

Validation Article

## Serum Folate in Asthma: Does it Correlate to Severity? A Single Center Experience

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### Abstract:

**Background:** Bronchial asthma is the most common chronic childhood illness. It is characterized by airway inflammation and episodic airflow obstruction. Low serum folate has been inconsistently reported as a risk factor for bronchial asthma severity. Other studies reported an increased risk of asthma in children whose mothers received prenatal folic acid supplementation.

**Aim of the work:** To compare serum folate level in asthmatic patients to non-asthmatic healthy controls, and to demonstrate whether folate level correlates to severity &/or frequency of asthma exacerbations and pulmonary function parameters.

**Methods:** Forty-five asthmatic Egyptian children and an equal number of healthy controls were included. Serum folate level was measured in both groups and spirometry was performed for the asthmatic children.

**Results:** Serum folate levels were significantly lower among patients ( $7.83\text{ng/ml} \pm 2.47$ ) versus controls ( $9.84\text{ng/ml} \pm 3.47$ ), ( $p$  value 0.002). Serum folate levels also had an inverse correlation with severity of asthma exacerbations ( $r = -0.482$ ), ( $p = 0.001$ ) and their frequency ( $r = -0.418$ ), ( $p = 0.004$ ). Serum folate levels inversely correlated as well with severity of asthma as a chronic illness ( $r = -0.315$ ), ( $p = 0.035$ ). The cutoff point of serum folate level that was found to increase the severity of asthma exacerbations was calculated at  $\leq 8.84\text{ng/ml}$ . Only 7 patients fell below the current normal reference range for serum folate. On the other hand, serum folate level did not significantly correlate with any of the pulmonary function test variables measured.

**Conclusions:** Serum folate levels were lower in asthmatic children when compared to healthy controls, and it negatively correlated with asthma severity and exacerbations, but not to pulmonary function parameters. The cutoff serum folate level for increased severity of asthma exacerbations fell within the normal folate range for children. Further research is recommended to assess any possible beneficial effects for folate supplementation in asthma.

### Level of Evidence of Study: IIB (1).

**Keywords:** Asthma; exacerbations; folic acid; serum folate; severity

**Abbreviations:** AUC: area under the curve; BA: bronchial asthma; FEF<sub>25%</sub>, 50%, 75%: forced expiratory flow at 25%, 50% and 75% of the FVC respectively; FEV<sub>1</sub>: forced expiratory volume in the first second of expiration, FEV<sub>1</sub>/FVC: ratio of FEV<sub>1</sub> to FVC; FVC: forced vital capacity; PEF: peak expiratory flow; PF(T): pulmonary function (tests); ROC: receiver operating characteristics; SD: standard deviation

### Introduction

Bronchial asthma (BA) is a common and potentially serious chronic illness in childhood. It imposes a substantial burden on the community, patients, and their families. Asthma causes respiratory symptoms, limited activity, and exacerbations that may require urgent medical



intervention and could be fatal, but are typically reversible (2). Documented variability and post-bronchodilator reversibility in pulmonary function tests (PFT) is an important tool for diagnosis in conjunction with the clinical picture. A reduced forced expiratory volume in the first second of expiration (FEV<sub>1</sub>) in comparison to forced vital capacity (FVC) in spirometry (i.e. a low FEV<sub>1</sub>/FVC ratio) is typical of an obstructive defect such as BA (3).

Folate is an essential water-soluble vitamin that occurs naturally in some foods as well as in a synthetic form (folic acid) in supplements and food fortifiers (4). Folate is a single carbon carrier essential for nucleic acid synthesis. Folate, as well as other nutrients working as methyl donors, affect deoxyribonucleic acid (DNA) methylation and, eventually, gene expression and thus could contribute to asthma risk (5). DNA methylation is a form of epigenetic regulation, and hence plays a role in gene-environment interactions of some complex diseases such as BA (6). Alterations in DNA methylation can increase or decrease the expression of disease-susceptibility genes, consequently affecting the pathogenesis of asthma (7).

A deficiency of folate or even a low level has been linked to increased risks of wheeze and atopy, and that a diet rich in folate or folic acid can decrease the incidence of BA or asthma exacerbations (8). It has been hypothesized that folate deficiency significantly correlates with severe asthma exacerbations, as well as with increased atopy in children (9). It has also been suggested that low serum folate and impaired folate metabolism are likely risk factors for the development of BA.9 However, folate level has not been associated with PFT impairment (10, 11).

On the other hand, some studies found that prenatal folate supplementation to mothers has been associated with an increase in childhood asthma and other allergies (12, 13). This study aims to assess the serum folate level in a group of asthmatic patients and to compare it to non-asthmatic healthy controls. Also, whether the folate level correlates to the severity of asthma and exacerbations and their frequency, as well as to pulmonary function parameters.

## Subjects and Methods

This cross sectional study was carried out at Cairo University Children's Hospitals. The study was approved by the Pediatric Department Committee of Research and Higher Studies Research Committee of Faculty of Medicine, Cairo University in compliance to Helsinki declaration guidelines (14). The study was designed to evaluate serum folate level in ninety Egyptian children; 45 asthmatics enrolled from the Allergy and Pulmonology Clinic of New Children's Hospital, Cairo University, and 45 healthy age and sex matched non-asthmatic healthy children, during the period from January 2018 to September 2018.

### Participants

Children aged 6 to 12 years, with a satisfactory nutritional history were included in the study. Any child with clinical evidence of malnutrition, malabsorption or any associated other chronic illness was excluded from the study. Other exclusion criteria also included any history of intake of alcohol containing drugs, sulfonamides, trimethoprim or immunotherapeutic drugs such as methotrexate, as well as history of folic acid supplementation (alone or in combination with iron therapy) as these medications would alter the patient's serum folate level. Asthmatics who could perform an acceptable PFT and were not in exacerbation at the time of sampling and PF testing were enrolled. The asthmatic children included both, those not on, and those on controller medications (with the exception of systemic steroids). All degrees of asthma control (according to NHLBI, 2007) (15) were included (figure 1).

An informed consent was obtained from all participants' guardians. All patients were subjected to full history taking and clinical examination, focusing on chest examination and ensuring absence of clinical evidence of malnutrition. Asthma severity and severity of exacerbations were determined according to NHLBI, 2007 (15).

### Methods

Pulmonary functions tests (PFT) were performed using a spirometer (Jaeger™ Master Screen PET system) in The Pulmonary Function Lab, Allergy and Pulmonology Unit, Pediatric Department, Faculty of Medicine, Cairo University. The child was allowed to take a few tidal

breaths, then was asked to inspire maximally, followed by complete expiration as rapidly and forcefully as possible. The best of three trials was calculated as a percentage to the predicted value for the child's sex, age and height.

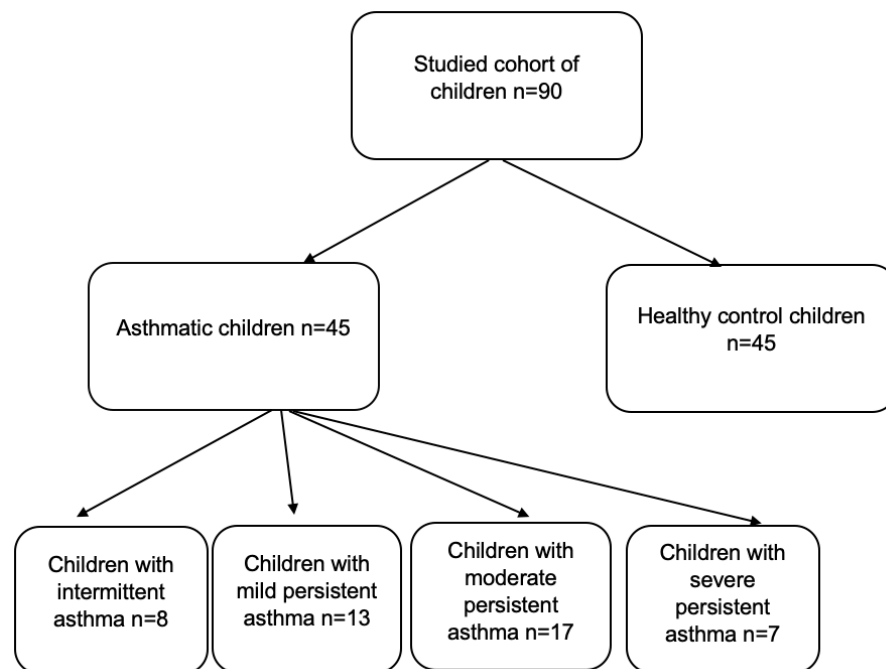
Folate level was assessed in the serum of both cases and controls using the folate (FOL) kits supplied by Siemens Diagnostics. It was done on ADVIA Centaur® CP system using the competitive chemi-luminescence immune-assay technique. It was performed in the Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University.

### Statistical Analysis

Statistical analysis was performed with the statistical package for social science (SPSS) version 18. Numerical data were summarized using mean and standard deviation, or medians and ranges. Categorical data were summarized as per centiles. Comparisons between the two groups were calculated using the student T test. Correlation between variables was evaluated using Pearson's correlation coefficient. Receiver Operating Characteristics (ROC) curve was done by plotting sensitivity against specificity of the diagnostic value of serum folate levels.

### Results

Forty-five children with BA were recruited and compared to a control group of 45 healthy children of similar age and sex distribution and similar residential background (table 1).



**Figure 1 :** The studied group of children with asthma and the control group.

The enrolled children were attendants of the Pulmonology Clinic, Children's Hospital, Cairo University, Egypt. At enrollment the children with asthma had mean +/- standard deviation (SD) age of 8.16 +/- 2.1 years, while the control group mean age at enrollment was 8.24 +/- 1.8 years ( $p=0.832$ ). The group with asthma comprised 19 girls and 26 boys, while the control group comprised 21 girls and 24 boys ( $p=671$ ). There was also no statistically significant difference regarding their residential background ( $p=0.517$ ). Asthma symptoms were pronounced in winter among 26 (57.85%) of patients, 11 (24.4%) of patients had perennial variation with seasonal flare, 7 (15.6%) of patients had their symptoms perennially and only one patient (2.2%) had the symptoms in summer. Thirty-five asthmatic (77.8%) patients had positive family history of asthma while 10 (22.2%) had no family history of asthma. Precipitating factors, other atopic manifestations and asthma severity are listed in table 2.

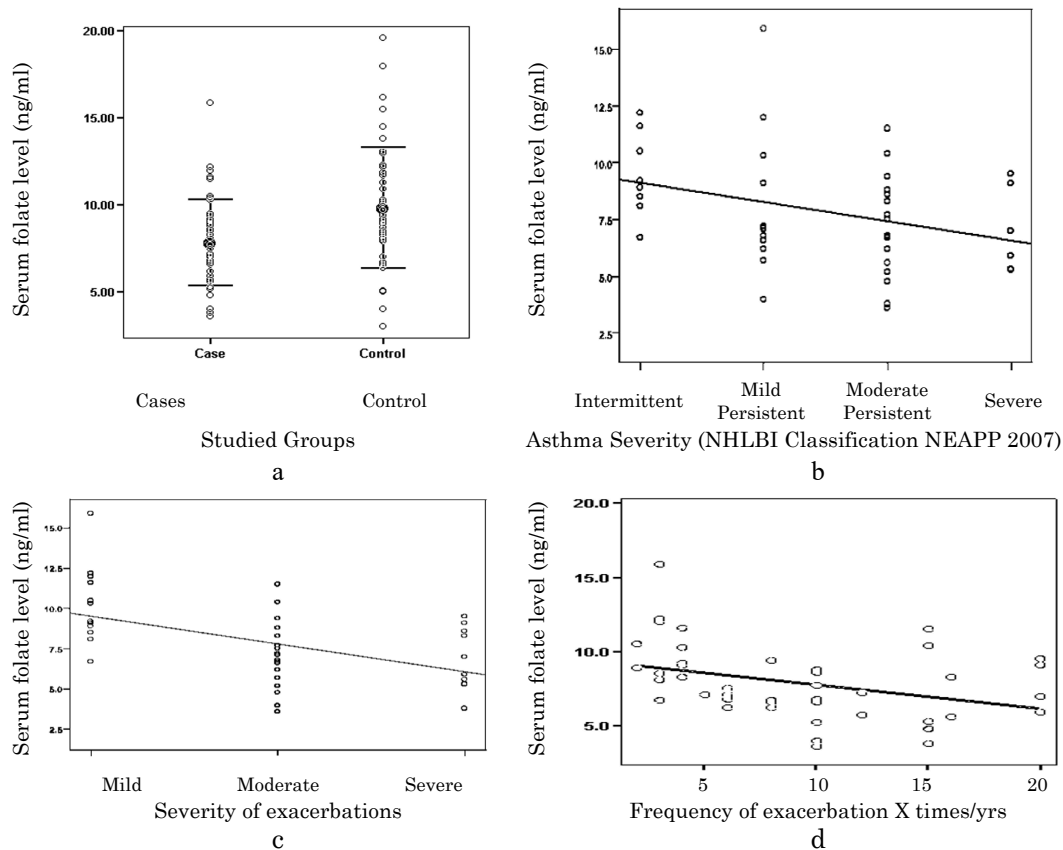
There was a statistically significant difference between cases and controls regarding serum folate level ( $p$  value 0.002), where the mean folate level was 7.83 ng/ml, compared to 9.84 ng/ml among the control group (figure 2.a). Also, the mean serum (+/- SD) folate level in asthmatic



patients with history of atopy was 6.64 ng/ml +/- 1.8 ng/ml, which was significantly lower than among patients without history of atopy 9.62 +/- 2.22 ng/ml ( $p > 0.001$ ).

**Table 1:** Demographic Findings of the Studied Group with Asthma.

	Cases (n=45)	Control Group (n=45)	P value
<b>Age in years (mean+/- SD)</b>			
Mean	8.16 (+/- 2.1)	8.24 (+/- 1.8)	0.832
<b>Gender</b>			
Females	19 (42.2%)	21 (46.7%)	0.671
Males	26 (57.8%)	24 (53.3%)	
<b>Residence</b>			
Rural	19 (42.2%)	16 (35.6%)	0.517
Urban	26 (57.8%)	29 (64.4%)	



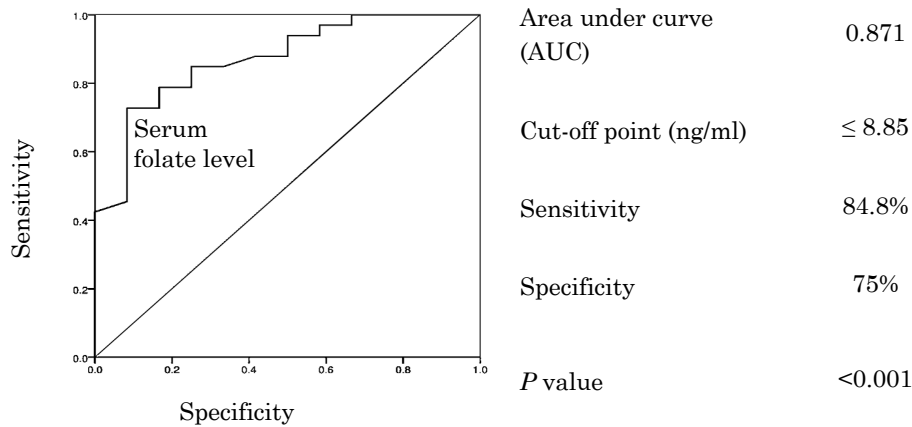
**Figure 2. :** Folate level, and its correlations with asthma exacerbation severity and frequency.

2.a. shows that mean and standard deviation of serum folate level in cases (7.83 ng/ml +/-2.47) was less than in controls (9.84 ng/ml +/-3.47). A statistically significant difference was present ( $p$  value 0.002). 2.b. shows that serum folate level correlated negatively with asthma severity as a chronic illness ( $r = -0.315$ ), ( $p = 0.035$ ). 2.c. shows that serum folate level correlated negatively as well with severity of asthma exacerbations ( $r = -0.482$ ), ( $p = 0.001$ ). 2.c. shows that serum folate level also correlated negatively with frequency of exacerbations ( $r = -0.418$ ) ( $p = 0.004$ ).

Asthma severity (as a chronic illness) correlated negatively with serum folate level ( $r = -0.315$ ) ( $p = 0.035$ ), as well as with severity of asthma exacerbations ( $r = -0.482$ ), ( $p = 0.001$ ) and



the frequency of exacerbations ( $r = - 0.418$ ) ( $p = 0.004$ ), (figure 2.b, c and d respectively). Pulmonary functions test parameters did not correlate with folate level (table 3).



**Figure 3:** ROC curve of serum folate as a diagnostic marker for severity of asthma exacerbation.

Receiver-operating characteristic (ROC) curve analysis revealed that the cut-off point of serum folate level that increases the risk of severity of asthma exacerbation was levels  $\leq 8.85$  ng/ml. Sensitivity: probability that a test result will be positive when the disease is present. Specificity: probability that a test result will be negative when the disease is not present.

**Table 2:** Precipitating factors, atopic manifestations and asthma severity among the studied asthmatic children.

	Number	%
<b>Precipitating Factor</b>		
Respiratory tract Infection	36	80
Exercise Induced Asthma	35	77.8
Passive Smoking	33	73.3
Dust	32	71.1
Associated food allergy	28	62.2
Animal Contact	9	20
<b>Associated Atopic Manifestation</b>		
Atopic Dermatitis	18	40
Allergic rhinitis	17	37.8
Allergic Conjunctivitis	10	22.2
<b>Asthma Severity*</b>		
Intermittent	8	17.8
Mild persistent	13	28.9
Moderate persistent	17	37.8
Severe persistent	7	15.6
Total	45	100

\*Asthma Severity, according to NHLBI, 2007. 11

The receiver-operating characteristic (ROC) curve analysis was used to examine the diagnostic value of folate for discrimination between mild asthma exacerbation and



moderate/severe asthma exacerbation (figure 3). The cut-off point of serum folate level that increases the risk of asthma exacerbation was  $\leq 8.85$  ng/ml with a p. value of  $< 0.001$  and a sensitivity of 84.8% and a specificity of 75%. Only 7 (15.56%) patients fell below the current accepted normal reference range for serum folate in children (5-21 ng/ml) (16).

**Table 3:** Parameters of pulmonary function tests and their correlations with serum folate among the studied children.

	Mean	SD	Minimum	Maximum	Correlation Coefficient (r)	P value
FVC%	91.18	19.46	45.8	134.1	0.036	0.816
FEV <sub>1</sub> %	86.04	19.32	32.2	115.6	0.061	0.692
FEV <sub>1</sub> /FVC%	95.43	16.44	59	116.7	0.082	0.593
FEF <sub>25</sub> %	69.20	38.17	7.70	171.4	- 0.064	0.675
FEF <sub>50</sub> %	65.49	27.64	12.2	130.3	- 0.065	0.671
FEF <sub>75</sub> %	64.73	21.79	20.3	112.9	- 0.119	0.436
PEF%	63.59	18.53	25.4	102.9	- 0.135	0.376

FEF<sub>25</sub>%, 50%, 75%: forced expiratory flow at 25%, 50% and 75% of the FVC respectively, FEV<sub>1</sub>: forced expiratory volume in the first second of expiration, FEV<sub>1</sub>/FVC: ratio of FEV<sub>1</sub> to FVC, FVC: forced vital capacity, PEF: peak expiratory flow, SD: standard deviation.

## Discussion

In the present study, the level of serum folate was found to be lower in the studied children with asthma than in the healthy control group. The relative folate deficiency among asthmatic children might be the result of inadequate dietary intake of folate or due to inadequate folate absorption. Actually, 80% of children with asthma were reported to have hypochlorhydria since 1931 (17). Hypochlorhydria is known to reduce folate absorption and accumulation of folate in the liver (18). The specific dietary intake for folate and hypochlorhydria were not assessed in our studied group. Hence, the relation of dietary intake/ intestinal absorption to the noted decrement of folate in bronchial asthma awaits future research validation.

On the other hand, this relative deficiency might also be due to folate being over utilized in asthma. This overconsumption of folate might happen in the rapid regeneration of the bronchial epithelial cells damaged by the inflammatory processes. Moreover, folate might be consumed in the production of lymphocytes responsible for the immune inflammatory process associated with the asthma pathogenesis (19). Another possibility might be the channeling of folate to produce histidine and histamine (20). It is not clear what factors govern the distribution of folate as a substrate in specific pathways. In any case, histidine is increased in urine of those with chronic asthma, which provide further evidence that histidine- histamine pathway is activated in asthma (21).

Among our studied cohort of asthmatic children, folate level inversely correlated with severity of asthma (as a chronic illness) ( $p= 0.035$ ), frequency of exacerbations ( $p=0.004$ ), as well as severity of exacerbations ( $p= 0.001$ ), but did not correlate with pulmonary function test parameters. Findings from other studies have been controversial, some showing similar results (9, 10) while others did not demonstrate them (11). Folate level is a dynamic one (it has a relatively short half-life of almost 2 hours, and 98.7% +/- 11.5% of the administered folic acid is cleared in the urine within 10 hours) (22). Hence the serum folate level that was estimated reflects a timely and not a cumulative level. Hence, the time of sample does not really reflect the chronic maintenance of folate level, and that in between asthma attacks (which was the case for our patients) regeneration sets in. The use of the limited folate might be prioritized to maintain the integrity of the bronchiolar epithelium, hence assuring regeneration and improved PFT, while the folate levels would be low in serum.





Since the serum folate level is related to feeds (23), and our daily diet is variable, thus daily serum folate levels varies from day to day. So if asthma exacerbations are predisposed to by low serum folate levels, then exacerbations would probably have variability in their recurrence rate. This may explain why the lower folate levels correlated with higher frequency of asthma exacerbations.

However, it is worth noting that other studies show that this variability in serum folate levels is not noted in red blood corpuscles (RBCs) folate levels, where RBCs half-life of folate is 60 days. It is interesting however, that it takes up to 40 weeks of regular folic acid intake to reach steady conditions for RBCs synthesis (23). Another peculiar fact is that mast cells life span is almost 12 weeks, hence its histamine content is not really subject to the hour-to-hour fluctuation of folate in blood (24).

The cutoff serum folate level that increased the risk for moderate to severe asthma exacerbations was  $\leq 8.85$  ng/ml (p. value  $< 0.001$ , sensitivity 84.8%, specificity 75%). Interestingly this cutoff level lies in the accepted normal range for serum folate in children (5-21 ng/ml) (16). Also only 7 (15.56%) of our patients had serum folate levels below that range. This cutoff level needs further validation by larger scale studies, as it might lead the medical community to reconsider the accepted folate levels and the recommended daily allowance values of folic acid for asthmatic children.

There is also a need to verify the effect of folate supplementation in asthma. Our study suggests a need for folate supplementation, but whether the required supplementation needs adherence for long durations to get a consistent measurable outcome (23), or whether short term folate supplementation could be adequate during exacerbations necessitates further research that eliminates the limitations in the present study. These limitations include the small sample size and its cross-sectional nature. The study also did not include measurement of the dietary folate, presence of hypochlorhydria, nor of histamine or histidine levels. We believe that the serum folate might represent the tip of the iceberg or is a simple confounder among other factors yet to be defined. Thus, further research is needed in the future to provide insight whether the relatively low folate levels in asthma are due to a deficient intake/absorption, or due to the fact that asthma itself increases the daily requirements of folic acid.

## Conclusion

Serum folate levels were significantly lower in asthmatic children than in controls. Serum folate also had significant inverse correlations with the severity and frequency of asthma exacerbations, as well as with severity of asthma as a chronic illness. The cut-off point of serum folate level, that increases the severity of asthma exacerbation, was found to fall in the accepted normal range for children. These findings may prompt further research to assess the need for folate supplementation in asthmatic children.

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## Author Contributions:

C.W.S.B.: conceptualization, supervising, data curation, data analysis, writing original draft and corresponding, M.A.K.: Data interpretation, writing original draft, supervising and revising. N.F.E.: Data curation and interpretation, investigation and methodology. N.E.A.T.: Data curation and analysis, investigation and methodology. A.K.A.: Conceptualization, methodology, data analysis, revising and writing. All authors reviewed the final manuscript. All authors have read and agreed to the published version of the manuscript.



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## CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the study.

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