

The Effect of Passive Smoking during Pregnancy on the Central Nervous System Structure and Behaviour of the Offspring

أثر التدخين السلبي خلال الحمل على تركيب الجهاز العصبي المركزي وسلوك المواليد

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Received: (10/2/2014), Accepted: (17/9/2014)

Abstract

Exposure to tobacco smoke during pregnancy is considered a major preventable risk factor for morbidity and mortality of the mother and the foetus. This study aims to investigate the effect of passive smoking during pregnancy on the structural integrity of the central nervous system (CNS) of the offspring, and to assess their neurobehavioral functions including short term memory, anxiety and social interactions. For this 35 pregnant hamsters were divided into 5 groups, based upon the pregnancy trimesters they were exposed to smoke in (during the 1st trimester or the 2nd and 3rd trimesters together) as well as the duration of exposure (4 or 6 hours). The 35 pregnant hamsters produced 78 offspring, which were evaluated at age 8 weeks for memory function by T-maze test, anxiety using marble burying test and social behaviour by social dominance test. They were also examined by CT scan subsequently for gross structural abnormalities in the CNS. The results demonstrated that there is a significant correlation between exposure to passive smoking during the 1st trimester and the presence of short-term memory impairment ($p=0.047$), as well as increasing anxiety level ($p=0.024$). Exposures during

the 2nd and 3rd trimesters were also associated with short-term memory dysfunction, with no effect on anxiety level. CT reports showed lumbar canal stenosis in a significant number of offspring who were exposed to 6 hours of cigarette smoke during the 1st trimester of pregnancy. Our results conclude that passive smoking during pregnancy is dangerous to the CNS development of the foetus as was evident by its effect on the behaviour of the offspring and structural integrity of their CNS.

Keywords: passive smoking, behavioral development, CNS integrity.

ملخص

يعتبر التدخين السلبي في فترة الحمل أهم المخاطر على صحة الأم وحياة الجنين التي يمكن تجنبها. تهدف هذه الدراسة إلى إبراز أثر التدخين السلبي خلال الحمل على تركيب الجهاز العصبي المركزي وسلوك المواليد بما في ذلك تقييم الذاكرة قصيرة المدى ومستوى التوتر ومدى التفاعل الاجتماعي. لقد تم تقسيم 35 أنثى من حيوان الهامستر إلى 5 مجموعات بناء على الفترة التي تعرضت لها للتدخين السلبي خلال فترة الحمل (خلال الثلث الأول أو خلال الثلثين الثاني والثالث معا) وكذلك بناء على مدة التعرض (4 ساعات أو 6 ساعات). نتج عن حمل هذه المجموعة 78 ابناً، وتم فحصهم على عمر 8 أسابيع لتقييم الذاكرة قصيرة المدى باستخدام (T-maze test) ومستوى التوتر باستخدام (marble burying test) ومدى التفاعل الاجتماعي باستخدام (social dominance test). وقد تم أيضاً فحص وجود التشوهات الخلقية العظمية في الجهاز العصبي المركزي باستخدام جهاز التصوير الطبقي. أظهرت النتائج وجود علاقة مهمة بين التدخين السلبي خلال الثلث الأول للحمل ووجود ضعف في الذاكرة قصيرة المدى ($p=0.047$) وكذلك مع الزيادة في نسبة التوتر ($p=0.024$). أما التعرض طوال الثلثين الثاني والثالث فقد كان له أثر مهم على الذاكرة قصيرة المدى دون التأثير على نسبة التوتر. أظهرت تقارير التصوير الطبقي للجهاز العصبي المركزي علاقة مهمة بين التدخين السلبي لمدة 6 ساعات خلال الثلث الأول للحمل وحدوث تضيق في قناة الحبل الشوكي القطنية. يستنتج من هذه النتائج أن التدخين السلبي خلال الحمل يشكل خطراً على تطور الجهاز العصبي للجنين، كما تبين من أثره على سلوك الأبناء وتركيب الجهاز العصبي المركزي.

الكلمات المفتاحية: التدخين السلبي، التطور السلوكي، سلامة الجهاز العصبي المركزي.

Introduction

Passive smoking is described as the inhalation of smoke exhaled by an active smoker within the same environment or proximity (mainstream smoke), and burns from the end of a

cigarette (side stream smoke) (WHO, 2005). The smoke itself contains over 4000 chemicals of which at least 250 are toxic including; tar, carbon monoxide, ammonia and cyanide that may result in respiratory, heart and disability complications leading to death (WHO, 2013).

Passive smoking during pregnancy is considered a major preventable risk factor for both pregnancy-related morbidity and perinatal complications including preterm delivery, low birth weight, intrauterine growth restriction, as well as sudden infant death syndrome (WHO, 2005). During fetal development, the nervous system is more susceptible to environmental toxicants, such as tobacco smoke, which is known to increase the risk of certain childhood tumors especially central nervous system (CNS) tumors, leukemia and lymphoma (Filippini *et al.*, 2000; Filippini *et al.*, 1994). The neural tube, an integral embryological part, is affected by passive smoking of the mother during pregnancy, leading to death, degeneration and cell loss, which can be detrimental to the development of the fetus (Papers, 2010b). Also, infants of mothers who were exposed to passive smoke are at a higher risk for neurodevelopmental delay (Statistics, 2009). A study showed that prenatal exposure to tobacco smoke has a neurotoxic effect on the newborn's neurobehavior (Law *et al.*, 2003). Another animal based study concluded that passive smoking during pregnancy has a teratogenic effect on various organs including the CNS by causing neural tube defect through its effects on the expression of apoptosis-associated genes (Papers, 2010a).

A literature search revealed a limited number of studies conducted to evaluate the effects of tobacco exposure during various trimesters of pregnancy on the CNS development (Gospe *et al.*, 2009).

According to the Palestinian Central Bureau of Statistics (PCBS), 22.5% of the Palestinian populations are smokers. About 37% of the smokers are males compared to 2.6% females (MoH, 2010). This suggests that pregnant women are likely to be exposed to passive smoking instead of being active smokers.

This research aims to study the effect of passive smoking exposure during different periods of pregnancy on the CNS structural integrity of the hamsters' offspring. Additionally, it aims to study the effect of this exposure on their neurobehavioral functions including short-term memory, the level of anxiety and social interaction.

1. Experimental Procedure

2.1 Confirming pregnancy and grouping

A non-equivalent quasi-experimental study was used. The study population consisted of dwarf hamsters type *Phodopus Campbelli*. 35 females and 10 males were prepared for mating. The date of detecting positive vaginal wash for sperms was considered to be the date of conception. Hamsters that were confirmed to be pregnant were arranged in study groups and placed in cages according to their conception date. The pregnant females were divided into five groups according to the period and dose of exposure as the following; group A1: Exposed in the 1st trimester for 4 hours per day, group A2: Exposed in the 1st trimester for 6 hours per day, group B1: Exposed in the 2nd and 3rd trimesters for 4 hours per day, group B2: Exposed in the 2nd and 3rd trimesters for 6 hours per day and group C: Not exposed to passive smoking.

2.2 Passive smoking exposure

The subjects of each group were transferred to the exposure room during the period designed for each one. The rate of smoking was one cigarette per 10 minutes with 5 minutes ventilation in smoke-free room before lighting the next cigarette. Since the gestation period for these hamsters is 21 days (Susan *et al.*, 2000); Smoking exposure for the 1st trimester was stopped 7 days after the date of conception, while exposure for the 2nd/3rd trimester was stopped after noticing the presence of offspring in each cage. The hamsters were exposed to both the mainstream and side stream smoke using a smoking machine.

2.3 Neurobehavioral Tests

Of the 78 offspring; 66 studies versus 12 controls, were included in the neurobehavioral tests. The offspring were allowed to grow up to the age of 8 weeks, which is the age of puberty, when the neurobehavioral tests were carried out.

2.4 Spontaneous Alteration Test (T-Maze Test)

Each subject was entered the T-maze twice daily, at least one hour in between, for five constitutive days. Each round measures if the hamster did a spontaneous alternation or not between the right and the left arms of the maze (Bublitz & Stroud, 2012; Lee *et al.*, 2011). After recording the results of spontaneous alternation, the percentage of alternation was calculated for each subject (Mallon *et al.*, 2008). The normal value of the alternation was the average alternation of the control group.

2.5 Marble Burying Test (MBT)

The test examined the presence of possible anxiety-related behavior of the rodents. The test consisted of 16 marbles placed evenly in 4 rows over 5-cm thickness layer of sawdust bed in squared shape box. The number of buried marbles was counted after leaving each hamster (age 9 weeks) in the box for 20 min (Lee *et al.*, 2011).

2.6 Social Dominance test:

The test consisted of a glassy rectangular tunnel that was divided into two 14 cm areas from both ends and a central 4 cm neutral area. At age 10 weeks, two hamsters in each test were chosen from study and control groups to be in the same age, gender and size. Each subject of the study groups was left against another one from the control group for 5 minutes. The frequency and duration of occupying the counterpart area were documented (Lee *et al.*, 2011).

2.7 Central Nervous System Structural Study

In the 11th week, the CT scans were performed for only the 69 remaining hamsters, rest of which have died before the procedure. All the hamsters were killed using high dose anaesthesia injected

intraperitoneally to assume rapid complete loss of consciousness (Susan V. Gibson & Alan G. Brady, 2000), which is needed for clear CT imaging. Two hours later, computed tomography CT scanning of the whole hamster was conducted in order to look for morphology of the brain and spinal cord. The hamsters were examined in the prone position by a 128-slice CT scanner (Somatom Sensation AS, Siemens) with field of view (FOV) 30-15 x 7 cm, slice thickness 5mm, reconstruction 1 mm. The images were evaluated for structural abnormalities findings of the central nervous system like brain atrophy, hydrocephalus, brain lesions, spinal cord anomalies and spinal canal stenosis.

2. RESULTS

2.1 Spontaneous alternation (T-maze Test)

Results of the T- maze test were considered normal when the average percentage of alternation for each subject was above 72.5%, which is the average alternation of the control group. Table (1) shows that the average alternation of each group and the number of normal and abnormal subjects. There is a significant correlation between passive smoke exposure during the 1st trimester of pregnancy and the presence of negative alternation compared with the control group ($p = 0.047$). There was also a significant relationship for those exposed during the 2nd and 3rd trimesters and the presence of negative alternation ($p=0.002$), without significant difference between different doses of exposure during each period of pregnancy.

Table (1): Results of spontaneous alternation of the study groups.

Group *	Average percentage of alternation	No. of subjects with normal test	No. of subjects with abnormal test
A1	60%	4	12
A2	60%	3	14
B1	51.4%	1	13
B2	55.3%	3	16
C	72.5%	6	6

Groups*: exposure to passive smoking; A1 exposed in the 1st trimester for 4 hours, A2 exposed in the 1st trimester for 6 hours, B1 exposed in the 2nd trimester for 4 hours, B2 exposed in the 2nd trimester for 6 hours, C not exposed to passive smoking.

3.2 Marble Burying Test (MBT)

Anxiety level of the experimental group was correlated with the control group, where the normal anxiety level was taken as 11.6, seen in table (2). There is a significant correlation between the exposure during the 1st trimester and the number of buried marbles ($p=0.024$), not affected by the dose. There is no significant association with exposure during the 2nd and 3rd trimesters and the number of buried marbles.

Table (2): Results of marble burying test of the study groups.

Group*	Average no. of Buried Marbles	No. of more anxious hamsters	No. of less anxious hamsters
A1	13.6	12	4
A2	14.1	15	2
B1	11.9	7	7
B2	11.5	8	5
C	11.6	7	5

Groups*: exposure to passive smoking; A1 exposed in the 1st trimester for 4 hours, A2 exposed in the 1st trimester for 6 hours, B1 exposed in the 2nd trimester for 4 hours, B2 exposed in the 2nd trimester for 6 hours, C not exposed to passive smoking.

3.3 Social Dominance Test

Table (3) demonstrates the number of dominant, subordinate and equivocal hamsters. There is no significant relation between the exposure to passive smoke during any trimester of pregnancy and the social dominancy results, regardless of the dose of exposure.

Table (3): Results of social dominance of the study groups.

Group[*]	No. of Dominant Hamsters	No. of Subordinate Hamsters	No. of Equivocal Hamsters
A1	6	7	2
A2	6	7	4
B1	5	3	5
B2	6	6	4
C	4	4	4

Groups^{*}: exposure to passive smoking; A1 exposed in the 1st trimester for 4 hours, A2 exposed in the 1st trimester for 6 hours, B1 exposed in the 2nd trimester for 4 hours, B2 exposed in the 2nd trimester for 6 hours, C not exposed to passive smoking.

3.4 CT Scanning

Brain scans of the offspring revealed no structural abnormality, including hydrocephalus, brain atrophy or tumours, induced by cigarette smoke. However, spinal canal stenosis was found in 9 out of the 13 hamsters who were exposed to 6 hours of passive smoking in the first trimester of pregnancy. This stenosis was mainly in the lumbar region of the canal, and often extending to the coccyx.

3. DISCUSSION

Focused neurobehavioral tests can be used to evaluate the particular functions controlled by individual areas of the brain.

3.1 Spontaneous alternation (T-maze test)

From the T-maze test results used to evaluate short-term memory in offspring exposed to passive smoke in the 1st trimester, we can deduce that such exposure leads to significant memory dysfunction. Similar exposure during both 2nd and the 3rd trimesters carries a greater degree of harm to memory compared to just exposure during the 1st trimester. Such impact on memory function are most likely to be due to the effect of nicotine on nicotinic receptors; alpha 4, beta 2 and alpha 7, located in the

ventral hippocampus and basolateral amygdale, all of which are involved in short term (working) memory function (MacLeod *et al.*, 2010). Little difference is noticed when comparing the nicotine effects on the 2nd versus the 3rd trimesters.

4.2 Marble burying test

Marble burying test is used for assessment of the level of anxiety in the animal subjects. This means that there is a significant association between passive smoking exposure during the 1st trimester of pregnancy and increasing anxiety behaviour of the offspring, which is indicated by burying more marbles compared to that of the control group. However, the exposure to passive smoking during the 2nd and 3rd trimesters together does not show a significant effect on the hamsters' anxiety behaviour. These results are in agreement with results of other studies on smoking effects which showed that smoking can cause alterations of anxiety level and mood by modulation of nicotinic acetylcholine receptors involved in stress response, anxiety and depression (Picciotto *et al.*, 2002).

4.3 Social dominance test

Social dominance test is a measure of social interactions of the rodents with their peers that can be used to measure the level of aggressiveness and violence. The results revealed no strong evidence concerning the effect of passive smoking during pregnancy on the dominance behaviour neither during the 1st trimester nor the 2nd and 3rd trimesters together.

4.4 CT scanning of the CNS

CT scan results didn't show any presence of gross anomalies, hydrocephalus, tumours or abnormal calcifications in the brain. However, by examination of spinal canal structure, which contains the spinal cord, stenosis was detected on the lumbar region, reflecting abnormal development of the spinal canal as a result of passive smoking exposure during the 1st trimester of pregnancy. Therefore, the structure of spinal

canal seems to be affected due to exposure to tobacco smoke at this stage of development.

4. Conclusion

The development of the central nervous system during embryological life can be affected in a negative way by passive smoking of the mother. This exposure during the 1st trimester can affect both the working memory function and the anxiety behaviour of the offspring. Concurrently, the same exposure during the 2nd and 3rd trimesters together can affect only the working memory. Spinal canal stenosis is associated with passive smoking during the 1st trimester.

Recommendations

According to the study results showing the negative effect of passive smoking during pregnancy on the nervous system development of the incoming offspring, we recommend conducting other studies to examine the effect of passive smoking exposure during pregnancy on other behavioral modalities of the incoming offspring such as cognition, learning, reward and punishment. We also recommend dissecting the central nervous system of the hamster's offspring after exposure to passive smoking during pregnancy in order to study the brain and spinal cord at tissue and cellular levels.

Acknowledgement

The authors would like to express their great regards and warm thanks to the medical research center (MRC) – University of Oxford – The United Kingdom of Great Britain, for their support and advices regarding animal lab work. Many thanks also to Rachel Loh for her help in English language review and advices in statistical analysis.

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