SERUM FOLATE LEVEL AND ASTHMA EXACERBATION IN CHILDREN

By

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ABSTRACT

Background: *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, pulmonary function tests variability and post-bronchodilator reversibility are useful diagnostic tools. Folate is an essential water-soluble vitamin found in various foods and in supplements and food fortifiers as folic acid. A diet high in folate or folic acid can reduce the likelihood of asthma exacerbations and wheezing. Folate deficiency is linked to severe asthma exacerbations and increased atopy in children.*

Aim and objectives: *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.*

Subjects and methods; *This a case control study that was conducted in Pediatrics department at AL Hussein and El-Sayed Galal university hospitals during the period from Feb 2022 to November 2022 on Sixty children were admitted at Pediatrics department at AL Hussein and El-Sayed Galal university hospitals with newly diagnosed as asthma exacerbation.*

Result: *Cases compared to controls. Serum folate was significantly lower in severe asthma exacerbation cases compared to moderate patients compared to mild patients. There is a positive significant correlation between serum folate with FEV1, FVC, and FEV1/FVC among cases group.*

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.*

Keywords: *Serum Folate, Asthma, Children.*

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 **(GINA, 2019)**.

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 (FEVi) in comparison to forced vital capacity (FVC) in spirometry (i.e., a low FEVi/FVC ratio) is typical of an obstructive defect such as BA **(Miller M. R., et al., 2010)**.

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 **(Crider K. S., et al., 2012)**.

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 **(Lovinsky-Desir S., R. L. Miller, 2012)**.

DNA methylation is a form of epigenetic regulation, and hence plays a role in gene-environment interactions of some complex diseases such as BA **(Nicholson A. et al., 2017)**.

Alterations in DNA methylation can increase or decrease the expression of disease-susceptibility genes, consequently affecting the pathogenesis of asthma **(Crider K. S. et al., 2013)**.

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 **(Blatter J. et al., 2013)**.

It has been hypothesized that folate deficiency significantly correlates with severe asthma exacerbations, as well as with increased atopy in children **(Blatter J. et al., 2016)**.

It has also been suggested that low serum folate and impaired folate metabolism are likely risk factors for the development of BA. However, folate level has not been associated with PFT impairment

(Thuesen B. H. et al., 2010) (Lin J. H. et al., 2013).

On the other hand, some studies found that prenatal folate supplementation to mothers have been associated with an increase in childhood asthma and other allergies (Parr C. L. et al., 2017)

Ethical considerations:

1. A written informed consent was obtained from parents or the legal guardians before the study.
2. An approval by the local ethical committee was obtained before the study.
3. The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
4. All the data of the patients and results of the study are confidential & the patients have the right to keep it.
5. Financial Disclosure/ Funding: The authors received no financial support for the research, authorship and/or publications of this article.
6. The patients have the right if withdrawn from study at any time.

Sample size calculation:

- According to Epi. Info 2000, the sample size was computed with a precision of (2%), a 95% confidence interval, and a sample size of 30 children vith asthma exacerbation and 30 healthy controls. In order to address issues with non-responses and missing data, the sample size was expanded by 10%. 85% of the study's power was used in the analysis. Convenient samples composed its
- A sample type: (CDC Epi info V7.0.8.3)

PATIENTS AND METHODS

This is a case-control study was conducted in the Pediatrics department at AL Hussein and El-Sayed Galal university hospitals.

Inclusion criteria:

Children aged 6 to 12 years of both sex, with a satisfactory nutritional status were included in the study, and documented asthmatic child.

Exclusion criteria:

- Any child with clinical evidence of malnutrition, malabsorption or any associated other chronic illness.
- Any history of intake of alcohol-containing drugs, sulfonamides, or trimethoprim.

- Any history of immunotherapeutic drugs such as methotrexate.
- History of folic acid supplementation (alone or in combination with iron therapy).

Tools of Assessment: all the studied children were subjected to the following:

A. Complete History including:

1. Personal history.
2. Past history of a recurrent chest infection or hospital admission, Current medications, known allergies (not just drugs).
3. Family history of allergic diseases and smoking exposure.

B. Clinical Examination:

- Vital signs.
- General with stress on nutritional staus: Wt, Ht, BMI.
- Systemic examination: including chest, heart, and abdomen with emphasizing on signs of obstructive airway manifestation.
- All children were undergone Pulmonary Function Test by Spirometry to determine FVC, FEV1.
- Asthma severity and severity of exacerbations were determined according to **NHLBI, 2007**.
- Folate assessment was done for all studied patients by Enzyme Linked Immuosorbent Assay (ELISA) method.

Statistical Analysis:

All data were collected, tabulated and statistically analyzed using SPSS 24.0 for windows (SPSS Inc., Chicago, IL, USA).

Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD (Standard deviation) for parametric and median and range for non-parametric data.

Independent T test and Mann Whitney test were used to calculate difference between quantitative variables in two groups for parametric and non-parametric variables respectively. One way ANOVA test was used to compare between more than two dependent groups of normally distributed variables.

Pearson's or Spearman's correlation coefficient was used for correlating normal and non-parametric variables respectively. The (+) sign was considered as

indication for direct correlation i.e., increase frequency of independent lead to increase frequency of dependent & (-) sign as indication for inverse correlation i.e., increase frequency of independent lead to decrease frequency of dependent, also we consider values near to 1 as strong correlation & values near 0 as weak correlation.

All statistical comparisons were two tailed with significance Level of P-value ≤ 0.05 indicates significant, p <0.001 indicates highly significant difference while, P> 0.05 indicates non-significant difference.

RESULTS

Table (1): Demographic characteristics of the two studied groups

		Cases (n=30)	**Controls** (n=30)	**t / χ^2**	**p**
Age (years) Mean ± SD		8.65 ± 1.65	8.43 ± 1.72	.506	.615
Sex	Female	13 (43.3%)	12 (40%)	.069	.793
	Male	17 (56.7%)	18 (60%)		
Weight (kg) Mean ± SD		55.63 ± 4.19	57.39 ± 4.82	1.51	.137
Height (cm) Mean ± SD		141.88 ± 10.18	145.3 ± 11.29	1.23	.223
Residence	Rural	12 (40%)	14 (47.7%)	.272	.602
	Urban	18 (60%)	16 (53.3%)		

This table shows that there is no significant difference between the two groups regarding age, sex, weight, height, and residence.

Table (2): Risk factors distribution between the two studied groups

	Cases (n=30)	**Controls** (n=30)	**χ^2**	**p**
Smoking exposure	13 (43.3%)	6 (20%)	3.77	.052
Positive family history of asthma	18 (60%)	4 (13.3%)	**14**	**<0.001**

This table shows that there is a significant difference between the two groups regarding family history of asthma.

Table (3): Precipitating factors distribution among cases group

	Cases (n=30)	
	N	**%**
Respiratory tract infection	23	76.7%
Exercise induced asthma	21	70%
Dust	19	63.3%
Food allergy	14	46.7%
Animal contact	6	20%

This table shows that the most prevalent precipitating factor was respiratory tract infection (76.7%) followed by exercise induced asthma (70%).

Table (4): Comparison of Respiratory function between the two studied groups

Variables	**Cases** (n=30)	**Controls** (n=30)	**T**	**p**
FEV1% Mean ± SD	67.83 ± 5.43	80.12 ± 7.32	**7.39**	**<0.001**
FVC% Mean ± SD	81.62 ± 3.89	85.47 ± 2.36	**4.65**	**<0.001**
FEV1/FVC Mean ± SD	85.12 ± 7.41	93.27 ± 5.23	**4.92**	**<0.001**

This table shows that FEV1, FVC, and FEV1/FVC were significantly lower in cases compared to controls.

Table (5): Comparison of Serum folate Level between the case and control

Variables	**Cases** (n=30)	**Controls** (n=30)	**T**	**p**
Serum folate (ng/ml) Mean ± SD	18.54 ± 5.84	22.37 ± 5.96	**2.78**	**.007**
Folate deficiency				
Deficient	14 (46.7%)	7 (23.3%)	3.59	.058
Sufficient	16 (53.3%)	23 (76.7%)		

This table shows that serum folate was significantly lower in cases compared to controls.

Table (6): Asthma severity according to NHLBI Classification among cases group

	Cases (n=30)	
	N	**%**
Intermittent	5	16.7%
Mild persistent	9	30%
Moderate persistent	12	40%
Severe persistent	4	13.3%

This table shows that 40% of the patients were moderate persistent, 30% were mild persistent, and 13.3% of the patients were severe persistent.

Table (7): Serum folate distribution among cases group according to asthma exacerbation severity subgroups

	Mild (n=9)	**Moderate** (n=14)	**Severe** (n=7)	**p**
Serum folate (ng/ml) Mean ± SD	19.35 ± 3.49	16.29 ± 4.12	12.87 ± 4.36	**.012**
Folate deficiency				
Deficient	3 (33.3%)	6 (42.9%)	5 (57.1%)	.294
Sufficient	6 (66.7%)	8 (57.1%)	2 (42.9%)	

This table shows that serum folate was significantly lower in severe asthma exacerbation cases compared to moderate patients compared to mild patients.

Table (8): Correlation of Serum folate with different parameters among cases group

Parameters	**Serum folate**	
	R	**P**
Age	**-0.353**	**.001**
Weight	.105	.634
FEV1	**.432**	**<0.001**
FVC	**.378**	**<0.001**
FEV1/FVC	**.416**	**<0.001**

This table shows that there is a positive significant correlation between serum folate with FEV1, FVC, and FEV1/FVC among cases group. Meanwhile, there is a negative significant correlation between serum folate and age among cases group.

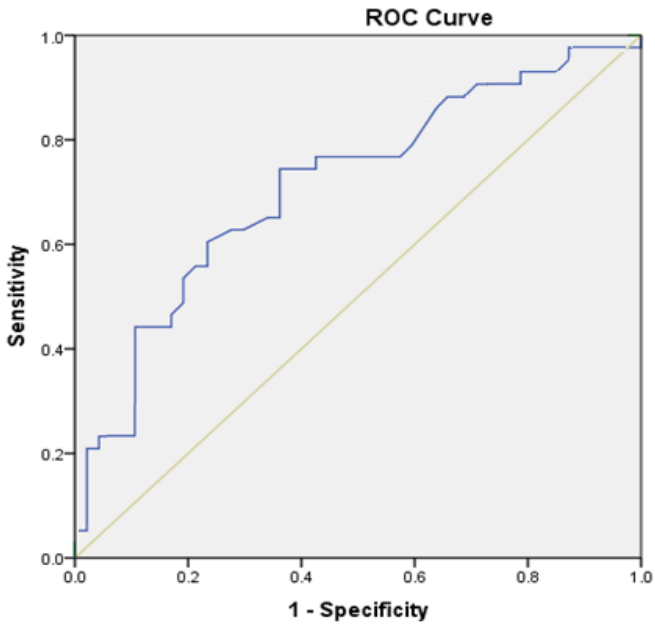

Figure (1): ROC curve for serum folate as a marker for asthma exacerbation

Variables	AUC	S.E.	Sig.	95% Confidence Interval
Serum folate	.811	.052	**.000**	.704 - .918

Variables	Cutoff	Sensitivity	Specificity	PPV	NPV
Serum folate	≤17.52	88.2%	76.4%	86.9	78.5

This table shows that serum folate yielded significant level at cut off level ≤17.52 ng/ml with sensitivity of 88.2%, specificity of 76.4%, PPV of 86.9 and NPV of 78.5 as a marker for asthma exacerbation.

DISCUSSION

Our results showed that there is no significant difference between the two groups regarding age, sex, weight, height, and residence.

Similarly, in the study of **Basanti et al., 2021** 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) with no statistically significant difference regarding age and sex.

There was also no statistically significant difference regarding their residential background (p= 0.517).

The present study showed that there is a significant difference between the two groups regarding family history of allergy.

Our results were supported by **Stanford et al., 1999** they reported an increase in the prevalence of asthma among first degree relatives with asthma to 20-25% compared with a general population prevalence of 4%. While **Gruchalla et al., 2005** found increasing the risk of asthma with positive family history of allergy or chronic rhinitis. These results were supported by **Broms et al., 2012** who stated that positive family history of asthma was risk factor for asthma.

Viral and bacterial infections are important causes of recurrent wheezing in children. It precipitates acute exacerbations of asthma and was the most common reason for hospital admissions.

The current study showed that the most prevalent precipitating factor was respiratory tract infection (76.7%) followed by exercise induced asthma (70%).

Our results were in line with study of **Blatter et al., 2016** as they reported that the most prevalent precipitating factor was respiratory tract infection (80%) followed by exercise induced asthma (77.8%).

Similarly, **Ali et al., 2019** demonstrates the precipitating factors of asthma, history of atopy and asthma severity in the studied patients. The most commonly reported precipitating factor was viral infection (40%), followed by exercise (22%), exposure to cold (20%), fumes (16%) and the least was food (in one case, 1%).

In the study in our hands, there is no significant difference between the two groups regarding laboratory parameters. FEV1, FVC, and FEV1/FVC were significantly lower in cases compared to controls.

Similarly; in the study of **Elsehaimy & Hassan, 2019** the respiratory function tests (FEV1% predicted, FVC% predicted and FEV1/FVC% were significantly reduced in study than control group.

In addition, **Blatter et al., 2016** demonstrated that compared with control subjects, cases were slightly younger and more likely to have lower spirometric measures of lung function (FEV1 and FEV1/FVC).

Our results showed that serum folate was significantly lower in cases compared to controls.

Our results were in agreement with study of **Basanti et al., 2021** as they reported that 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.

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. Hypochlorhydria is known to reduce folate absorption and accumulation of folate in the liver. 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.

Also, in the study of **Kim et al., 2022** they observed the serum folic acid level in quintiles to assess the risk of asthma. Similarly, the asthma risk significantly increased when the serum folic acid level decreased. Therefore, serum folic acid levels were inversely associated with the risk of physician-diagnosed asthma.

Furthermore, **Elsehaimy & Hassan 2019**, revealed that 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%).

The present study showed that regarding asthma severity according to NHLBI Classification among cases group; 40% of the patients were moderate persistent, 30% were mild persistent, and 13.3% of the patients were severe persistent. Regarding Severity of asthma exacerbation among cases group; 46.7% of the patients were moderate, 30% were mild, and 23.3% of the patients were severe.

The current study showed that serum folate was significantly lower in severe asthma exacerbation cases compared to moderate patients compared to mild patients. There is a positive significant correlation between

serum folate with FEV1, FVC, and FEV1/FVC among cases group. Meanwhile, there is a negative significant correlation between serum folate and age among cases group. Using ROC curve for serum folate as a marker for asthma exacerbation; serum folate yielded significant level at cut off level ≤17.52 ng/ml with sensitivity of 88.2%, specificity of 76.4%, PPV of 86.9 and NPV of 78.5 as a marker for asthma exacerbation.

Our results were supported by study of Elsehaimy & Hassan, (102), as they reported that 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. In their 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).

Furthermore, **Ali et al., 2019** revealed that the mean folic acid level was lowest in the severe group (1.2 ±0.1), followed by the moderate (2.4 ± 0.3) then the mild group (3.4 ± 0.4), with a significant difference when all the groups were compared with each other ($p<0.001$) .There were significant ($p <0.001$), positive strong correlations between folic acid level and each of FEV1% ($r = 0.885$) and FEV1/FVC% ($r=0.895$) (i.e. in most cases, folic acid level tended to decrease with the decrease in FEV1% and FEV1/FVC% ratio).

In agreement of our study on pediatric **Blatter et al., 2016** report that folate deficiency is significantly associated with severe asthma exacerbations among Puerto Rican children with asthma.

Also, **Brehm et al., 2012** hypothesized that Folate deficiency is significantly associated with severe asthma exacerbations in children with asthma.

CONCLUSIONS

We Conclude from our study that:

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. 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.

RECOMMENDATIONS

- Further multicenter studies on large sample size are needed to confirm the current results.
- Folic acid supplement of 400 micrograms per day to mother with history of allergy planning a pregnancy until 12 weeks gestation.
- Our study suggests a need for folate supplementation, to the patients with excacerbation whether the required supplementation needs adherence for long durations to get a consistent measurable outcome.

Limitations:

- The inability to determine the cause-effect relationship between folate deficiency and asthma exacerbation.
- The small sample size and its cross-sectional nature.
- The study also did not include measurement of the dietary folate.

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