

The Role of Genetic Polymorphism of IL-4 (C-589T) and TNfa (G-308A) and Regular Passive Smoking in Clinical Manifestations of Pneumonia in Infants

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Received date: April 16, 2016; Accepted date: June 06, 2016; Published date: June 13, 2016

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Abstract

Objective: This study set out to investigate the relationship between molecular-genetic mechanisms that modulate cytokine in infants whose parents expose them to cigarette smoke and those that do not expose their infants to cigarette smoke with the possible effects on the severity of pneumonia at presentation to hospital.

Methods: This cross sectional study recruited infants and parents from a regional Children's Hospital. The infants were classified into three groups:

1. Where the infant was exposed to cigarette smoke through the mother directly (N=50).
2. The mother did not smoke but the infant was exposed to tobacco smoke from the relatives in the family (N=50).
3. The infant was in a family in which no one smoked. Analysis of genetic polymorphisms (regional frequencies) and determination of CRP and IgE concentrations were performed to investigate if exposure to cigarette smoke could be linked to the severity of pneumonia.

Results: Exposure to cigarette smoke in infants changed the likelihood of the severity of pneumonia. The results suggest a possible link between the duration of clinical manifestation of pneumonia within infants that are exposed to cigarette smoke. Infants who are exposed to cigarette smoke had higher plasma concentrations in both IgE and CRP. Among infants with the mutant variant of the TNF- α (G-308A) gene, infants from households where both parents smoked or had a father who smoked had longer duration of clinical signs of pneumonia than did infants with no exposure to tobacco smoke. The plasma concentration of IgE in infants in families where one or both parents smoke was significantly higher than in infants with no smoke exposure.

Conclusion: The study begins to develop evidence that if the newborn infant is exposed to tobacco smoke either directly from their mother or from others in the household including automobiles, the severity of pneumonia is increased. Both nurses and pediatricians should reinforce the consequences of smoke around the baby to all parents at every opportunity.

Keywords: Passive smoking; Mother smoking; Modulate cytokines; Infants; Pneumonia

Introduction

Tobacco smoking has a pronounced toxic effect on the human body. The increase in the prevalence of smoking among women is a human tragedy because smoking not only adversely affects the woman herself: if she smokes during pregnancy it also affects the health of future generations. Over the past 10 years in Ukraine, smoking prevalence among the female population has tripled, which leads to pathology in pregnancy and childbirth, and unassociated increase in perinatal mortality and morbidities in infants [1-4].

Globally, about 40% of children are exposed to the harmful effects of passive smoking. According to a survey conducted in Kiev, 49% of children suffer from exposure to tobacco smoke at home and 71% are

exposed to tobacco smoke in public places [5]. The presence of smokers in the family is a risk factor for intrauterine damage to fetal endothelium and, as a consequence, lung disease in the postnatal period of the child's development [6]. Secondhand exposure to smoke is also a very significant risk factor for sudden infant death syndrome [7,8].

A previous study investigated the probability of hospitalization for respiratory diseases in 4486 infants whose mothers smoked. Compared with the absolute risk of hospitalization for children whose parents had never smoked in rooms where the child was present, the absolute risk of hospitalization for children with a smoking parent present in the room was 56% higher than if never been exposed to tobacco smoke. If the mother smoked while holding the baby in her arms, the risk increased to 73% greater than the clean air infant. If the mother smoked while breastfeeding the baby, the risk of hospitalization increased to 95% [9-11].

The higher frequency of respiratory diseases in these children can, in part, be explained by the mucous membranes of the upper respiratory tract being most affected by the toxins in tobacco smoke [12,13]. The cytomorphological and functional integrity of these membranes an important element of the first line of defense against the effects of unfavorable environmental factors associated with exposure to tobacco smoke [14-16]. Tobacco smoke can trigger allergic sensitization that directly affects immunoglobulin-E at the cellular level and can increase the permeability of the epithelium of the airways, further reducing its function as a protective barrier [17-19].

Cytokines are important in the communication between the immune system and other cells involved in the immune response, and the cytokines C-reactive protein (CRP) and tumor necrosis factor (TNF- α) play a central role in the regulation of inflammatory responses [20,21]. Thus the concentration of these cytokines in the blood can indicate the functional activity of various types of immune cells, the severity of inflammation, its transition to a system level, and the prognosis [22]. Tobacco smoke is known to increase the content of inflammatory mediators in endothelial cells. Blood levels of C-reactive protein, TNF- α , anti-inflammatory cytokine (IL-4), and inflammatory cytokines IL-6 and IL-8 are also increased [23]. These changes in cytokine concentrations may increase the risk and severity of pneumonia. Thus young children exposed to tobacco smoke may be at increased risk of severe pneumonia.

The molecular-genetic mechanisms that modulate cytokine status have not been studied in infant passive smokers with pneumonia of various severities. Specifically, the relationship between polymorphisms of the genes for cytokine IL-4 (C-589T) and TNF- α (G-308A) and severe pneumonia in infant passive smokers is unknown. The IL-4 gene is involved in the immune response: it plays an important role in the interaction of cellular and humoral immune factors and inflammatory reactions. The most important functional polymorphism of the IL-4 gene is C-589T. This gene polymorphism is the most studied the most common and the most informative.

A human may have following variants of IL-4 (C-589T): C/C – the normal variant in homozygous form; C/T – the heterozygous variant; and T/T – the mutated variant, which is associated with increased risk of disease in the homozygous form. The international databases HuGEN and Gene Cards provide information on the various polymorphisms of the TNF- α gene and their possible association with inflammatory autoimmune diseases and malignant and benign tumors. The most widespread polymorphism of the TNF- α gene is G308–A. human may have following variants of TNF- α (G-308A): G/G – the normal variant in homozygous form; G/A – the heterozygous variant; and A/A – the mutated variant.

Objectives

The purpose of the study was to investigate in a cross sectional manner the role of genetic polymorphism of IL-4 (C-589T) and TNF- α (G-308A) and regular passive smoking in clinical manifestations of pneumonia in infants. Specifically, we sought to (i) determine the frequency of polymorphism in two genetic determinants of the inflammatory response [IL-4 (C-589T) and TNF- α (G-308A)] among a population of infants with pneumonia, and (ii) investigate the effects of passive smoking on the severity of pneumonia in these infants in the context of the relationship with both the genetic variants of IL-4 (C-589T) and TNF- α (G-308A) and plasma levels of IgE and CRP.

Methods

The project was approved by the Ethics Committee of the Odessa Regional Children's Clinical Hospital. Children of the 1st year of life: from 28 days to 1 year (150) with pneumonia of various severities were recruited representing 76.5% of all infants with pneumonia admitted to the pediatric department of the hospital. Women were eligible if they could read the informed consent, agreed to participate, were willing to let the investigators to take blood from the infant for the purpose of the study, and allow investigators to collect recorded data from the mother and infants medical records. The severity of pneumonia was classified according to the system adopted by the 12th Congress of Pediatricians of Ukraine.

The infants in this cross sectional study were categorized into three groups:

1. Infant passive smokers (50) whose mothers smoked.
2. Infant passive smokers (50) whose mothers did not smoke, but who were exposed to tobacco smoke from other relatives in the family.
3. Infants (50) in families in which no-one smoked.

To determine the level of IgE and CRP were used a biomaterial - blood serum. Levels of IgE and CRP have been carried out by immune turbidimetric method using the analyzer Cobas 6000. To determine gene polymorphism were used a biomaterial - buccal scraping. Molecular genetic studies have been carried out by polymerase chain reaction. Analysis of genetic polymorphisms (regional frequencies) and determination of CRP and IgE concentrations were performed in the German research center HERMEDTEH in Odessa, Ukraine.

Statistical Analysis

The data were processed using statistical software (STATISTICA 7.0) [24,25] and the statistical component of Microsoft Excel TM 2003; with the integration program Atte Stat 13.5, Internet-calculator SISA (Simple Interactive Statistical Analysis). Descriptive statistics (mean, 95% confidence intervals) are presented for the frequencies of genetic variants. Duration of clinical manifestations of pneumonia are presented as mean \pm standard error of mean (SEM). In all procedures when testing the null hypotheses, the critical level of significance (P) was set at 0.05. Three methods were used to check for normal distribution: graphics, Kolmogorov-Smirnov tests and Shapiro-Wilk tests. The relationship between pairs of discrete quantitative variables as examined by analysis of paired contingency tables, where the estimated values of the statistic Pearson's chi-square (χ^2) achieved the level of significance (P) and odds ratio (OR) with 95% Confidence intervals.

Results

We have studied the regional frequency of gene polymorphisms IL-4 (C-589T) and TNF- α (G-308A) among 150 infants who were hospitalized to the pediatric department of the Odessa Regional Children's Hospital (N/150).

The frequency of IL-4 (C-589T) gene variants among infants differed significantly (Table 1). The heterozygous variant (C/T) was more frequent than the mutant variant (T/T) (OR=2.98, 95% CI 1.78-4.98). The normal variant (C/C) was also more frequent than the mutant variant (T/T) (OR=2.38, 95% CI 1.42-4.01).

| IL-4(C-589T) Polymorphism | Gene | The detection rate in the sample |
|---|------|-------------------------------------|
| | | n=150 (n/%) (95%CI) |
| C/C | | 56/37 ^a (22.36-51.15) |
| C/T | | 64/43 ^a (31.34-55.15) |
| T/T | | 30/20 (10.18-34.4) |
| ^a Significant different in groups (p<0.05) | | |

Table 1: Distribution of infants depends on different variants of IL-4 (C-589T) gene polymorphism.

| TNF-α (G-308A) gene polymorphism | The detection rate in the sample |
|---|-------------------------------------|
| | n=150 (n/%) (95%CI) |
| G/G | 62/41 ^a (28.93-51.6) |
| G/A | 74/49 ^a (38.14-59.12) |
| A/A | 14/9 (3.79-21.18) |
| ^a Significant different in groups (p<0.05) | |

Table 2: Distribution of infants depends on different variants of TNF-α (G-308A) gene polymorphism.

The frequency of TNF-α (G-308A) gene variants among infants also differed significantly (Table 2). The normal variant (G/G) was more frequent than the mutant variant (A/A)(OR=6.84, 95 % CI 3.61-12.97). The heterozygous variant (G/A) was also more frequent than the mutant variant (A/A)(OR=9.46, 95% CI 5.01-17.88).

The average duration of clinical manifestations of pneumonia in infants with the normal and heterozygous variants of the IL-4 (C-589T) gene was longer in infants in families where one or both parents smoked than in infants with no smoke exposure (Table 3). Similarly, the average duration of clinical manifestations of pneumonia in infants with the normal and heterozygous variants of the TNF-α (G-308A) gene was longer in infants in families where one or more parents smoked than in infants in families with no smoke exposure (Table 4). In infants with the mutant variant of the IL-4 (C-589T) gene, there were no significant differences in duration of clinical signs between infant passive smokers and infants with no smoke exposure (Table 3). However, among infants with the mutant variant of the TNF-α (G-308A) gene, infants from households where both parents smoked or had a father who smoked had longer duration of clinical signs than did infants with no exposure (Table 4).

| IL-4(C-589T) gene polymorphism | The duration of clinical manifestations of pneumonia | | |
|---|--|--------------------------------|--------------------|
| | Both parents are smokers (n=50) | Only father is a smoker (n=50) | No exposure (n=50) |
| | Mean ± SEM | Mean ± SEM | Mean ± SEM |
| C/C | 15.46 ± 2.12 ^a | 12.23 ± 1.87 ^a | 7.27 ± 0.72 |
| C/T | 20.21 ± 3.25 ^a | 16.07 ± 2.36 ^a | 10.04 ± 1.22 |
| T/T | 24.04 ± 2.56 | 22.36 ± 2.01 | 20.07 ± 1.73 |
| ^a Significant different in groups (p<0.05) | | | |

Table 3: The average duration of clinical manifestations of pneumonia in infants, depending on degree of cigarette smoke exposure in the family and identified IL-4 (C-589T) gene polymorphisms.

| TNF-α (G-308A) gene polymorphism | The duration of clinical manifestations of pneumonia | | |
|---|--|-------------------------------|--------------------|
| | Both parents are smokers (n=50) | Only father is a smoker(n=50) | No exposure (n=50) |
| | Mean ± SEM | Mean ± SEM | Mean ± SEM |
| G/G | 14.25 ± 2.32 ^a | 13.12 ± 1.37 ^a | 8.32 ± 0.64 |
| G/A | 20.31 ± 3.46 ^a | 17.08 ± 3.12 ^a | 11.12 ± 1.43 |
| A/A | 25.13 ± 3.57 ^a | 19.48 ± 1.89 | 17.59 ± 1.84 |
| ^a Significant different in groups (p<0.05) | | | |

Table 4: The average duration of clinical manifestations of pneumonia in infants, depending on degree of cigarette smoke exposure in the family and identified TNF-α (G-308A) gene polymorphisms.

The plasma concentration of IgE in infants in families where one or both parents smoke was significantly higher than in infants with no smoke exposure. The concentration of CRP in infants in families where both parents smoked was higher than that of both infants in families where only the father smoked and infants with no smoke exposure (Table 5).

Discussion

This study provides an analysis of the frequency of IL-4 (C-589T) and TNF-α (G-308A) gene polymorphisms, and of their association with clinical manifestations of pneumonia, among infants in the Odessa region. The findings show that the severity of pneumonia in infants with normal and heterozygous forms of both the IL-4 (C-589T) and TNF-α (G-308A) genes are of longer duration in the infants if the

infant is exposed to tobacco smoke either directly from the mother or in the presents of other smoking. The differences were not as marked among infants with the mutant forms of both genes. The results also suggest a possible link between the duration of clinical manifestations of pneumonia (within smoking categories) and the presence of the mutant form of both genes although this was not statistically demonstrated in the study.

| Parameter | Smoking status | | |
|---|---------------------------------|-------------------------------|--------------------|
| | Both parents are smokers (n=50) | Only father is a smoker(n=50) | No exposure (n=50) |
| | Mean ± SEM | Mean ± SEM | Mean ± SEM |
| IgE (ME/ml) | 21.05 ± 2.36 ^a | 16.04 ± 1.26 ^a | 13.42 ± 0.28 |
| CRP(mg/l) | 2.62 ± 0.87 ^a | 1.24 ± 0.42 | 0.31 ± 0.25 |
| ^a Significant different in groups (p<0.05) | | | |

Table 5: Levels of IgE and CRP in relation to degree of cigarette smoke exposure (Mean ± SEM).

In addition, the results showed that infant passive smoking is associated with higher plasma concentrations of both IgE and CRP. This was most marked in infants whose parents both smoked and lead to a more severe course of pneumonia.

The observed frequencies of the variants of the IL-4 (C-589T) and TNF-α (G-308A) genes in this population of infants with respiratory diseases corresponds to those found in other studies [1,7].

New data on the effects of passive smoking and efficiency of predicting the severity of acute pneumonia in infants who are exposed to passive smokers can improve the results of diagnostics, treatment and prevention of respiratory diseases in children. If the clinician knows the infant is exposed to tobacco smoke in the home, a most aggressive treatment may be the better course of action. The severity of pneumonia will be greater for infants coming from households where tobacco smoke is in the environment.

This study provides a strong case that all parents at the birth of their child should be made aware of the direct consequences of their smoking to their new baby. Both nurses and pediatricians should reinforce this information of the consequences of smoke around the baby to the parents on every visit to a clinic for well-baby checkups. It is the duty of health care practitioners to encourage mothers to quit smoking during pregnancy and to encourage them to remain non-smoking for the health of their infants. This is currently not the practice of doctors [26]. Further pediatricians in training should be provided a greater education on the harmful effects of tobacco smoking to the fetus and new born infant. This additional training should include how to discuss and plan a smoke free environment for the infant with their patients.

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