Prenatal tobacco exposure shortens telomere length in children

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ABSTRACT (242/250 WORDS)

Introduction: Preliminary evidence suggests a possible association between prenatal tobacco exposure and telomere length in children. This study was conducted to investigate whether maternal smoking during pregnancy was associated with telomere shortening in their children, and whether prenatal and childhood exposure to environmental tobacco had any impact on this association.

Methods: This is a population-representative study on the association between prenatal tobacco exposure and telomere length in children. 98 Hong Kong Chinese children aged under 15 years with prenatal tobacco exposure and 98 age- and gender-matched controls were recruited from a population health study with stratified random sampling.

Results: Telomere length in children with prenatal tobacco exposure was significantly shorter than in those with no exposure (mean T/S ratio = 24.9 [SD = 8.58] in exposed vs. 28.97 [14.15] in control groups; p = 0.02). A negative dose-response relationship was observed between the T/S ratio and tobacco exposure duration: the longer the duration of maternal smoking in pregnancy, the shorter the child's telomere length. The association between the child's telomere length and prenatal tobacco exposure remained significant after considering the influence of family socioeconomic status and exposure to environmental tobacco smoke during pregnancy and childhood.

Conclusions: Prenatal tobacco exposure was associated with telomere shortening in children. As this may impose significant health impacts through fetal genetic programming, more efforts should be made to reduce fetal tobacco exposure by educating pregnant women to not smoke and motivating smokers to quit in early pregnancy.

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IMPLICATIONS

As reflected by telomere shortening, prenatal tobacco exposure in children can cause premature aging and increased health risks, which we suggest is entirely preventable. Not smoking during pregnancy or quitting smoking is critical to improving the health outcome of our future generations as prenatal tobacco exposure may affect children's biological programming.

MAIN TEXT (3312 WORDS)

INTRODUCTION

Prenatal tobacco exposure is associated with detrimental child health outcomes including premature birth,[1][2] low birth weight,[2-4] sudden infant death syndrome,[4] allergic disorders,[5-8] and neurodevelopmental disorders such as attention deficit hyperactivity disorder and externalizing behaviors.[9-11] In spite of these potential risks, smoking during pregnancy is still very common in China and worldwide. It is estimated that 12.8% of women in U.S.[12] and 5.4% in China[13] actively smoke during pregnancy.

Telomeres are specialized nucleoprotein complexes located at the distal ends of linear eukaryotic chromosomes that have critical roles in maintaining chromosomal structures during mitotic cell division by protecting the chromosomal ends from abnormal fusion and nucleolytic degradation, and shielding the chromosome from recognition by the DNA damage-repair system.[14] Telomeres are essential components that protect against the potential loss of genetic information due to replication flaws.[15-18] The maintenance of telomere length is strongly associated with long-term human health. Telomere shortening is associated with adverse health outcomes across the lifespan including type 2 diabetes,[19] cardiovascular diseases,[20][21] cancer,[22][23] Alzheimer's disease,[24] and early mortality.[25][26] Besides being a biomarker for cellular and biologic aging, telomere shortening also reflects the cumulative exposure to oxidative stress and subsequent disease development.[19][26-28]

Interestingly, the rate of telomere shortening was found to be accelerated by exposure to environmental chemicals such as tobacco smoke.[29][30] Empirical studies found that smoking was associated with telomere shortening in adults.[29][31] Compared to adults who have never been exposed to tobacco smoke, the telomere length of exposed adults shrank at a more significant

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rate over time, which suggests tobacco exposure may accelerate the aging process.[29] Several

studies found a dose-response relationship between cumulative lifetime tobacco exposure and

telomere length in adults: those with longer exposure to cigarette smoke had shorter telomere

lengths.[1 22 29]

Recent animal studies investigating the potential effect of smoking on telomere length found that

developing mouse embryos exposed to cigarette smoke condensate (CSC) and cadmium (a major

component in tobacco) resulted in telomere shortening and significant reduction in the cleavage

of mouse embryos.[29] This appeared to be related to chromosomal instability and oxidative

damage to embryonic stem cells induced by different levels of stress intensity.[30,31]

In spite of these empirical findings from animal studies, the actual relationship between tobacco

exposure in women during pregnancy and the telomere length in their children is still largely

unknown. Previous studies on telomere length in children were more focused on the effect of a

deprived environment in early childhood.[25 32 33] We still do not know whether maternal smoking

during pregnancy leads to telomere shortening in their offspring, and whether this telomere

shortening can be a possible mechanism to explain maternal smoking-related detrimental health

outcomes in children. Findings from a retrospective study on a small group of disadvantaged US

children suggested that infants born to mothers who smoked during pregnancy had shorter

telomere lengths, but the study had major limitations in its small sample size and combined

measurement in ETS and maternal active smoking.[34] Meanwhile, another study measuring a

newborn's telomere length in the cord blood found a possible relationship between exposure to

ETS during pregnancy and shortening of neonatal telomere length.[35] However, this study did not

consider the dosage effect of maternal smoking during pregnancy.[35] A robust study that considers

the effect of the duration of prenatal tobacco exposure and ETS during pregnancy and in

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childhood is still lacking. We have conducted the first such study to investigate the impact of prenatal tobacco exposure on a child's telomere length. We hypothesize that prenatal tobacco exposure is associated with telomere shortening in children.

METHODS

Study population

This cross-sectional study examined the association between prenatal tobacco exposure (women actively smoking during pregnancy) and telomere length in children recruited from the Hong Kong Child Health Survey (CHS), a population-based household survey conducted in September 2005 to August 2006 on Chinese children under 15 years of age. The CHS selected households using systematic replicated sampling based on the Register of Quarters, a record of all permanent household quarters maintained by the Hong Kong Census and Statistics Department.[36-38] A total of 7393 children under 15 years were recruited from all 18 districts of Hong Kong. Among them, 59 children were active smokers who were excluded from this study and 146 children were non-smokers whose mothers smoked during pregnancy. The exposed group comprised of 98 randomly selected non-smoking children whose mother smoked during pregnancy; the control group comprised of another 98 age- and gender-matched children randomly selected from all non-smoking children whose mothers never smoked during pregnancy.

A previous study identified an effect size of 0.55 between prenatal exposure to tobacco and children's telomere length.[34] We adopted a more conservative effect size of 0.41 (75% of that reported in the above study), which required an effective sample size of 190 children (95 exposed and 95 control). Three additional participants per group were included to give a final sample size of 196 (98 exposed and 98 control), which has at least 80% statistical power to detect the effect size at a significance level of 0.05.

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Smoking exposure measures

Face-to-face interviews were conducted with the parents of all participating children to obtain information on the children's tobacco exposure, general health status, and sociodemographics. The interview was anonymous and did not collect any tracable personal identifiers (e.g. name, address, and identity number) to avoid social desirability bias. If the interview respondent was not certain regarding a certain question, he/she was suggested to discuss with family members.

There were four questions related to the children's tobacco exposure during pregnancy and in childhood, namely "*Did the mother smoke during the pregnancy? If yes, for how long?*", "*Has the mother been exposed to second hand smoking during pregnancy?*", "*What is the mother's current (or in the past 7 days) smoking habit?*", and "*What is the mother's current (or in the past 7 days) smoking habit?*".

Children's prenatal exposure to tobacco was categorised into: 1) mother did not smoke during pregnancy, 2) mother smoked initially but quit in first trimester of pregnancy, and 3) mother smoked throughout the pregnancy. Children in the last two categories were classified as being exposed to prenatal tobacco. Children's prenatal ETS exposure was categorised into: 1) mother not exposed to ETS during pregnancy, 2) exposed to prenatal ETS for less than 1 h daily, and 3) exposed to prenatal ETS for at least 1 h daily. Children's ETS exposure was categorised according to the current smoking status of the parents as: 1) non-smoker, 2) smoked ≤ 5 cigarettes per day, and 3) smoked > 5 cigarettes per day.

Family socioeconomic status (SES)

Two key family variables were included to reflect the family SES, family monthly income adjusted for household size and whether the family received Comprehensive Social Security

Assistance (CSSA), which is a direct cash subsidiary provided by the government to low income families. Family income adjusted for household size was computed using the Organisation for Economic Co-operation and Development (OECD) square-root scale to account for the fact that the household economic need may not be linearly proportional to household size.[39]

DNA extraction and telomere length

The telomere length was measured in buccal epithelial cells from children participating in the study. Buccal swab samples were collected from each child during a household visit. Genomic DNA was isolated and extracted using the Gentra Puregene Buccal Cell Kit (Qiagen) according to the manufacturer's instruction. The isolated DNA samples were diluted to a concentration of 5 ng/μL in the buffer solution (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and quantified using a Nanodrop 2000c spectrophotometer (Applied Biosystems).

The relative telomere length was determined by adopting the protocols described by Drury et al. and Cawthon.[33 40] For each sample, the telomere length was represented by the relative ratio of the telomere repeat copy number (T) to the single copy gene 36B4 copy number (S). The T/S ratio was determined by quantitative polymerase chain reaction (qPCR) using a 7900HT Thermocycler (Applied Biosystems). It can then be calculated by the formula $T/S = 2^{(-dCt)}$, where dCt is the difference in threshold cycle obtained by subtracting the average 36B4 Ct value from the average telomere Ct value.

Statistical analysis

Reliability of the T/S ratio values was tested using the generalized extreme studentized deviate (GESD) test,[41] which is an extension of the Grubbs test for outlier detection that allows testing of multiple potential outliers without inflating type I error. Detected outliers were regarded as

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missing data in the analysis. Missing data was handled using multiple imputations (MI), which is a more robust technique for handling missing-at-random data than mean imputation and complete data analysis.[42] Imputations were repeated five times in this study and all the analysis results were based on the pooled estimates from the five imputed datasets.[42]

The T/S ratio difference between the exposed (mother smoked during pregnancy) and control (mother did not smoke during pregnancy) groups was examined using paired t-test. The dose-response relationship between T/S ratio and prenatal tobacco exposure (not exposed, exposed only in first trimester of pregnancy, and exposed throughout pregnancy) was tested using a linear regression model adjusted for the child's age and gender (the crude model). To eliminate the potential confounding effect of SES, family income and CSSA status were additionally controlled (the adjusted model). Similarly, the relationship between T/S ratio and exposure to prenatal ETS and childhood ETS (from paternal and maternal smoking) were tested using crude and adjusted models. Finally, a full model was fitted with all the covariates, including age, gender, family income, CSSA status, prenatal tobacco exposure, prenatal ETS exposure, and childhood ETS exposure from paternal and maternal smoking. In other words, there were four smoking exposure variables in the crude, adjusted, and full models: (i) prenatal tobacco (mother smoking status during pregnancy), (ii) ETS during pregnancy (maternal exposure to ETS), (iii) ETS in childhood due to paternal smoking, and (iv) ETS in childhood due to maternal smoking. Statistical significance was determined using the two-tailed p-value of the regression coefficients. A p-value of less than 0.05 was deemed statistically significant. All analyses were performed in *R Statistical Package* version 3.2.1 with the package *MICE*.[43]

Ethics Approval

The study and consent procedures were approved by the ethical committee of the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. Written informed consent was obtained from the parents of each participant.

RESULTS

A total of 196 children were included in this study (98 exposed, 98 age- and gender-matched control). Telomere lengths of eight children, (three from the control and five from the exposed group) were deemed as outliers by the GESD tests. Detailed descriptions of the respondents' tobacco exposure and family SES are shown in Table 1. The overall mean age of participants was 6.33 ± 3.85 years. Among children with direct prenatal tobacco exposure, 90.8% were exposed throughout pregnancy and 84.7% were also exposed to ETS during pregnancy. In the control group, 39.8% of children were exposed to prenatal ETS. Children in the exposed group had much longer ETS exposure during pregnancy and childhood, and 68% of fathers were current smokers compared to 26.5% in the control group. Concerning their socioeconomic status, the exposed group had significantly lower family income and more were receiving CSSA than in the control group.

The overall mean telomere length of children participating in this study as represented by the mean T/S ratio was 26.96 ± 11.88. There was a significant difference in telomere lengths between children in the exposed and control groups. Figure 1 shows the distribution of the T/S ratios among the sampled children. The mean T/S ratio of children with prenatal tobacco exposure was significantly lower than that of children in the control group (24.90 [SD 8.58] in exposed vs. 28.97 [14.15] in control groups; $p = 0.016$), indicating there was a crude association between prenatal tobacco exposure and the telomere length in children.

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As shown in Table 2, the crude model with child's gender and age as the only covariates indicated a significant and negative association between the child's T/S ratio and the maternal smoking throughout the pregnancy (β = -4.49; 95% CI -7.88 to -1.10; p < 0.01). Moreover, the adjusted model with monthly family income and CSSA recipient status as additional covariates confirmed that the T/S ratio was negatively associated with direct tobacco exposure during pregnancy (β = -4.82; 95% CI -8.30 to -1.35; p < 0.01). Upon fitting all four exposure variables with the previously included covariates in the full model, we found only direct tobacco exposure due to maternal smoking during pregnancy had a significantly negative association with the child's T/S ratio (β = -6.02; 95% CI -11.14 to -0.89; p < 0.05). In all three models, both ETS exposure during pregnancy (mother exposed to ETS) and ETS exposure in childhood related to paternal and maternal smoking did not have any significant association with the child's T/S ratio.

Moreover, prenatal tobacco exposure and children's telomere length had a dose-response relationship. The telomere length of children had a decreasing trend with the increasing duration of direct maternal smoking (p = 0.02), the longer the mother smoked during pregnancy, the shorter the child's telomere length (Figure 2).

DISCUSSION

Prenatal tobacco exposure and telomere shortening

This is one of the first studies to demonstrate that smoking during pregnancy causes telomere shortening in children. Our study differed from previous studies[34,35] in that we considered both the duration of maternal smoking during pregnancy and the influence of ETS during pregnancy and childhood. The study also involved a much larger and representative sample recruited through a population health study.

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A recent study provided evidence of an inverse relationship between foetus's intrauterine tobacco exposure and the cord blood telomere length at birth.[35] Our findings provide further evidence to support this association, which persisted beyond the newborn period and suggested a longer lasting effect due to prenatal tobacco exposure. Meanwhile, we also found a gradient relationship between the duration of maternal smoking during pregnancy and children's telomere length (Figure 2). However, ETS exposure during pregnancy and childhood did not affect this relationship.

Tobacco smoke exposure and telomere shortening

Tobacco smoke is a prevalent systemic human mutagens and leads to significant genotoxicity. In utero exposure to tobacco smoke can disrupt the foetus's intrauterine programming and results in the elevated frequencies of hypoxanthine phosphoribosyltransferase (HPRT) mutations, translocations, and DNA strand breaks in newborns.[44] On the other hand, telomeres are an important biological marker for aging and have been linked with cancer development.[45] Children diagnosed with neuroblastoma and leukaemia were found to have significant telomere shortening.[46] Although tobacco smoke exposure has been shown to result in telomere shortening in adults, its effect on telomere length and related potential health implications in children are largely unknown.

Richter and Zglinicki found that oxidative stress-mediated DNA damage was an important determinant of telomere shortening.[15 28 47 48] Laboratory studies investigating the effects of cigarette smoke condensate (CSC) on animal cells and mouse embryos showed that cigarette smoke increased cellular reactive oxygen species load that caused oxidative stress in cells.[49] Mouse embryonic stem cells exposed to low doses of CSC or cadmium resulted in shortened

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telomere length and DNA damage.[50] Whole mouse exposed to cigarette smoke increased reactive oxygen species in cells leading to telomere shortening and apoptosis.[51]

The high G-C content of the telomeres renders them susceptible to oxidative stress. Oxidative stress was found to affect the rate of telomere attrition during DNA replication under a range of stress intensities via intensifying the replication problem at different extent. This suggests that cigarette smoke can lead to telomere shortening at different levels of exposure.[49] This could also explain the dose-gradient relationship between the duration of prenatal maternal smoking and child's telomere shortening found in this study.

Environmental tobacco smoking and telomere length

Although ETS exposure has been linked to adverse effects on child health,[52-55] we found that ETS exposure during pregnancy and childhood were not significantly associated with telomere shortening or with the relationship between maternal smoking during pregnancy and telomere shortening in children.

Implications of the findings

Our original findings support the hypothesis that maternal smoking during pregnancy shortens the telomere length in children. As reflected by telomere shortening, in utero exposure to tobacco smoke could have more profound harmful effects than previously expected by affecting the biological programming of foetus, which may lead to adverse long-term outcomes in childhood and adulthood.[20-23 25 27] Our findings demonstrated that harmful effects of prenatal tobacco exposure could be observed well before clinical manifestations. As smoking is still relatively common among pregnant women, these findings have important implications for health care planning and related policy making.[56] Policy makers should recognize that smoking during

pregnancy is a key public health issue, and should educate pregnant women and public about the potential harmful effects of prenatal tobacco exposure on the long-term health of children. Successful intervention programs to prevent/reduce smoking among pregnant women should comprise of multiple components with different dimensions.[57-59] Nonetheless, the power of evidence-based information in convincing pregnant smokers to quit smoking should not be underestimated. The novel finding of telomere shortening in children directly exposed to prenatal tobacco implies that offspring of mothers who smoke might experience additional health risks in early life, and this can be a strong argument to educate pregnant women to persuade them not to smoke during pregnancy. More effective smoking-cessation and counselling programs during pregnancy could be developed based on evidence from this original study.

Limitations of this study

The findings from this study should be interpreted with several caveats. First, this observational study did not determine the causal relationship between prenatal tobacco exposure and telomere shortening in children, even though the reverse is not likely. Second, parents' tobacco use was not measured by biomarkers and the self-report figures may subject to recall and social desirability bias. To minimize recall bias, we encouraged the mothers to discuss with family members concerning their smoking history if there were not certain for this question. In addition, mothers may have been unwilling to disclose the information for fear of social stigmatization. We attempted to overcome this issue by keeping the questionnaire anonymous without any personal identifiers (e.g. name and identify numbers). Third, parents' telomere lengths were not measured in this study. It would have been helpful to have this information from parents as there may be a high correlation between parents' and children' telomere length.[60] Nevertheless, the correlation may be partially due to the shared living environment as both the parents and children

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are exposed to similar environmental stressors. Fourth, maternal substance abuse and alcohol use was not measured in this study. Since smoking, alcohol use, and substance abuse was mildly associated, the current effect size could have been overestimated. Nevertheless, this is not very likely to alter our conclusion because of the low prevalence in substance abuse among women in Hong Kong.[61][62]

Conclusion

This is the first study to demonstrate a dose-response relationship between the duration of prenatal smoking exposure and telomere length in children. Exposure to ETS during pregnancy and childhood did not affect telomere length in children. As reflected by telomere shortening, prenatal tobacco exposure in children can cause premature aging and increased health risks, which we suggest is entirely preventable. Not smoking during pregnancy or quitting smoking is critical to improving the health outcome of our future generations as prenatal tobacco exposure may affect children's biological programming.

The relationship between tobacco exposure and biological aging could be further studied. First, it would be important to understand how prenatal smoking at various stages of pregnancy affects telomere length in childhood so that the critical point of intervention could be determined. Second, the potential synergistic or additive effects of prenatal and postnatal tobacco exposure on telomere length should also be investigated. Last but not least, longitudinal study on the long-term health outcomes of children with telomere shortening might provide further information concerning the significance of using telomere as a biological marker in children.

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FUNDING

COMPETING INTERESTS

The authors declare no competing interest.

ACKNOWLEDGEMENTS

CONTRIBUTOR STATEMENT

PI and BHYC conceptualised the study, interpreted the data, and critically revised the manuscript. FKWH analysed the data, drafted a part of the manuscript, and critically revised the manuscript. GCFC, WD, WHSW, SLL, and YLL interpreted the data and critically revised the manuscript. DY and WLW conducted the experiment, interpreted the data, and critically revised the manuscript. PYTC and KTST interpreted the data and drafted a part of the manuscript.

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Table 1. Characteristics of the study participants

	Overall	Exposed	Control	p-value
Gender, n (%)				-[a]
Female	98 (50.0)	49 (50.0)	49 (50.0)	
Male	98 (50.0)	49 (50.0)	49 (50.0)	
Mean (SD) age (years),	6.33 (3.85)	6.33 (3.86)	6.33 (3.86)	-[a]
T/S ratio, mean (SD)	26.96 (11.88)	24.90 (8.58)	28.97 (14.15)	0.02
Prenatal exposure to tobacco, n (%)				-[a]
None	98 (50.0)	0 (0.0)	98 (100.0)	
In first trimester of pregnancy	9 (4.6)	9 (9.2)	0 (0.0)	
Throughout pregnancy	89 (45.4)	89 (90.8)	0 (0.0)	
Prenatal ETS exposure, n (%)				< 0.001
None	74 (37.8)	15 (15.3)	59 (60.2)	
Exposed to < 1 hour per day	92 (46.9)	55 (56.1)	37 (37.8)	
Exposed to ≥ 1 hour per day	29 (14.8)	27 (27.6)	2 (2.0)	
Missing	1 (0.5)	1 (1.0)	0 (0.0)	
Current smoking status of mother, n (%)				< 0.001
Non-smoker	116 (59.2)	25 (25.5)	91 (92.9)	
Smoked ≤ 5 cigarettes per day	54 (27.6)	48 (49.0)	6 (6.1)	
Smoked > 5 cigarettes per day	22 (11.2)	22 (22.4)	1 (1.0)	
Missing	4 (2.0)	3 (3.1)	0 (0.0)	
Current smoking status of father, n (%)				< 0.001
Non-smoker	103 (52.6)	31 (31.6)	72 (73.5)	
Smoked ≤ 5 cigarettes per day	43 (21.9)	29 (29.6)	14 (14.3)	
Smoked > 5 cigarettes per day	46 (23.5)	35 (35.7)	11 (11.2)	
Missing	4 (2.0)	3 (3.1)	1 (1.0)	
Adjusted family income, mean (SD), HKD	19,770 (13,490)	17,300 (9,960)	22,110 (15,850)	0.11
CSSA, n (%)				0.09
Yes	10 (5.1)	8 (8.2)	2 (2.0)	
No	165 (84.2)	77 (78.6)	88 (89.8)	
Missing	21 (10.7)	13 (13.3)	8 (8.2)	

[a] Not available due to study design

Table 2. Association between exposure to tobacco and T/S ratio

	Crude models[a]			Adjusted models[b]			Full model[c]	
	Coefficient (95% CI)	p		Coefficient (95% CI)	p		Coefficient (95% CI)	p
Direct prenatal exposure to tobacco (mother actively smoked)								
None	0 (Reference)	-		0 (Reference)	-		0 (Reference)	-
Only in first trimester of pregnancy	-2.44 (-10.48 to 5.59)	0.55		-2.53 (-10.62 to 5.55)	0.54		-2.16 (-10.97 to 6.65)	0.63
Throughout pregnancy	-4.49 (-7.88 to -1.10)	0.01	**	-4.82 (-8.30 to -1.35)	0.007	**	-6.02 (-11.14 to -0.89)	0.02 *
ETS exposure during pregnancy (maternal exposure to ETS)								
None	0 (Reference)	-		0 (Reference)	-		0 (Reference)	-
Exposed to < 1 h per day	-2.15 (-5.83 to 1.52)	0.25		-2.14 (-5.86 to 1.58)	0.26		-0.72 (-4.89 to 3.45)	0.73
Exposed to ≥ 1 h per day	-2.27 (-7.43 to 2.90)	0.39		-2.38 (-7.62 to 2.86)	0.37		0.05 (-6.19 to 6.30)	0.99
ETS exposure in childhood related to maternal smoking								
Not exposed	0 (Reference)	-		0 (Reference)	-		0 (Reference)	-
Smoked ≤ 5 cigarettes per day	-1.65 (-5.54 to 2.24)	0.40		-1.81 (-5.71 to 2.09)	0.36		2.21 (-3.56 to 7.97)	0.45
Smoked > 5 cigarettes per day	-3.38 (-8.88 to 2.12)	0.23		-3.65 (-9.32 to 2.01)	0.20		-0.96 (-8.20 to 6.29)	0.79
ETS exposure in childhood related to paternal smoking								
Not exposed	0 (Reference)	-		0 (Reference)	-		0 (Reference)	-
Smoked ≤ 5 cigarettes per day	-2.24 (-6.48 to 2.01)	0.30		-2.26 (-6.53 to 2.01)	0.30		-0.62 (-5.71 to 4.48)	0.81
Smoked > 5 cigarettes per day	-0.04 (-4.16 to 4.07)	0.98		-0.12 (-4.29 to 4.04)	0.95		2.80 (-2.67 to 8.27)	0.31

[a]Crude models were constructed for each of the four exposure variables with the adjustment of children's age and gender

[b]Adjusted models controlled family monthly income and CSSA status for each exposure variable, in addition to children's age and gender

[c]The full model included all exposure variables, children's age, gender, adjusted family monthly income, and CSSA status

** P < 0.01; * P < 0.05

Figure 1. Distribution of T/S ratio by direct prenatal exposure to tobacco

Exposed children whose mothers smoked during pregnancy; control children whose mothers did not smoke during pregnancy.
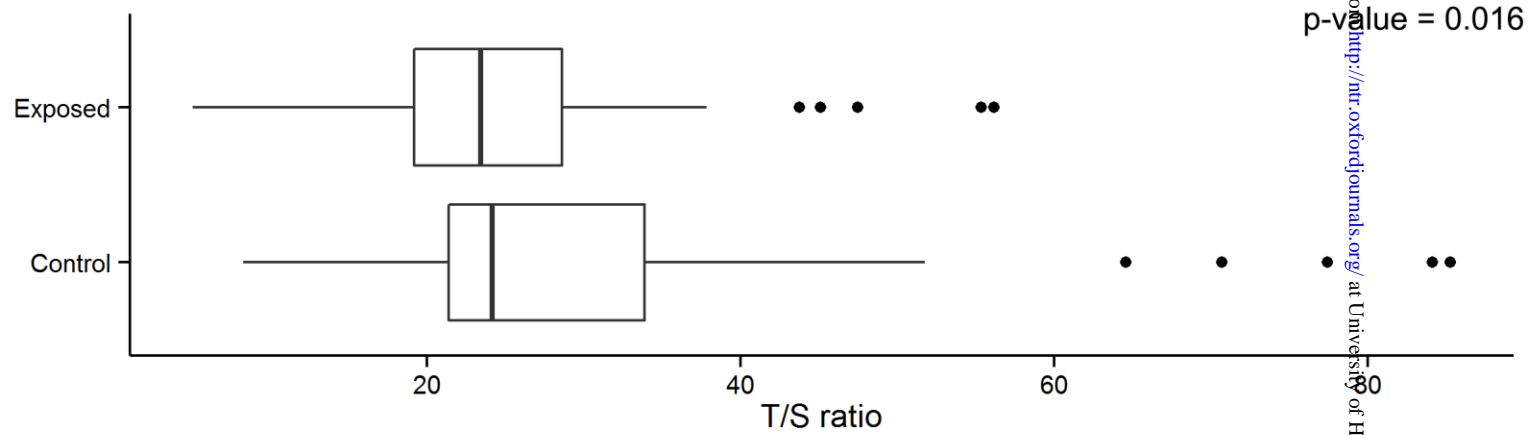
Figure 2. Mean T/S ratio according to different levels of prenatal direct exposure of tobacco

Error bar shows the 95% CI.

Figure 1

Figure 2