensatory mechanism includes an increase in peripheral surface area due to the increase in number of foetal capillaries and profuse branching.

CONCLUSION

In conclusion this study has shown that passive smoking has an effect on placenta and significant diffusive conductance was found in different part within the cotyledon. Previous works have used the entire block of cotyledon to estimate oxygen-diffusing capacity in various condition of foetal hypoxic stress where as we divide the cotyledon into three parts namely maternal, materno-fetal and foetal part. Further investigation is needed to explore the compensatory mechanism of placenta in overcoming the hypoxic stress. The small number of the sample size warrants comment since we used convenient sampling method in a single hospital based environment. Most of our samples

are low income group patients which do not represent the whole population. Factors such as short duration of project, funding and facilities are limiting factors to look into large number of placentae with ethnic stratification.

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AUTHOR'S NOTE

Ramesh KN conceived paper, conducted research, oversaw data collection, wrote manuscript and approval final version. Nasaruddin AA participated in study design, data analysis and interpretation, revised manuscript and approval final version. Stanslas J participated in study design, interpretation of data and revision of manuscript and approval final version. Sivalingam N provided oversight to study, participated in data interpretation, and critically revised manuscript and approval final version. Mathavan I participated in study design, interpretation of data and

revision of manuscript and approval final version. The authors declare that they have no conflict of interest.

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Table 1: Maternal, neonatal and placental variables in exposed and non-exposed subjects

Variable	Exposed	Non-exposed	df	p level
Maternal				
Height (m)	1.57(1.54-1.60)	1.56(1.53-1.59)	22	0.726
Weight (kg)	62.6(57.7-67.5)	65.0(58.1-72.0)	22	0.565
BMI (kg/m ²)	25.5(22.9-28.0)	26.6(24.2-28.9)	22	0.498
Neonatal				
Gestational age(days)	276.4(270.0-282.8)	278.2(273.4-282.9)	22	0.621
Birth weight (kg)	3.17(2.99-3.36)	3.16(2.94-3.38)	22	0.92
Length (m)	0.47(0.46-0.50)	0.48(0.46-0.50)	22	0.648
BMI kg/m ²	14.1(13.1-15.1)	13.7(12.8-14.6)	22	0.501
Head circumference (cm)	32.9(32.3-33.5)	33.0(32.3-33.8)	22	0.852
Urine cotinine(ng/ml)	17.1±15.65	10.11±7.50	22	0.824
Surface area m ²	324.4(292.3-355.7)	287.4(242.6-334.3)	22	0.197
Volume(cm ³)	491.2(446.7-535.6)	411.5(363.6-459.3)	22	0.016*
Weight (g)	497.7(457.0-538.5)	447.0(392.5-501.4)	22	0.135
Specific Weight	23.3(22.3-24.4)	22.0(20.8-23.1)	22	0.077

BMI-Body mass index; * Significant at p<0.05.

Table 2: Coherent set of structural variables for determining oxygen diffusive conductance of the placenta

Variables	Exposed	Non-exposed	df	p level
Exchange surface area (cm²)				
Maternal erythrocytes				
Cotyledon region				
Upper	4.7 E5	2.4 E5	8	0.013*
Middle	4.0 E5	2.5 E5	8	0.277
Lower	3.2 E5	3.7 E5	8	0.781
Maternal trophoblast				
Upper	1.8 E5	1.4 E5	8	0.15
Middle	1.6 E5	2.8 E5	8	0.398
Lower	1.8 E5	1.1 E5	8	0.017*
Harmonic mean thickness				
Foetal plasma				
Upper	1.5 E4	9.6 E5	8	0.012*
Middle	1.1 E5	9.2 E5	8	0.286
Lower	9.6 E5	1.0 E4	8	0.732
Partial diffusive conductance (ml/min/kPa)				
Maternal plasma				
Upper	401.2	193.3	8	0.041*
Villous tissues				
Lower	352.8	197.3	8	0.027*
Foetal erythrocytes	1149.4	815.3	8	0.049*
Lower	1336.4	717.6	8	0.039*
Partial relative resistances (%)				
Foetal Plasma				
Upper	14.4	7	8	0.009*
Number of villi	22	16	8	0.022*
Lower	22.4	15.4	8	0.007*

*Significant at p <0.05. E: exponent to the power of (10^x)