Maternal stress during pregnancy and neurodevelopmental outcomes of children during the first two years of life

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What is already known on this topic

- A growing body of literature documents associations between maternal stress in pregnancy and child development, but findings across studies are often inconsistent.
- The effects depend to a large extent on the kind of stressors, their objective intensity and duration, time of occurrence and mother's subjective perception of a stressor related threat.

What this paper adds

- Current prospective cohort study design enables longitudinal assessment of a variety of stressors that may influence a child's neurodevelopment.
- The study indicated that child neurodevelopment is differentially affected by different kinds of maternal stressors
- Children prenatally exposed to maternal life stress had decreased cognitive functions
- Occupational stress and satisfaction with family functioning were not significantly associated with child psychomotor development

Abstract

Aim: A growing body of literature documents associations between maternal stress in pregnancy and child development, but findings across studies are often inconsistent. The aim of this study was to estimate the association between exposure to different kinds of prenatal stress and child psychomotor development.

Methods: The study population consisted of 372 mother-child pairs from Polish Mother and Child Cohort. The analysis was restricted to the women who worked at least one month during pregnancy period. Maternal psychological stress during pregnancy was assessed based on: the Subjective Work Characteristics Questionnaire, Perceived Stress Scale and Social Readjustment Rating Scale. The level of satisfaction with family functioning and support was evaluated by APGAR Family Scale. Child psychomotor development was assessed at the 12th and 24th months of age by Bayley Scales of Infant and Toddler Development.

Results: Negative impact on child cognitive development at the age of two was observed for the Perceived Stress Scale (β=-0.8; p=0.01) and the Social Readjustment Rating Scale (β=-0.4; p=0.03) after adjusting for the variety of confounders. Occupational stress, as well as satisfaction with family functioning were not significantly associated with child psychomotor development (p>0.05).

Conclusions: The study supports the findings that prenatal exposure to maternal stress is significantly associated with decreased child cognitive functions. In order to further understand and quantify the effects of prenatal stress on child neurodevelopment further studies are needed. This will be important for developing interventions that provide more assistance to pregnant women, including emotional support or help to manage psychological stress.

Key words: child psychomotor development, life stress, occupational stress, family functioning, life events stress

Introduction

Psychological stressors, a generic term that includes a wide range of different types of events, can be acute - resulting from external disasters, as well as chronic - such as daily hassles. Negative health effects of stress depend on many factors including its duration and/or magnitude.[1] The following outcomes are associated with prenatal stress: congenital malformations, lower birth weight or shorter gestation, and neurodevelopmental as well as psychopathological outcomes.[2-11]

While there is general agreement that maternal stress is a risk factors for future child's health, various factors contribute to the inconsistencies reported in epidemiological studies including differences in stress measurements, and the lack of consensus on the definition of maternal stress.[6,12,13] Existing studies have focused on different aspects of stress covering general, occupational, pregnancy-related stress, daily hassles and relevant life events. Stress results from a perceived imbalance between acute and chronic stressors and individual resources including personality, social support and lifestyle, leading to the increased risk of maladaptive cognitive, emotional and behavioural responses. In addition, instruments used to assess stress of a general population might not be appropriate for the period of pregnancy.[13] Other difficulties in the studies evaluating the impact of prenatal stress on child neurodevelopment are related to inadequate control for confounding factors and, selection of appropriate tests for child development assessment.

The current study intends to address the above mentioned criticalities in human studies. Prospective cohort study design enables longitudinal assessment of a variety of stressors that may influence a child’s neurodevelopment. Additionally, the analysis includes several potential confounding factors and a well-standardized test evaluating neurodevelopmental effects in young children.[14]

The aim of this study was to estimate the association between exposure to different kinds of maternal stress during pregnancy and child psychomotor development.

Materials and methods

Study design and population

The mother-child pairs constitute a part of Polish Mother and Child Cohort (REPRO_PL) - a multicentre prospective cohort established in 2007 with the aim to evaluate environmental factors contributing to the pregnancy outcomes, children's health and neurodevelopment.[15-,19] Briefly, women were recruited if they fulfilled the following inclusion criteria: single pregnancy up to 12 weeks of gestation, no assisted conception, no pregnancy complications, and no chronic diseases as specified in the study protocol.[15] The participants were interviewed once in each trimester of pregnancy to collect and update socio-demographic data, medical and reproductive history, information about environmental, occupational and lifestyle factors. Children were assessed for their exposure, health status and neurodevelopment at 1 and 2 years of age.[16-19] Finally, out of 781 eligible children, 547 (70%) were examined at least once within the first two years of life. The current paper is intended to assess the impact of prenatal stress on child neurodevelopment, considering: occupational stress, perceived levels of stress in the daily life, family functioning and occurrence of stressful life events. Therefore, the analysis was restricted to the population of mothers working at least one month during pregnancy (85% of the population). Complete data on stress during pregnancy were available for 377 of the 464 women who indicated being employed during pregnancy.

The study was approved by the Ethical Committee of the Nofer Institute of Occupational Medicine, Lodz, Poland.

Assessment of maternal stress during pregnancy

Between the 20th and the 24th week of gestation the study participants completed 3 self-administered questionnaires to measure occupational stress, life stress and family functioning.

A 4th questionnaire measuring life events stress was completed between the 30th and 34th week of gestation.

Occupational stress was assessed using the Subjective Work Characteristics Questionnaire (SWCQ) by Dudek et al.[20] This widely used scale for diagnosis of occupational stress in Poland consists of 55 items describing potential occupational stressors. The subjects were asked to state if a given characteristic is present in their work position and how stressful they find it to be. Each item had the following answers: 1) a given psychosocial factor is not present; 2) it is present but it is neither irritating nor disturbing; 3) it is irritating or disturbing from time to time; 4) it is quite often irritating or disturbing; and 5) it is irritating all the time. The general indicator of the level of stress was the sum of points marked by each woman. In the present analysis an additional indicator of occupational stress – the number of stressful work characteristics as pointed out by each subject – was used.

Life stress was assessed by means of the Perceived Stress Scale (PSS, a 10-items scale) which measures an individual's level of perceived stress in the past month.[21] The items are designed in such a way to tap into how unpredictable, uncontrollable and overloaded the respondents find lives. The response options for each item indicate the frequency with which it occurs from 0 – never to 4 - very often. The results were the sum of points. The higher the score obtained by the respondents, the higher the level of their global stress.

Satisfaction with family functioning and support was assessed using Polish adaptation of the APGAR Family Scale by Smilkstein.[22,23] The scale consists of 7 statements related to help, acceptance or support from the family. The women indicated the frequency of experiencing positive behavior from their family on a 3-point scale, from 1 almost never to 3 very often. The higher the overall score, the higher the level of support experienced from the family.

Life events as stress factors were measured by a modified version of the Social Readjustment Rating Scale (SRRS).[24] Not only was the presence of critical life events during pregnancy

measured but its impact on women's emotional well-being was also assessed. Thus, we obtained 2 indices of stress: a sum impact scores assigned to the pointed life events and an individual perception of stress related to indicated events, which was the sum of the scores calculated from the answers to the question "To what extent did the event have impact on your emotional well-being?" ranging from "no" to "extreme impact" (0-3).

Child neurodevelopment assessment

Bayley Scales of Infant and Toddler Development (3rd edition) was used to assess children's neurodevelopment at around 12- and at 24- months of age.[14] The details regarding child psychomotor assessment has been published elsewhere.[16-19]

We excluded data from 5 children, since their Bayley tests were of poor quality because of pathologies or less-than-optimal cooperation of the child. Finally, based on availability of the data on prenatal stress exposure and psychomotor development test results the analysis was performed for 372 children (337 children were examined at 12 months of age, 184 had the examination repeated at 24 months and 35 were examined only at 24 months of age).

Confounding variables

The evaluated covariates that were identified from the literature were as follows: parental age and education; marital status, place of residence, SES and their changes over the study period; child gender; major pregnancy complications which appeared after inclusion into the study; type of delivery; gestational age and birth outcomes; breastfeeding; number of siblings; day care attendance; active/passive smoking and alcohol consumption during pregnancy; child ETS exposure after birth; child health status and hospitalizations. Socio-demographic data and information about alcohol consumption during pregnancy were obtained based on questionnaires. SES of the family was measured based on the following question: 'What is the financial status of your family?' Women who declared that they have sufficient money for current expenses and that it is possible for them to put a substantial sum aside were allocated

into the high income category. Those who indicated sufficient money for current expenses, with possibility to put aside some money were allocated into the medium and those who declared insufficient money for current expenses into the low income category. Prenatal exposure to tobacco constituents was assessed based on cotinine level in saliva and child ETS exposure after birth based on cotinine level in urine. The biomarker levels were measured using LC-MS/MS-ESI+.[25] As the cotinine levels in each trimester of pregnancy were highly correlated (the $1^{st}/2^{nd}$ and the $2^{nd}/3^{rd}$ trimester: $r=0.8$; the $1^{st}/3^{rd}$ trimester: $r=0.7$; $p<0.001$) the samples from the first trimester were included in analyses. The cotinine levels were log transformed. Child health status was assessed by means of frequency of respiratory diseases and otitis media.

Statistical analysis

First, univariate analysis was performed to assess the effect of each potential confounding variable on the Bayley test results. The following confounders ($p\leq0.1$) were identified to be included in the multivariate model: child gender ($p\leq0.08$), parental age ($p\leq0.03$) and education ($p\leq0.08$), prenatal ETS exposure ($p\leq0.05$), child ETS exposure ($p<0.06$), child health status ($p<0.1$) and, in the case of cognitive development, marital status ($p<0.03$), changes of marital status during pregnancy and after delivery ($p=0.09$) and day care attendance ($p<0.08$). As some confounders, namely mother and father's age as well as mother and father's education were highly correlated, sensitivity analysis was performed and finally only maternal age and education were selected in the final model. The second stage of the analysis focused on the evaluation of the impact of prenatal stress exposure on child psychomotor performance at one and two years of age. In the first model, confounding effects of the examiner who administered the test were taken into account. The second model was conducted with additional adjustment for potential confounders. Statistical significance was specified as $p<0.05$. The analysis was performed using the R software.

Results

Child and parental characteristics

Demographic and exposure characteristics of mothers and children are summarized in Table 1. About 51% of the children were girls. On average, the children were born at the 39th week of gestation (±1.5 week) with the mean birth weight of 3367 g (±478 g). About 58% of the children did not have siblings and 7% of mothers indicated child day care attendance at one and 23% at two years of age. The mean maternal and paternal age was 31(±4.3) and 33(±5.6) years, respectively. Most of the mothers and 43% of the fathers had a university degree. About 9% of the women indicated alcohol consumption and 10% were classified as active smokers during pregnancy.

The mean composite scores for psychomotor development were average or high average at one- and two-year-old assessments (Table 2). About 39% of the children were hospitalized at least once and 54% of them were ill at least once within the two-year period.

Characteristics of the maternal stress and family functioning during pregnancy

Occupational stress measured by the SWCQ with the mean of 93 points (range 57-180) and the mean number of stressful psychosocial factors at work equal 22 (range 2–50) can be interpreted as moderate (Table 2). The level of stress measured by PSS (mean 18, range 2-34) was also medium. The mean for SRRS, which was 100 (range 40-435), indicated a low level of stress in life and low probability of developing a stress-related disorder.

The mean APGAR Family score was 10 points (range 7-19), which indicates moderate satisfaction with family support and functioning.

Maternal stress, family functioning and child neurodevelopment

Table 3 presents the associations between prenatal stress and child psychomotor development (adjusted for examiner). Life stress assessed by PSS was negatively associated with child cognitive development at the age of 2 (β=-0.7; p=0.01). The same pattern was observed when

considering life events (β=-0.3; p=0.04). No association was observed between occupational stress and the analyzed domains of child development. Mother's satisfaction with family functioning and support during pregnancy was not significantly associated with the child's psychomotor development.

The final model confirmed the results observed in Model 1 (Table 4). Negative impact on child cognitive development at the age of two was observed for PSS (β=-0.8; p=0.01) and SRRS (β=-0.4; p=0.03).

Discussion

A number of studies report that prenatal stressors influence brain development. The effects depend to a large extent on the kind of stressors, their objective intensity and duration, time of occurrence and mother's subjective perception of a stressor related threat.[11] We show here that child neurodevelopment is differentially affected by different kinds of maternal stressors. In this study prenatal exposure to maternal stress measured by the PSS and SRRS was associated with decreased cognitive functions at two years of age. Occupational stress and satisfaction with family functioning were not significantly associated with child psychomotor development.

It is worth noting that we did not find any associations between prenatal stress and children's development at the age of one. Moreover, stress related effects occurred only for cognitive development. Our results might be partly attributable to the characteristics of Bayley Scales at the age of 12 and 24 months. Cognitive development in the first year of life is measured mainly by observation of the child and obviously by non-verbal tasks. At the age of two years, tasks become more complex and the attention is paid to exploration activities and adaptation to new situations. Moreover, according to Piaget's theory, our study covered the first 2 stages of child cognitive development: sensorimotor stage (0-24 months of age) and early phase of preoperational stage (between the ages of 2 and 7).[26,27] Some of the toddlers in

our study at the time of the 2nd measurement might have already been at the beginning of preoperational stage characterized by dynamic language and further cognitive development. Thus, our results support the concept that maternal stress does not appear to be significant for coordination of sensation through reflexive behaviours and primary circular reactions, visual-motor coordination but seems to be an important factor for development of cognitive schemata, mental operations and insight.

Our findings are consistent with those of Zhu at al., (2014) who reported that stressful life events predicted a lower mental development but had no impact on psychomotor development index of 16- to 18-month-old children.[12] Also in the study of Quebec women who were pregnant during a severe ice storm, the level of objective stress the mother experienced was related to poorer intellectual and language abilities in 2-year-old toddlers.[28] High amounts of daily hassles in early pregnancy were associated with lower development in the study by Huizink et al.[29]

The methods used for assessment of exposure and outcome of interest play important role in the studies evaluating the association between maternal stress and child neurodevelopment. Firstly, tools for assessment of infants and toddlers neurodevelopment are less reliable and more situation-sensitive than the methods used for older children, which may lead to a bigger measurement error. Secondly, the way of stress exposure measurement may be crucial.[30] The recently published review of psychometric instruments to assess stress during pregnancy indicated that, among the four different tests for measuring disturbances of daily hassles, PSS is the best.[13] Limitation of the PSS is that the scores obtained by the respondents reflect life stress intensity only in the preceding month. Of the five different instruments to measure life events as a stress factor, Prenatal Life Events Scale (PLES) was identified by the authors as the best available.[13] In our study, we selected SRRS with the adaptation of asking respondents to report how undesirable or negative a given event was. As concerns measurement of

occupational stress, the advantage of SWCQ is the availability of the norms for the Polish population. In our study low/medium level of stress was indicated by 79% of the respondents. It needs to be pointed that in Poland less than 20% of women continue working until late pregnancy.[31] Therefore, a relatively small proportion of pregnant women was exposed to occupational stress during entire pregnancy. Taking this into account, it seems that the working condition is of less importance than the other sources of stress during pregnancy period. In our analysis prenatal perceived family support was not associated with any of the measured scores of child psychomotor development. It is noteworthy that the mean score obtained by the respondents in our study indicated that they were generally pleased with family support.

The current study has several strengths. First, the prospective study design with well assessed exposure is the main advantage of the current analysis. Prenatal exposure to maternal stress was determined taking into account different sources of stress. Second, a series of detailed questionnaires allow for a reliable assessment of confounding variables. By restricting our population to healthy women, we were able to eliminate additional confounding factors. Finally, in the current analysis we assessed multiple aspects of child development.

Limitations of the study also need to be considered. Firstly, although we have measured different aspects of stress, we did not cover maternal anxiety, depression and stress related to pregnancy and performing parental roles, which can also be related to child neurodevelopment. Secondly, we cannot exclude the possibility that confounding by unmeasured risk factors (e.g. maternal IQ, children's maternal relationship and home environment, maternal stress after child birth) produced associations between exposure of interest and child development. In addition, the questionnaires measuring stress were applied during the 2nd and 3rd trimesters of pregnancy so the exact time of exposure allowing for assessment of critical window was not possible.

Conclusions

In summary, our study found associations between prenatal exposure to maternal stress and child decreased cognitive functions. In order to further understand and quantify the effects of prenatal stress on child neurodevelopment further studies are needed. The future studies should be based on prospective study design with assessment of all potential sources and aspects of stress.[13] Important issue is related to selection of the appropriate instruments for the assessment of specific stress with the possibility to include physiological measures. Identification of periods of increased vulnerability during pregnancy, using repeated measurements, on child psychomotor development is also recommended.[6] In addition, crucial aspect which needs to be pointed is related to adequate controlling for confounders. Further follow-up studies are also needed to explore the long-term outcomes of prenatal stress. Lastly, investigation of the effectiveness of interventions that provide more assistance to pregnant women, such as emotional support or help to manage psychological stress through positive emotion-focused or problem-focused coping, could be essential.

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Table 1 Child and parental characteristics

Variable	n, mean or median	%, SD or range
Gender of the child (N=372)		
Boys	181	48.7
Girls	191	51.3
Birth weight (g); mean, SD (N=356)	3369.5	478.0
Gestational age (weeks); mean; SD (N=356)	39.4	1.5
Length (cm); mean, SD (N=355)	55.2	3.6
Head circumference (cm); mean, SD (N=355)	34.4	1.5
Chest circumference (cm); mean, SD (N=354)	33.4	1.8
Breastfeeding (months) (N=371)		
No	33	8.9
<6	130	35.0
≥6	208	56.1
Day care attendance at 12 months (N=337)		
Yes	24	7.1
No	313	92.9
Day care attendance at 24 months (N=202)		
Yes	47	23.3
No	155	76.7
Age at Bayley test administration (months)		
For one year old; mean, SD (N=337)	12.8	1.4
For two years old; mean, SD (N=219)	24.4	2.8
Hospitalizations within two years after birth (frequency) (N=198)		
0	121	61.1
1	60	30.3
>1	17	8.6
Diseases within two years after birth (frequency) (N=198)		
0	91	46.0
1	78	39.4
>1	29	14.6
Number of siblings (N=372)		
0	217	58.3
≥1	155	41.7
Maternal age (years); mean, SD (N=372)	30.8	4.3
Paternal age (years); mean, SD (N=344)	32.8	5.6
Maternal education (N=372)		
Primary/vocational	16	4.3
Secondary	102	27.4
University	254	68.3
Paternal education (N=365)		
Primary/vocational	58	15.9
Secondary	149	40.8
University	158	43.3
Socio-economic status (N=372)		
Low	39	10.5
Medium	260	69.9
High	73	19.6

Marital status (N=370)		
Married	285	77.0
Unmarried	85	23.0
Maternal pre-pregnancy BMI (kg/m^2); mean, SD (N=368)	22.5	3.6
Major pregnancy complications (N=336)		
Yes	17	5.1
No	319	94.9
Type of delivery (N=336)		
Cesarean	120	35.7
Vaginal	216	64.3
Saliva cotinine level in pregnancy (ng/ml); median, range (N=335)	1.2	<LOD-376
Alcohol consumption during pregnancy (N=369)		
Yes	33	8.9
No	336	91.1

Table 2 Characteristics of the exposure and outcome variables

Variables	N	Mean	SD	Median	Min	Max
Subjective Work Characteristics Questionnaire						
Sum in points (potential range: 55-275)	372	93.1	23.9	89.0	57.0	180.0
Number of psychosocial factors at work (potential range: 0-55)	372	21.6	9.4	21.0	2.0	50.0
APGAR Family Scale						
Sum in points (potential range 7-21)	372	9.7	2.4	9.0	7.0	19.0
Perceived Stress Scale						
Sum in points (potential range: 0-40)	372	17.5	5.6	17.0	2.0	34.0
Social Readjustment Rating Scale						
Sum of wages (potential range: 40-1398)	367	100.7	78.8	78.0	40	435.0
Subjective stress related to events (sum of scores) (potential range: 0-120)	367	4	5	2	0	26
Composite score for one year old children; N=337						
Cognitive		107.2	10.5	110.0	80.0	145.0
Language		108.7	13.6	109.0	68.0	141.0
Motor		105.8	14.0	107.0	73.0	151.0
Composite score for two years old children; N=219						
Cognitive		113.0	16.3	110.0	80.0	145.0
Language		102.5	13.0	100.0	74.0	144.0
Motor		112.3	14.3	110.0	73.0	154.0

Table 3 Association between prenatal stress exposure and child cognitive, language and motor development - adjusted for examiner

	One year old children β (95% CI)			Two years old children β (95% CI)		
	Cognitive	Language	Motor	Cognitive	Language	Motor
Subjective Work Characteristics Questionnaire						
Sum in points	0.02 (-0.02 to 0.07)[a]	0.01 (-0.04 to 0.06)[a]	0.03 (-0.02 to 0.08)[a]	0.001 (-0.06 to 0.06)[b]	0.04 (-0.02 to 0.1)[b]	0.05 (-0.01 to 0.1)[b]
Number of psychosocial factors at work	0.06 (-0.04 to 0.2)[a]	0.05 (-0.06 to 0.2)[a]	0.06 (-0.05 to 0.2)[a]	-0.02 (-0.1 to 0.1)[b]	0.07 (-0.06 to 0.2)[b]	0.1 (-0.01 to 0.3)[b]
APGAR Family Scale						
Sum in points	-0.1 (-0.6 to 0.4)[a]	-0.3 (-0.8 to 0.3)[a]	-0.3 (-0.8 to 0.3)[a]	0.4 (-0.2 to 1.1)[b]	-0.3 (-1.0 to 0.4)[b]	-0.6 (-1.2 to 0.1)[b]
Perceived Stress Scale						
Sum in points	-0.09 (-0.5 to 0.3)[a]	-0.3 (-0.7 to 0.1)[a]	-0.3 (-0.7 to 0.2)[a]	**-0.7 (-1.2 to -0.2)[b] ***	-0.2 (-0.7 to 0.4)[b]	0.05 (-0.5 to 0.6)[b]
Social Readjustment Rating Scale						
Sum of impact scores	0.001 (-0.01 to 0.02)[c]	0.01 (-0.001 to 0.03)[c]	0.001 (-0.02 to 0.02)[c]	-0.02 (-0.04 to 0.001)[d]	-0.01 (-0.04 to 0.01)[d]	-0.01 (-0.03 to 0.01)[d]
Subjective stress related to events	0.1 (-0.2 to 0.4)[c]	0.2 (-0.07 to 0.5)[c]	0.06 (-0.2 to 0.3)[c]	**-0.3 (-0.7 to -0.02)[d] ***	-0.3 (-0.6 to 0.1)[d]	-0.1 (-0.5 to 0.2)[d]

[a] N=337; [b] N=219; [c]=332; [d]=214

*p≤0.05

Table 4 Association between prenatal stress exposure and child cognitive, language and motor development – multivariate model

	One year old children β (95% CI)			Two years old children β (95% CI)		
	Cognitive	Language	Motor	Cognitive	Language	Motor
Subjective Work Characteristics Questionnaire						
Sum in points	0.006 (-0.05 to 0.06)	-0.02 (-0.08 to 0.04)	-0.01 (-0.07 to 0.05)	-0.06 (-0.1 to 0.02)	-0.002 (-0.08 to 0.07)	0.04 (-0.04 to 0.1)
Number of psychosocial factors at work	0.01 (-0.2 to 0.2)	-0.04 (-0.2 to 0.09)	-0.05 (-0.2 to 0.08)	-0.01 (-0.3 to 0.3)	-0.03 (-0.2 to 0.1)	0.08 (-0.1 to 0.2)
APGAR Family Scale						
Sum in points	-0.6 (-1.5 to 0.3)	-0.4 (-0.9 to 0.2)	-0.3 (-0.9 to 0.3)	-1.4 (-3.3 to 0.4)	-0.3 (-1.1 to 0.4)	-0.6 (-1.3 to 0.2)
Perceived Stress Scale						
Sum in points	-0.2 (-0.6 to 0.2)	-0.02 (-0.9 to 0.8)	-0.01 (-0.8 to 0.8)	**-0.8 (-1.3 to -0.2)***	-0.3 (-0.6 to 0.05)	0.1 (-0.5 to 0.2)
Social Readjustment Rating Scale						
Sum of impact scores	-0.02 (-0.05 to 0.02)	0.001 (-0.02 to 0.02)	-0.003 (-0.02 to 0.02)	-0.03 (-0.08 to 0.03)	-0.01 (-0.04 to 0.02)	-0.02 (-0.04 to 0.01)
Subjective stress related to events	-0.3 (-0.9 to 0.3)	0.02 (-0.3 to 0.3)	0.03 (-0.3 to 0.3)	**-0.4 (-0.8 to -0.05)***	-0.2 (-0.6 to 0.2)	-0.2 (-0.6 to 0.2)

Model adjusted for: examiner, child gender, maternal age, maternal education, cotinine level in maternal saliva during pregnancy, cotinine level in child urine, child health status and for cognitive development additionally marital status, changes in marital status during the study period, and child day care attendance

*$p \leq 0.05$