Original Article

Investigating the Link Between Dietary Vitamins and Lung Cancer: The Influence of Folic Acid, Cobalamin, and Female Sex Hormones

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Abstract

Objective: The objective of this study was to investigate how the supplemental use of Folic Acid and Cobalamin impacts the histomorphology of the lung tissue of female Sprague Dawley rats.

Methodology: A 4-week laboratory-based experimental control trial was conducted at the Department of Anatomy, CMH Multan Institute of Medical Sciences Pakistan, after getting approval from the Institutional Review Board and Ethical Committee. The study, conducted from September 2019 to March 2021, involved 120 female Sprague-Dawley rats, divided into four groups of 30. Group A received distilled water, Group B1 received folic acid (0.2 mg/kg), Group B2 received cobalamin (45µg/kg), and Group B3 received both folic acid and cobalamin. Treatments were administered orally for 28 days, followed by euthanasia, tissue preparation, and H&E staining for microscopic analysis. Data was analyzed using SPSS version 26.

Results: In this abstraction, control group A exhibited normal lung histology, while experimental groups B1 (Folic Acid) and B2 (Cobalamin) showed significant histopathological alterations, thickening of the interalveolar septa and proliferation of fibroblasts caused by invasion of macrophages and lymphocytes. The B3 group (Folic Acid + Cobalamin) demonstrated milder changes. Ashcroft scoring revealed a statistically significant difference among groups ($p = 0.000$). Serum estradiol levels were normal in groups A and B1, but significantly elevated in groups B2 and B3 ($p = 0.000$).

Conclusion: Cobalamin, either alone or in combination with folic acid, induces significant histopathological lung changes or elevates serum estradiol levels.

Keywords: Ashcroft Score, Estradiol, Folic Acid, Lung Diseases, Vitamin B12

Introduction

While lung cancer is the second most frequent kind of cancer, it is also the main cause of mortality that is directly related to cancer. Lung cancer is equally common in men and women, although men have had a higher incidence rate in the past. The gender disparity in lung cancer rates has been tapering, and there has been an uptick in the incidence of lung cancer in women.[1] Female non-smokers face a lung cancer risk that is approximately three times higher than male non-smokers. Despite a dramatic rise in the incidence of lung cancer in women over the past few decades—particularly lung adenocarcinoma (LUAD)—the exact causes of this disease remain unknown, with the exception of environmental factors and smoking. Female patients are more likely to get lung cancer, and this is thought to be due in part to reproductive and hormonal factors.[2,3]

Differential occurrence of cancer based on gender suggests the possible causal influence of hormonal factors. The significant involvement of sex hormones in the pathogenesis of cancers in non-reproductive organs and tissues such as the lung, esophagus, colon, and kidney has been well verified.[4] The fact that considerably more women than men who are diagnosed at the same age (under 50 years old) are primarily premenopausal is another indicator of the role that female sex hormones play in lung cancer.[5] Studies conducted in East Asia have also revealed a three-fold higher risk of lung cancer in women with shorter menstrual cycles, suggesting that there are more hormone levels during the reproductive phase.[6]

Folic Acid and Cobalamin are dietary essential vitamins. As a result of the significance of these vitamins, nutritional supplements are added to food. They are widely used to treat anemia and they prevent neural tube defects (NTDs).[7]

Despite the vitamins' obvious benefits, recent studies have connected folic acid and cobalamin overconsumption to changes in DNA methylation of many genes that may play a role in cancer formation.[8] It is proved that these vitamins cause endocrine chemical disruption; hence, they disrupt different endocrine hormonal levels.[9]

The one-carbon metabolism pathway involves interactions between homocysteine, methionine, folic acid, and cobalamin, according to an additional human study. The progression of cancer may be accelerated if this process is disrupted.[10] An increased risk of lung cancer was associated with using vitamin B6 and cobalamin supplements in high doses, according to a retrospective cohort study.[11]

This research had two primary objectives. To address the existing knowledge gap, this study explored the potential link between these vitamins and lung cancer, as there is currently no investigative research available to support this hypothesis.Secondly, to investigate the association between female sex hormones and the risk of lung cancer, aiming to understand the potential role these hormones play in the development of the disease.

Methodology

Before this new study was conducted in the anatomy department's research lab at Pakistan's CMH Multan Institute of Medical Sciences, it was approved by the institutional review board and ethical committee. It was a laboratory based experimental study. The University of Veterinary and Animal Sciences (UVAS) in Lahore is a well-known study institution with excellent veterinary institutes; One hundred and twenty healthy female Sprague-Dawley rats were acquired from them. Researchers all throughout the globe rely on Sprague-Dawley rats as reliable experimental subjects.[12]

None of the rats in the study were pregnant or otherwise unhealthy. A random sampling procedure was used to choose the rats. Four groups were involved in the study: three experimental (B1,2,3) and one control (A). There were thirty mature female rats in each group. Group A was administered 1ml/100g of pure distilled water orally every day for four weeks. Daily oral gavage of 0.2 mg/kg of folic acid in 1 ml/100 g of pure distilled water was administered to Group B1. For four weeks, members of Group B2 were administered 45µg/kg of cobalamin in 1ml/100g of pure distilled water through oral gavage once a day. Folic acid (0.2 mg/kg) and cobalamin (45µg/kg) were given orally to Group B3 as supplemental dosages for four weeks. For every 100 grams of distilled water, one milliliter was administered. We determined the ideal dosage with the use of a pilot technique and a reference paper.[13,14] All housing conditions were the same for the animals in the experiment and the control group. The experiment was deemed to have begun on Day 0. The rats in each of the four groups were sacrificed after 28 days of drug administration. Before each animal was sacrificed, its overall weight was ascertained. Placed on a sterile sheet of paper on a dissecting board, the rats were put to death by breathing in chloroform once they had ceased breathing and moving.[15] The muscles and skin covering the neck and ventral thoracic areas were delicately peeled off using scissors and forceps. A total of three incisions—one at the collarbone, one on either side of the ribcage, and two lateral incisions—were made to allow for a thorough examination of each lung lobe. During this procedure, the lungs and heart were exposed for closer inspection. Close to the jaw, the trachea was grasped using forceps. The trachea was entirely sliced through above the forceps using scissors. Following a careful lifting of the trachea with forceps and a meticulous cutting of the underlying tissue connections with scissors, the lungs, heart, and trachea,

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collectively talk about to as the "pluck," were taken out from the body without incident. After that, the lungs were laid out in a flat position on the surface of the work and submerged them in a fixative solution with a tissue-to-fixative ratio of around 20:1. For the purpose of histological analysis, the transverse segment of the left lobe was extracted.[16,17] It was placed in neutral buffered formalin (NBF) for 24 hours for fixation. It was passed through the ascending series of alcohol from 70% to 100% and cleared in xylene according to the schedule from Bancroft. Filtered paraffin wax with a melting point of 56-58°C was used for infiltration. The sections were cut at 5µm and stained with hematoxylin and eosin (H&E).

Ashcroft fibrosis score was used to score lung fibrotic changes. Slides were examined at 10x objective lens. With 0 representing normal lungs and 8 representing complete fibrosis, each field was given a score. The average of the scores received in each field was used to determine the section's fibrotic score.[18]

After completion of the study at four weeks all the animals were euthanized by chloroform inhalation. Terminal blood (2ml) was obtained to evaluate biochemical parameters by cardiac puncture method. Serum estradiol levels were measured in pg/ml by ELISA.

Data Analysis

Data analysis was done by using Statistical Package for the Social Sciences (SPSS) software version 26. Mean ± S.D. was used to express all the data. For quantitative variables, one-way ANOVA was used in conjunction with the Post Hoc Tukey test to identify significant differences between the experimental and control groups.

Results

The lung tissue sections of control group A showed normal histological structure (Figure IA). Experimental groups B1 (Folic Acid group) and B2 (Cobalamin group) showed marked histopathological changes in the lungs (Figure I B, C). These mice showed signs of fibroblast development and thickening of the interalveolar septa due to the infiltration of macrophages and lymphocytes. There were less noticeable changes in the histology in the B3 - F.A. + Cobalamin group compared with the other groups (Figure ID). For Ashcroft score the difference between the groups was statistically significant (p = 0.000) (Figure II).

Serum estradiol levels were normal in control group A and B1 (Folic Acid group), and the levels were elevated in group B2 (Cobalamin group) and B3 (Folic Acid + Cobalamin group). Pair-wise comparison by posthoc Tukey test showed that the Estradiol levels were significantly higher in group B2 (Cobalamin group) and B3 - F.A. + Cobalamin groups than group A – Control and B1 (Folic Acid group) (p=0.000). (Table I).

Discussion

The estrous cycle is essential for the reproductive health of female mammals, including rats, and can be influenced by various factors, such as dietary components and hormonal imbalance. Folic acid and cobalamin, commonly used to treat megaloblastic anemia and prevent neural tube defects, are often added to foods for fortification.[7] This has led to a dramatic rise in the amount of folate and cobalamin consumed by the general public. Fasting blood samples with folate values greater than 45 nmol/L (19.8 ng/mL) are often considered to have supraphysiological levels.[19] Raised up levels of folic acid and cobalamin yonder the physiologic range can function as endocrine-disrupting chemicals, potentially interfering with normal hormone signaling and function. This highlights the importance of carefully considering the implications of increased folate and cobalamin levels on endocrine health.

The present study provides evidence supporting the hypothesis that elevated levels of cobalamin, either alone or in combination with folic acid, may contribute to significant histopathological changes in lung tissue, as well as an increase in serum estradiol levels. These findings align with previous studies suggesting a link between excessive vitamin supplementation and potential endocrine disruption, which can influence the development of non-reproductive organ cancers, including lung cancer.[20]

Our results revealed that rats in the B2 (Cobalamin) and B3 (Folic Acid + Cobalamin) groups exhibited significantly advanced serum estradiol levels paralleled to the regulator group and the B1 (Folic Acid) cluster. This elevation in estradiol levels corresponds with the observed histopathological alterations in lung tissue, specifically, the cell division of fibroblasts and the thickening of interalveolar septa caused by the infiltration of macrophages and lymphocytes. These changes were most pronounced in the B2 group, where cobalamin was administered alone, suggesting a stronger correla-

Table 1. Pairwise comparisons - Significance values from post-hoc Tukey Test

Variable	A vs B1	A vs B2	A vs B3	B1 vs B2	B1 vs B3	B2 vs B3
Serum Estradiol Levels	0.439	0.000*	0.000*	0.000*	0.000*	0.977

p-value indicates significance level by Post-Hoc Tukey test.

p-value <0.05 was considered significant & marked by Asterix (*)

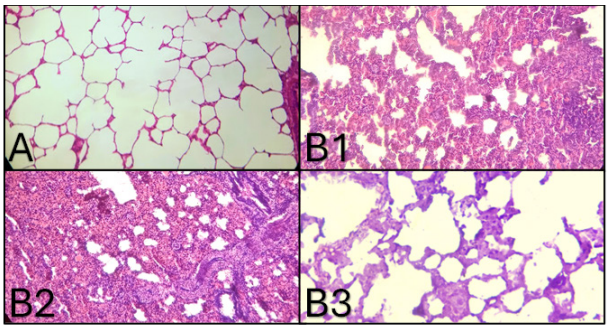

Figure 1: A photomicrograph of the lung tissue stained with hematoxylin and eosin (A) reveals the lung tissue in its normal histological state; B1 shows some thickening of the alveolar walls; B2 shows some developing fibrosis and obvious structural damage to the lung; and B3 shows some thinned alveolar walls caused by fibrous tissue, but not much.

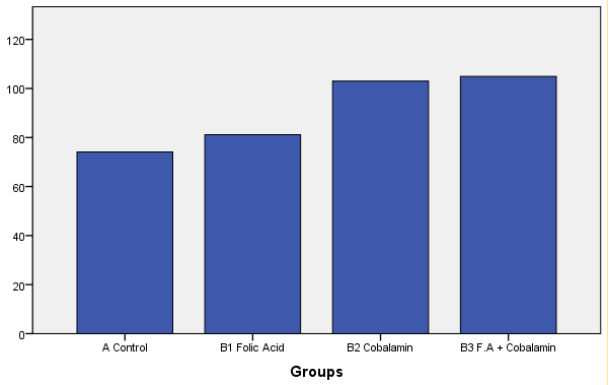

Figure 2: Serum Estradiol levels

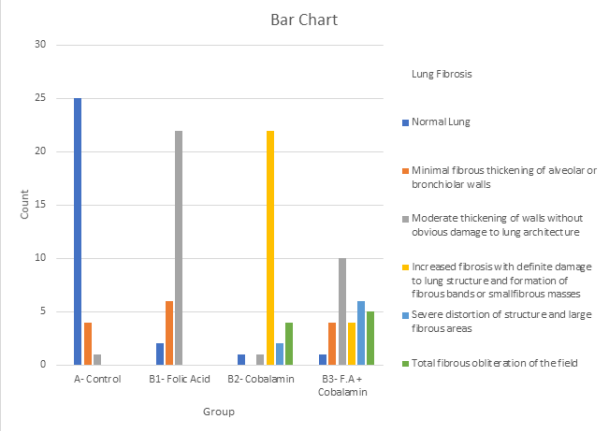

Figure 3: Ashcroft Score Distribution for Lung Fibrosis in Control and Experimental Groups

tion between cobalamin and lung tissue damage.

The correlation between high serum estradiol levels and lung fibrosis observed in this study could be explained by the endocrine-disrupting properties of cobalamin. Excessive intake of cobalamin may interfere with normal hormone signaling pathways, leading to an imbalance in estradiol levels. Elevated estradiol, in turn, has been implicated in various pathogenic processes, including the promotion of fibrotic responses in tissues. The presence of higher estradiol levels in the B2 and B3 groups suggests that cobalamin, either by itself or in conjunction with folic acid, may exacerbate these fibrotic processes within the lungs.[21]

Moreover, the interaction of cobalamin with the one-carbon metabolism pathway, particularly with homocysteine and methionine, might further contribute to carcinogenic processes, including lung cancer development. The disruption of this pathway by excessive cobalamin could lead to aberrant DNA methylation, a well-established mechanism in cancer pathogenesis.[22] Our findings support this notion, as the histopathological changes observed in the lung tissue of the B2 and B3 groups indicate a potential for cobalamin-induced carcinogenesis, mediated through both hormonal imbalance and epigenetic alterations.

The increased estradiol levels in the B2 and B3 groups may also have implications for the gender disparity observed in lung cancer incidence, particularly among female non-smokers. Given that estradiol is a primary female sex hormone, its elevation due to excessive cobalamin intake could partly explain the higher susceptibility of females to lung cancer in the absence of traditional risk factors such as smoking. This aligns with previous studies suggesting that hormonal and reproductive factors play a critical role in the development of lung cancer in women, especially in premenopausal individuals.

Limitations

Regarding the interpretation of the findings, it is important to take into account the fact that this study contains a number of limits. The study involved only female rats, which restricts the applicability of the findings to males and does not account for potential gender differences in response to these supplements. Further research is needed to clarify the molecular pathways involved.

Conclusion

Cobalamin, either alone or in combination with folic acid, induces significant histopathological lung changes and elevates serum estradiol levels.

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Authors' Contribution Statement

AI contributed to the conception, design, acquisition, analysis, interpretation of data, drafting of the manuscript, critical review, and final approval of the version to be published. HBS contributed to the design, acquisition, analysis, interpretation of data, and critical review of the manuscript. MAR contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. AH contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. BUKA contributed to the acquisition, analysis, and interpretation of data. KS contributed to the acquisition, analysis, and interpretation of data. All authors are accountable for their work and ensure the accuracy and integrity of the study.

Confilct of Interest

Authors declared no conflict on interest

Grant Suppport and Financial Disclosure

None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.