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*Full Length Research Paper*

# Association between Folate Deficiency and Asthma, Severity and Exacerbations in a sample of Egyptian Children

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***Abstract***

***Background:*** *Childhood asthma increased globally till it reach an epidemic state. Different pathophysiologic mechanisms were proposed. However, the role of folic acid did not extensively be investigated.* ***Aim of the work:*** *The current work was designed to check the association between folate deficiency and childhood asthma in a sample of Egyptian children.* ***Methodology:*** *Children aged 6 to 14 years, who have a new or old diagnosis of asthma were included. We screened 3800 children. Asthma identified among 196 children (5.16%). However, 171 of them were included in final analysis, and 196 healthy children were included as a control group. Patient demographics, general health, family history, and exposure to environmental toxins were collected, and blood samples were drawn to assess serum levels of folate. Spirometry was performed to assess the pulmonary function.* ***Results:*** *There was a significant increase of smoke exposure in the study than control group (46.8% vs 19.9%) and in parents' history of asthma (63.7% vs 15.8%), while there was a significant reduction of the serum levels of folate in the study than control group (18.97±5.65 vs 22.28±6.50 ng/ml, respectively). However, there was increased non-significant number of folate deficient children in the study than the control group (42.5% vs 35.7%). FEV1% predicted, FVC% predicted and FEV1/FVC% were significantly reduced in study than control group. Children with asthma and folate deficiency were significantly older in age (10.78±1.22 vs 9.00±1.10 years, respectively). Folate deficiency was associated with female gender and reduced pulmonary function tests, and asthma exacerbation was significantly higher among children with folate deficiency than those without folate deficiency. Finally, there was negative significant moderate correlation between folate deficiency and child age. However, the correlation between folate and pulmonary function tests (FEV1, FVC and FEV1/FVC) was proportional.* ***Conclusion:*** *Reduced folate is associated with asthma, asthma severity and exacerbations in children.*

***Keywords:*** *Bronchial Asthma; Children; Folic Acid; Exacerbations; Severe*

## Introduction

Asthma has been recognized as a broad term comprising several distinct disease conditions of multifactorial etiology. It is a result of complex interactions between environmental allergens, genetic and behavioral factors including diet. The hallmark characterization of asthma is the chronic airway inflammation and obstruction [(1)]. Asthma usually appeared around the age of 6 years in children, and persists into adulthood in about 60% of asthmatic children. It represented a substantial burden with significant morbidity, impaired school achievement, poor mental health, and increased economic burden for the family and medical care facilities. Thus, adequate treatment is crucial, with an aim to achieve early adequate control to improve patient's quality of life (QoL) and lung function, decrease associated morbidity, disease severity and shorten the overall disease duration from diagnosis to adulthood, with subsequent reduction of overall economic burden [(2)].

Irrespective of global efforts to control asthma in children, the prevalence witnessed continuous increase of the disease. This was attributed to adoption of a Western lifestyle, including dietary factors [(3)]. For example, different studies showed that, low levels of plasma diet-derived antioxidants (e.g.., vitamins C, D and E, beta-carotene, glutathione and glutamine) are associated with increased oxidative stress, reduced lung function and symptoms in asthmatic children [(4-7)]. Furthermore, the prenatal and childhood supplementation by dietary antioxidants (e.g., vitamins, zinc, and selenium). Evidence from systematic reviews and meta-analyses of observational studies examining the effect of prenatal and childhood exposure to dietary antioxidants and asthma in children suggest that a higher intake of antioxidant vitamins, zinc, and selenium during pregnancy, infancy, and childhood reduced the likelihood of asthma and wheezing in children <18 y, despite substantial risk for bias due to inconsistencies among study designs, sample size, exposure, and asthma assessment tools [(8-10)].

Folate is a water-soluble vitamin B. it had a crucial role in amino acid metabolism, one carbon transfer, synthesis of nucleic acid bases (purine and pyrimidine), and formation of S-adenosylmethionine (a key substance in DNA methylation). Thus, it could

directly influence tilt the immune response and its balance in favor of development of allergic disease, due to effect of DNA methylation on gene expression [11,12]. Food allergy is a well-known risk factor for development of asthma. Actually, both conditions can co-exist. However, studies evaluating the role of dietary deficiencies in development of food allergy had reported mixed results [13,14].

The potential association between folate and childhood asthma did not well-investigated in previous clinical trials. Thus, the current study was designed to investigate such association.

*The aim of the work*

The current work was designed to check the association between folate deficiency and childhood asthma in a sample of Egyptian children.

**Patients and Methods**

This was a case-control study. Children aged 6 to 14 years, who have a new or old diagnosis of asthma during the period from August 2017 to August 2018 were included in the study. Asthma defined as a child with physician-diagnosed asthma and wheeze in the previous year [15]. An equal number of healthy children were selected and included as a control group. We were able to investigate 3800 children during the study period. We could identify 196 children (5.16%) with asthma, 25 of them did not complete the study or parents refuse participation (8 did not complete the study and 17 refused to participate). Thus, the final number of asthmatic children included in the study was 171 children. However, 196 healthy children completed the study, as a control group.

The included children were a sufficient sample to give a power of 95% or greater, to detect odds ratio ≥ 4 for exposure with a prevalence >4% [the sufficient sample size per group equals 160]. A prepared questionnaire was completed by one of the parents (mainly the mother in more than 96%) and blood samples were drawn, centrifuged and serum was stored at -20$^{o}$C till the time of biochemical analysis. The questionnaire was an Arabic version of the slightly modified and validated questionnaire used by Blumenthal *et al.* [16]. The questionnaire collected data about child's demographics, his/her general health condition, respiratory health, family history, current exposure to environmental toxins mainly passive smoke, especially early exposure (in utero or during the first two years of life).

The child's height and weight were measured to the nearest centimeter and kilogram. In addition, all were submitted to general and local chest examination. Furthermore, spirometry was conducted by an EasyOne spirometer (NDD Medical Technologies, Andover, MA, USA). All participants underwent for spirometry after they were free of respiratory illnesses and instructed to stop (when possible) the use of inhaled short- and long-acting bronchodilators for at least 4 weeks. If not possible, asthma therapy was stopped 12 hours before testing. Forced expiratory measurements were obtained if they are in line or above the American Thoracic Society criteria modified for children [17]. The best FVC and FEV1 were included in data analysis. For FVC, the normal values were ≥80% of predicted, while values below 80.0% percent of predicted was considered abnormal. In addition, FEV1 more than or equal 80.0% of predicted was the level of normal function, 70-79% indicate mild abnormal function, 60-79% denote moderate abnormal function, 50-59% is moderate to severe abnormal function, 35-49% indicate severe abnormality and < 35% percent of predicted categorize the very severe abnormality. FEV1/FVC normal level was equal or more than 85.0%.

Severe asthma exacerbation was defined as a hospitalization or emergency department visit with systemic corticosteroids treatment or a course of systemic corticosteroids to treat asthma lasting at least 3 days. The serum values of folate were estimated by the use of the Folic Acid ELISA kit [catalogue no MET-5068-5] a product of Cell BioLabs Inc. [7758 Arjons Drive San Diego, CA 92126, USA]. It is a competitive enzyme immunoassay developed for rapid detection and quantitation of folic acid in serum, cell or tissue samples. The product manual was used to perform analysis, and values below 20 ng/ml were considered deficient according to Sachdev *et al.* [18].

*Ethical considerations:* An oral assent and a written informed consent were obtained from the child and his/her guardian respectively. In addition, the study protocol had been reviewed and approved by the local research and ethics committee. The research had been completed according to codes of Helsinki Declaration for research conduct and reporting.

*Statistical analysis:* the collected data were anonymized, organized, tabulated and statistical analysis was performed using statistical package for social sciences, version 12 (SPSS Inc., Chicago, Illinois, USA). Normally distributed, parametric data were presented by their mean (measure of central tendency) and standard deviation (measure of dispersion). Categorical data on the other side were presented by their relative frequency and percentages. The study and control groups were compared by independent samples "*t*" test. However, categorical association was tested by Chi square test. P value < 0.05 was considered significant.

**Results**

In the current work, there was statistically significant increase of smoke exposure in the study (asthmatic) children than control group (46.8% vs 19.9%) and in parents' history of asthma (63.7% vs 15.8%). On the other side, there was statistically significant reduction of the serum levels of folate in the study than control group (18.97±5.65 vs 22.28±6.50 ng/ml, respectively). However,

when children with folate deficiency were considered, there was increased non-significant number of folate deficient children in the study than the control group (42.5% vs 35.7%) (Table 1).

As expected, the respiratory function tests (FEV1% predicted, FVC% predicted and FEV1/FVC% were significantly reduced in study than control group (Table 1). However, both cases and controls were comparable regarding child age, gender, BMI z-score percentiles, and residence. Searching the association between folate deficiency and other variables in the study group revealed that, asthmatic children with folate deficiency are significantly older in age (10.78±1.22 vs 9.00±1.10 years, respectively). Folate deficiency was significantly associated with female gender and reduced pulmonary function tests. Finally, asthma exacerbation was significantly higher among children with folate deficiency than those without folate deficiency (Table 2).

In the current work, there was negative significant moderate correlation between folate deficiency and child age. Otherwise, there was positive correlation between folate and pulmonary function tests (FEV1, FVC and FEV1/FVC) (Table 3).

**Table (1):** Comparison between study (children with asthma) and control (healthy children) groups

| Variables | | Study group | Control group | Test | P value |
|---|---|---|---|---|---|
| **Age** | Mean±SD | 9.76±1.45 | 9.89±1.14 | 0.94 | 0.34 |
| **(years)** | Min.-Max. | 6-14 | 7-13 | | |
| **Gender** | Male | 102(59.6%) | 108(55.1%) | 0.77 | 0.22 |
| **(n,%)** | Female | 69(40.4%) | 88(44.9%) | | |
| **Smoke exposure (n,%)** | Yes | 80(46.8%) | 39(19.9%) | ***30.12*** | ***<0.001**** |
| | No | 91(53.2%) | 157(80.1%) | | |
| **BMI Z score percentile** | Mean±SD | 57.13±13.96 | 59.11±11.50 | 1.48 | 0.14 |
| | Min. – Max. | 30-90 | 40-85 | | |
| **One or both Parents** | Yes | 109 (63.7%) | 31(15.8%) | ***88.90*** | ***<0.001**** |
| **history of asthma** | No | 62(36.3%) | 165(84.2%) | | |
| **Residence** | Urban | 108(63.2%) | 110(56.1%) | 1.87 | 0.17 |
| **(n,%)** | Rural | 63(36.8%) | 86(43.9%) | | |
| **Folate (ng/ml)** | Mean±SD | 18.97±5.65 | 22.28±6.50 | ***5.17*** | ***<0.001**** |
| | Min. – Max. | 10-28 | 12-30 | | |
| **Folate deficiency** | Deficient | 73 (42.7%) | 70(35.7%) | 1.86 | 0.17 |
| **(n,%)** | Sufficient | 98(57.3%) | 126(64.3%) | | |
| **Respiratory function** | FEV1% predicted | 70.12±6.74 | 79.56±4.12 | ***16.38*** | ***<0.001**** |
| | FVC% predicted | 81.33±3.98 | 85.26±2.39 | ***11.58*** | ***<0.001**** |
| | FEV1/FVC% | 86.24±7.48 | 93.37±5.22 | ***10.68*** | ***<0.001**** |
| **Severe asthma exacerbation (n,%)** | | 93 (54.4%) | NA | - | - |

**Table (2):** Association between folate deficiency and other variables in the study group (children with asthma)

| Variables | | Folate deficiency (73) | Normal folate levels (98) | Test | P value |
|---|---|---|---|---|---|
| **Age (years)** | Mean±SD | 10.78±1.22 | 9.00±1.10 | ***9.94*** | ***<0.001**** |
| **Gender** | Male | 32(43.8%) | 70(71.4%) | ***13.23*** | ***<0.001**** |
| **(n, %)** | Female | 41(56.2%) | 28(28.6%) | | |
| **Smoke exposure (n, %)** | | 33(45.2%) | 47(48.0%) | 0.12 | 0.42 |
| **BMI Z score percentile** | | 56.64±13.69 | 57.50±14.21 | 0.40 | 0.69 |
| **One or both Parents history of asthma** | | 48(65.8%) | 61(62.5%) | 0.22 | 0.37 |
| **Residence** | Urban | 46(63.0%) | 62(63.3%) | 0.001 | 0.97 |
| **(n, %)** | Rural | 27(37.0%) | 36(36.7%) | | |
| **Respiratory** | FEV1% predicted | 65.02±4.31 | 73.91±5.64 | ***11.23*** | ***<0.001**** |
| **function** | FVC% predicted | 80.57±3.63 | 81.90±4.15 | ***2.18*** | ***0.030**** |
| | FEV1/FVC% | 80.79±5.48 | 90.30±6.07 | ***10.55*** | ***<0.001**** |
| **Severe asthma exacerbation (n, %)** | | 51(69.9%) | 42(42.9%) | ***12.29*** | ***<0.001**** |

**Table (3):** Correlation between folate levels and other variables

| | Folate | |
|---|---|---|
| | r | p |
| BMI Z Score percentile | .002 | .968 |
| Age | -.532** | <0.001* |
| FEV1 | .481** | <0.001* |
| FVC | .220** | <0.001* |
| FEV1/FVC | .455** | <0.001* |

**Discussion**

In the current work, there was significant reduction in the serum levels of folate deficiency in asthmatic children than healthy children. In addition, pulmonary function tests were significantly reduced in the asthmatic children with folate deficiency than those with normal folate levels. Furthermore, severe asthma exacerbation was significantly higher among those with folate deficiency, and serum folate levels were positively and significantly correlated with pulmonary function tests. This correlation proves the association (link) between asthma and serum folate levels. These results are in line with Matsui and Matsui **(6)** who reported an inverse association between folate levels and wheezing in children. However, Thuesen *et al.* (19) reported that, this association did not exist. The association between folate deficiency and severe asthma exacerbation may be attributed to the reduction of DNA methylation of genes regulating Th2 or Th17 immune response. However, folate deficiency may be considered a marker of nutrient deficiency (e.g., vitamins with antioxidants and anti-viral actions, mainly vitamins A, C and E) with increased risk of wheeze **(20).** This reflected reciprocal relationship between wheeze or asthma and folate deficiency. In line with current results, serum folate was significantly reduced in patients with higher total IgE (as a marker of atopy) in 120 patients with asthma **(21).**

Bueso *et al.* (22) also noted co-occurrence of vitamin D insufficiency with folate deficiency suggesting poor nutrition intake in association with reduction of sun exposure. The reduction of folate and vitamin D were observed in adolescents with asthma than those without asthma.

Current results are also in line with Nicholson *et al.* (23) who conclude that, lower serum folate levels were associated with a higher risk of asthma. On the extreme side, a study by Okupa *et al.* (24) included 138 children, aged 2- 8 years reported contradictory results to the current one, where higher folate levels in early childhood was associated with increased rates of allergic diseases and asthma. This discrepancy could be explained by different sample size and different age groups. Hollingsworth *et al.* (25) also reported that, methyl donors (like folate) supplementation during gestation led to increased allergic airway disease (AAD) in animal models. However, these results were defeated by the fact that "asthma epidemic" of industrialized countries were developed before fortification of foods or development of regular folic acid supplementation in pregnancy. Blatter *et al.* (26) and Crider *et al.* (27) reported that, they were unable to find evidence of association between prenatal folate status and asthma in childhood.

It must be confirmed that, folic acid supplementation during pregnancy is directed to prevention of neural tube defects. Values of serum folic acid in children of pregnant mothers with folic acid supplementation did not be traced in many trials. However, the current study could be considered as one of such studies, as the folic acid supplementation is regularly prescribed for the Egyptian Pregnant mothers for more than a decade. However, we found significant differences between groups, irrespective of the fact that, nearly all mothers had folic acid supplementation during their pregnancy of included children. It seems that, other factors could play a role. In addition, timing of supplementation during pregnancy yielded different results in children at different ages (28-32).

Interestingly, Lim *et al.* (33) reported that, the risk for asthma increased in children of asthmatic mothers with low serum folate levels than children of non-asthmatic mothers with low serum folate. They also reported that, the risk for asthma development in children for asthmatic mothers is higher than those of asthmatic fathers. This adds to the puzzle. However, folate reduction in the serum of asthmatic children remains the participating factor either as a sole or as a contributing factor with others. It seems that, the process is multifactorial and it confirms the definition of asthma per se. The value of the current work comes from the simplicity and wide availability of folic acid which could be supplied to asthmatic children and its value could be evaluated.

One of the limitation steps in the current work is the inability to determine the cause-effect relationship between folate deficiency and asthma exacerbation. However, the linear association between pulmonary function and folate levels is a strength point of the current work as it confirmed the association between asthma severity and folate levels. Finally, these results could not be generalized to non-Egyptian children. However, it is relevant for identification of children at risk for severe asthma, and associated morbidity.

**Conclusion**

Reduced folate levels is associated with asthma, its severity and exacerbations in children. Folate supplementation could be considered in children with asthma in future studies to investigate their potential therapeutic effect. In addition, studies of other nutrient deficiencies among growing children are warranted.

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