

A matched patient-sibling study on the usage of paracetamol and the subsequent development of allergy and asthma

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A number of studies have suggested that intake of paracetamol during pregnancy and during the first months of life is associated with an increased risk of childhood asthma. We aimed to determine the association between paracetamol usage during pregnancy and the first 6 months of life, and childhood allergy (i.e. positive skin prick tests), allergic asthma, and asthma, using a matched patient-sibling study comparing patients with allergic asthma with their healthy siblings without any symptoms of allergic diseases. Allergy in patients and their siblings was determined by skin prick tests. Children having at least one positive skin prick test were considered to be allergic. Intake of paracetamol was assessed by standardized, interviewer-administered, questionnaire. Nineteen pairs of allergic asthma patients vs. non-allergic siblings were compared to determine the risk factors for allergic asthma, while 15 pairs of allergic asthma patients vs. allergic siblings were compared to determine the risk factors for asthma. Moreover, 33 pairs of allergic asthma patients vs. non-asthmatic siblings (with and without allergy) were compared to determine the risk factors for asthma. In addition, 17 allergic siblings (without asthma) were compared with 19 non-allergic siblings (without asthma) to determine the risk factors for allergy. Usage of paracetamol during pregnancy was associated with allergic asthma ($p = 0.03$). Furthermore, usage of paracetamol between birth and 6 months of age, and between 4 and 6 months of age, was also found to be associated with non-allergic asthma ($p = 0.008$ and $p = 0.03$ respectively). Usage of paracetamol during pregnancy and during the early months of life may play a role in the development of allergic and non-allergic asthma in children. However, due to obvious ethical reasons, direct evidence for this association (i.e. a double-blind, prospective study) is not available.

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The prevalence of childhood asthma has been on the rise in many countries in recent years (1). Singapore is no exception, where the prevalence of doctor-diagnosed asthma is 20%, and the prevalence of reported symptoms of asthma is 31% (2). The rise in prevalence of asthma is unlikely to be due to genetic changes despite genetic factors being the major risk for asthma, and hence environmental factors are likely to play a major role. Many possible environmental

risk factors for asthma have been identified so far (i.e. summarized in the so-called 'Hygiene Hypothesis'), one of which is the usage of paracetamol during pregnancy and during early life (3, 4).

In this study we aimed to investigate the association between paracetamol use and childhood asthma/allergy by using a matched patient-sibling study design, in which a child with allergic asthma (case) was compared to his/her sibling without asthma, rhinitis and eczema (control).

Sibling pairs were preferentially selected in order to control for hereditary, socio-economic, familial and social environmental factors, which may be unobserved but relevant attributes in the association between usage of paracetamol and asthma/allergy. Only gestation and the first 6 months of life were studied, as it is now accepted that these periods are critical for alterations of the immune system. Furthermore, during these periods there is also rapid lung development, making the lungs more prone to environmental insults.

Methodology

Cases and controls

Cases were recruited from the paediatric outpatient clinics and the 'I CAN' (The Children's Asthma and Allergy Network) programme at the National University Hospital (NUH), Singapore. 'I CAN' is a holistic programme set up in 2002, to improve asthma and allergy management of patients through support and education. Recruitment of cases and controls took place between October 2005 and February 2006.

Recruitment process. In outpatient clinics, patients were eligible only if they fulfilled the following criteria (Fig. 1):

- Had active asthma (defined as having at least one asthma attack and/or using asthma medication in the past 1 yr)
- Had doctor-diagnosed asthma (defined as having had at least three wheezing episodes)

- Between 3 and 10 yr of age
- Had a healthy sibling

The siblings were eligible only if they fulfilled the following criteria:

- Between 3 and 10 yr of age
- No symptoms of asthma, wheezing, rhinitis, eczema, urticaria and angioedema either at present or in the past.

The health status of the sibling was determined by the following questions to the parent:

- 'Did your child ever had asthma or wheezing since birth?'
- 'Did your child ever had rhinitis, or often have sneezing, blocked or runny nose, especially in the mornings?'
- 'Did your child ever had eczema, or sensitive skin?'
- 'Did your child ever developed urticaria and/or angioedema?'

Asthma patients who took part in this research study were the cases and their siblings were their matched controls.

Telephone calls were also made to the parents of 278 asthmatic patients who were enrolled in the 'I CAN' programme and who were between 3 and 10 yr of age. Similarly, patients were eligible only if they had active asthma, and had a healthy sibling between 3 and 10 yr of age.

Parental consent was compulsory for the participation of the cases and controls in this

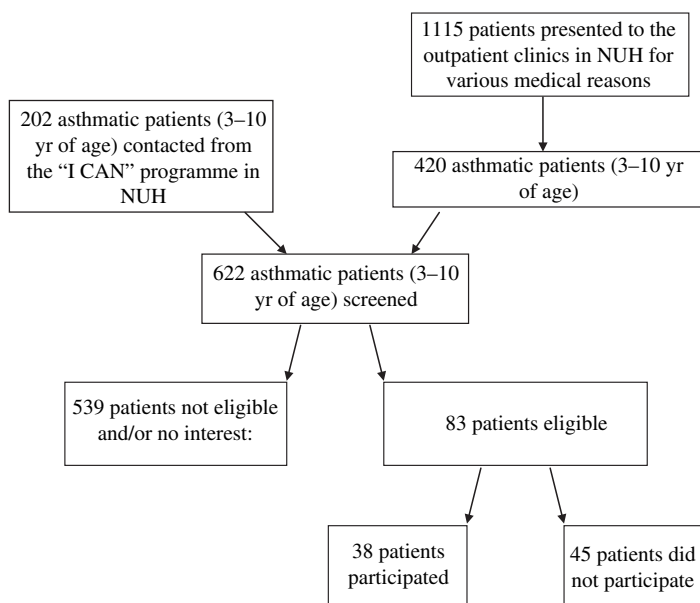


Fig. 1. The recruitment outcome of the cases and controls from the 'I CAN' programme and the outpatient clinics of NUH.

study, and the parents had to sign a written informed consent form.

Recruitment outcome. A total of 622 asthmatic patients who were between 3 and 10 yr of age were screened from the 'I CAN' programme and the paediatric outpatient clinics of NUH, out of which 520 patients (83.6%) were ineligible because (i) the patients had no sibling (ii) their siblings were not between 3 and 10 yr of age, (iii) their siblings had symptoms of atopic disease(s), (iv) the patients had inactive asthma. The parents of 19 patients, whose eligibility status was unknown, were not interested to hear about the study (3.1%). Only 83 patients (13.3%) were eligible, out of which 38 participated (Fig. 1). The main reasons for not participating were because the parents had no free time, were not interested, or had their misgivings about the procedure (skin prick tests).

Although 38 pairs of patient-sibling participated, an extra sibling was recruited for each of the four patients. This was to maximize the chance of finding a non-atopic sibling (i.e. as determined by skin prick tests), as this was the optimal control for comparison. Hence, a total of 38 patients and 42 siblings participated.

Skin prick testing

Both the cases and controls were subjected to skin prick testing. The allergens tested were *Dermatophagoides pteronyssinus* [house dust mites (HDM)], *Dermatophagoides farinae* (HDM), *Blomia tropicalis* (HDM), *Periplaneta Americana* (cockroach), *Blattella germanica* (cockroach), *Felis domesticus* (cat), *Canis famil-*

iaris (dog), egg white, cow's milk, soy and peanut. Histamine chloride and saline were used as positive and negative controls, respectively. Each of these was applied on the forearms and pricked with a lancet. Twenty minutes after the initial prick of histamine positive control, the weal reactions were measured. A weal diameter, taken as the mean of the length of the longest diameter and the length of its perpendicular diameter, was considered positive if it was 3 mm or more, both in the presence of a weal reaction of 3 mm or more to the histamine positive control and after the subtraction of the weal reaction to the saline negative control.

Cases who had a positive skin test response to any of the 11 allergens tested were considered as having allergic asthma. In contrast, non-allergic asthma was diagnosed if the cases were tested negative for all the allergens. Controls who had positive skin test response to any of the 11 allergens tested were considered as being allergic, those with negative skin prick tests for all allergens were considered as being non-allergic.

After skin prick testing on 38 cases and 42 controls, five cases and six controls were excluded because the cases had non-allergic asthma. The remaining cases who had allergic asthma were separated into separate groups (Fig. 2). The comparison of cases and controls, and the outcome variable of interest is shown in Table 1. We defined the outcomes of interest as follow:

1. Allergic asthma: having asthma symptom (history of ≥ 3 wheezing episodes) and positive skin prick tests.

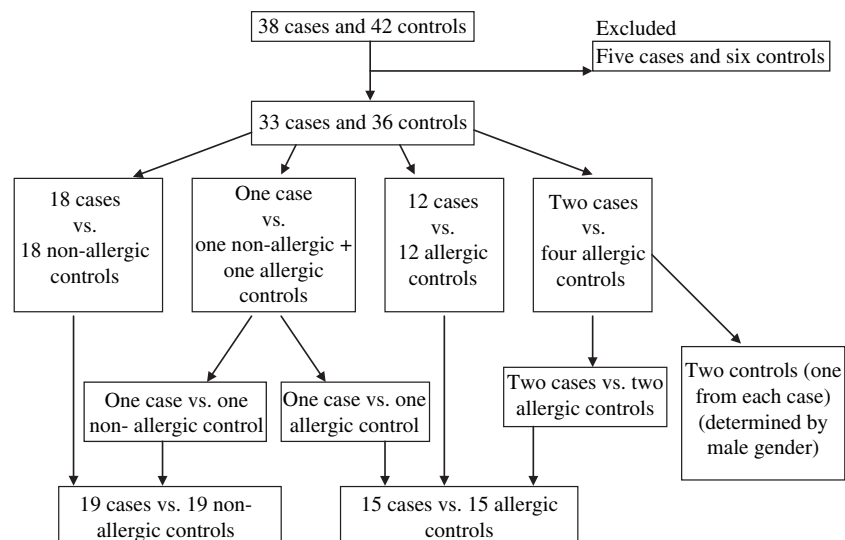


Fig. 2. The grouping of cases and controls according to skin prick tests.

Table 1. A summary of comparison between cases and controls in this study

No.	No. pairs	Cases	Controls	Outcome of interest
(i)	19	Allergic asthma	Non-allergic	Allergic asthma
(ii)	15	Allergic asthma	Allergic	Asthma
(iii)	33	Allergic asthma	Non-allergic and allergic	Asthma
(iv)	17	Allergic, non-asthmatic siblings	19 Non-allergic, non-asthmatic siblings	Allergy

(i), (ii) Refer to Fig. 2.

(iii) As the cases can only be matched once with their respective controls, there can only be 33 pairs of cases and controls.

(iv) Initially allergic and non-allergic controls, they are now compared with each other. Instead of 15 allergic non-asthmatic siblings, we now have 17 because the two allergic controls who were initially excluded are now included.

2. Asthma: having asthma symptom (history of ≥ 3 wheezing episodes), regardless of skin prick tests.
3. Allergy: sensitization to allergen(s) (positive skin prick tests) in the absence of any symptoms of asthma, rhinitis and eczema. It is used interchangeably with 'atopy'.

Questionnaire

Paracetamol intakes of the child in the first 6 months of life, of the mother during pregnancy and of the mother during the first 6 months of breastfeeding were assessed. If the response was positive for paracetamol intake, details regarding period of intake, the frequency, duration of intake, daily quantity (ml/day or no of tablets/day), daily dose and indications of paracetamol intake were asked. Similar questions were asked on the intakes of antibiotics during the first 6 months of life, of the mother during pregnancy and of the mother during the first 6 months of breastfeeding. The questionnaire was interviewer-administered to the mother of cases and controls and was based on parental report.

Ethics

The local ethical committee of National University of Singapore, National Healthcare Group DSRB, approved the study.

Sample size

We postulated that a difference of 35% between the asthmatics and their matched siblings on any risk factor would be deemed as clinically relevant (5). A sample size of 20 pairs would be sufficient to have a power of 80% with a two-sided test of 5% to achieve a significant result.

Statistical analysis

Statistical analysis was carried out using SPSS (Chicago, IL, USA) 13.0. The paired statistic McNemar's test, was used to assess the associations between pairs of cases (asthma patients) and their matched siblings (allergic and/or non-allergic siblings). Associations between allergic non-asthmatic siblings and non-allergic non-asthmatic siblings were performed using chi-squared test or Fisher's exact test as appropriate, and Odds Ratio (OR) and their respective 95% Confidence Intervals (CI) were also calculated. A p-value < 0.05 was considered as statistically significant.

Results

Table 2 lists the demographic data of the cases and controls in the study.

A number of questions that were asked during the interview were not analysed due to low response, mainly as a result of poor recall. Responses of 'not sure' and 'not applicable' for each question were removed for more accurate analysis. All results that showed statistical significance (p-value < 0.05) are reported (Table 3).

There were no differences in intake of antibiotics between cases and controls for any of the outcomes (i.e. non-allergic asthma, allergic asthma and allergy) (results not shown). For paracetamol intake the results were the following.

Allergic asthma cases vs. non-allergic controls (healthy siblings)

There were 19 pairs of cases and controls in this group, and they were compared to determine the potential risk factors for allergic asthma. More cases than controls had their mothers taking

Table 2. Demographic data on 33 allergic asthma cases and 36 non-asthma controls (allergic and non-allergic siblings) in the study

	Cases n (%)	Controls n (%)
Age (yr)		
3–6	11 (33)	22 (61)
7–10	22 (67)	14 (39)
Gender		
Male	21 (64)	17 (47)
Female	12 (36)	19 (53)
Age of clinical diagnosis of asthma (yr)		
<3	11 (33)	NA
3–6	15 (46)	NA
7–10	6 (18)	NA
Unknown	1 (3)	NA
Birth order		
1st	13 (39)	9 (25)
2nd	11 (33)	12 (33)
3rd, 4th, 5th	9 (27)	15 (42)

Table 3. A summary of significant findings in the study

Risk factors	Comparison pair	Outcome of interest	n	p-value	Differences between cases & controls		
					Responses	n	%
Paracetamol during pregnancy	Allergic asthma cases vs. non-allergic controls (healthy siblings)	Allergic asthma	19	0.03	Yes/no	6/17 vs. 0/17	35 vs. 0
Paracetamol in first 6 months of life	Allergic asthma cases vs. non-asthma controls (allergic & non-allergic siblings)	Asthma	33	0.008	Yes/no	8/28 vs. 0/28	29 vs. 0

paracetamol during pregnancy (6/17 (35%) vs. 0/17 (0%), $p = 0.03$).

Allergic asthma cases vs. non-asthma controls (Allergic and non-allergic siblings)

There were 33 pairs of cases and controls in this group. They were compared to determine the potential risk factors for asthma. Any paracetamol intake between birth and 6 months of age (8/28 (29%) vs. 0/28 (0%), $p = 0.008$) was associated with asthma in the child. We also calculated the frequency of paracetamol indication in the cases and controls, which is summarized in Table 4.

Discussion

In this study, a positive association between paracetamol consumption during pregnancy and subsequent allergic asthma was found, which is compatible with findings of Shaheen et al. (3, 4), who found that paracetamol consumption during pregnancy was associated with asthma and elevated serum IgE antibodies, although they found that the effects of paracetamol in late pregnancy (20–32 wk gestation) were more crucial than in early pregnancy (< 18–20 wk gestation). We also found a positive association between paracetamol consumption in the first 6 months of life and asthma. To our knowledge, there are only two studies on early life paracetamol consumption (first year and first 6 months, respectively), and both, too, found an increased risk for childhood asthma (4, 6). Our above findings are also in line with those of an ecological study, which found an increase of

0.52% in the prevalence of wheeze in children for each gram per capita increase in paracetamol sales (7).

However, from the different studies it seems that the evidence for this association is indirect, and therefore, conclusions are not strict forward and should be drawn with great caution. On the other hand, it will be impossible to obtain direct evidence (i.e. a prospective double-blind controlled study) due to obvious ethical reasons. Two possible associations can be put forward for the association between paracetamol intake and the development of allergic and non-allergic asthma: a causal link and reverse causation.

Causal link

There are many possible mechanisms to explain the causal link between paracetamol and asthma. The most common hypothesis is related to the imbalance of oxidant/antioxidant equilibrium. In the process of elimination, paracetamol gets oxidized to form *N*-acetyl-*p*-benzoquinoneimine (NABQI), a highly reactive metabolite which is neutralized by the antioxidant glutathione in the liver (8, 9). In animal studies, it was found that paracetamol also depleted pulmonary glutathione (10), resulting in increased susceptibility to oxidative damage, inflammation and bronchospasm, as seen in asthma (11). Paracetamol can also cross the placenta during pregnancy and is oxidized by the fetal liver to generate NAPQI (12).

Decreased levels of glutathione may also alter antigen presentation and recognition, thereby favouring a Th2 immune response (13). Another hypothesized mechanism is the lack of suppression of cyclooxygenase (COX) by paracetamol. COX-2 expression is upregulated during inflammation (e.g. childhood viral respiratory infections) and is involved in the production of prostaglandin E2 (PGE2), which in turn favours Th2 immune responses and inhibits Th1 immune responses (14).

By suppressing fever, paracetamol also lowers the patient's immune response, resulting in an increased viral load and a prolonged viral shedding (15). The increase in viral load has been

Table 4. Frequency of paracetamol indication in the cases and controls

	Cases (n = 23)	Controls (n = 18)	Cases + controls (n = 41)
Post-vaccination fever (%)	12 (52)	12 (67)	24 (59)
Infection-related fever (%)	7 (30)	3 (17)	10 (24)
Fever due to vaccination + infection (%)	4 (17)	3 (17)	7 (17)

suggested to provoke asthma (16), given that viral infections are common triggers of asthma in children.

It is also believed that avoidance of aspirin may explain the association between paracetamol use and asthma. As some asthmatics are sensitive to aspirin (17, 18), they may tend to avoid aspirin and use paracetamol instead (11). Alternatively, paracetamol user groups may contain asthmatics who are not only sensitive to aspirin but are also cross-sensitive to paracetamol (19).

Reverse causation

Although in our study the indication of paracetamol intake was asked to the parents, this was not analysed and controlled due to the poor response and small sample size. Migraines and viral respiratory tract infections have been thought to be more common in asthmatic mothers, which can lead to an increase in paracetamol use. However, Shaheen et al. (4) found that the association was still present even after controlling for these potential confounders.

It should also be considered that it is possible that infants with an atopic tendency may have increased susceptibility to acquire viral and/or bacterial respiratory infections early in life and hence are more likely to be treated with paracetamol. Although the latter is a plausible explanation, the majority of the children interviewed in this study actually took paracetamol for post-vaccination fever, hence reverse causation cannot entirely account for our findings.

Trends

Although we found only two significant findings as mentioned above, we also noticed some trends in our study (Table 5). Associations which were statistically insignificant (p -value > 0.05) but with differences between the cases and controls were reported as trends, because the differences between the cases and controls might be of clinical interest and/or significance. Also, we did

not want to exclude weak effects, which might have been obscured by the small sample size. However, further studies are needed to verify these trends.

Limitations of this study

The main weakness of our study is its small sample size. However, it is worth noting that in order to reach this sample size, we had to screen more than 600 patients, most of who were ineligible. Also, as the study population was selected from the asthma and allergy clinics, caution is required before relating these results to the general population.

Due to the retrospective nature of this study, recall bias may result in incomplete and incorrect reporting of exposures. However, pregnancy and the first 6 months of life of the child are generally lived as important periods in the mother's life, and hence this may reduce the likelihood of the mother forgetting about trivial events in these periods. Nevertheless, as this is particularly true for parents of 'sick' children, recall bias cannot be completely excluded.

There is also a possibility that healthy siblings, irrespective of their allergy status, may develop allergic disease later in life. If these siblings are compared with their matched asthma cases, it may result in a bias toward the null. However, most asthmatic children will usually have manifested their first episode of wheezing by the age of 3 yr according to the literature (20).

Strengths of this study

By matching an asthma case and a healthy control from the same family, we were able to control for hereditary, socio-economic, social and familial environments, which may be relevant factors in the association between paracetamol use and asthma/allergy. Even then, these relevant factors are not entirely identical between the two siblings (case and control), and hence the

Table 5. A summary of trends in the study

Risk factors	Comparison pair	Outcome of interest	n	p-value	Differences between cases & controls		
					Responses	n	%
Paracetamol in first 6 months of life	Allergic asthma cases vs. non-allergic controls (healthy siblings)	Allergic asthma	19	0.06	Yes/no	5/15 vs. 0/15	33 vs. 0
Paracetamol in first 6 months of life	Allergic asthma cases vs. allergic controls (allergic siblings)	Asthma	15	0.25	Yes/no	3/14 vs. 0/14	21 vs. 0
Paracetamol during pregnancy	Allergic asthma cases vs. non-asthma controls (allergic & non-allergic siblings)	Asthma	33	0.13	Yes/no	6/26 vs. 1/26	23 vs. 4

design of our study can only minimize these differences as much as possible.

Conclusion

It is concluded that there might be an association between usage of paracetamol, both during pregnancy and during the early months of life, and asthma. Although the association seems to be positive, it remains, however, uncertain whether this is due to a causal role or to reverse causation.

On the other hand, it is emphasized that the study has a number of potential limitations. Therefore, results require cautious interpretation and should only be considered suggestive of the association. At this point in time, it is too early to draw any conclusions on the exact nature of the association and further research will have to elucidate the exact nature of the association. An ideal research design would be a large prospective study of matched identical twins from birth. This will allow an accurate assessment of the various environmental risk exposures in the early years of life of the child, and at the same time will allow the control for genetic factors, gestational factors and other possible childhood exposures such as diet and allergen exposures.

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