

PREVALENCE OF SERUM FOLIC ACID DEFICIENCY AND DEMONSTRATION OF HAEMATOLOGICAL PARAMETERS AMONG POPULATION OF THARPARKAR SINDH, PAKISTAN

Suresh Kumar Langhani¹, Amna Khan², Vinita Kumari³, Salma Parween⁴, Faiza Zeeshan⁵, Shahida Kashif⁶

ABSTRACT

Background: Folate or vitamin B9 is essential for synthesis of DNA and maintenance of erythropoiesis. Deficiency of folic acid leads to macrocytic anaemia, cognitive impairments, cardiovascular diseases and obstetric complications. The present study was aimed to identify the prevalence of serum folic acid deficiency and to demonstrate haematological parameters among lactovegetarians and non-vegetarian population of village Chachro, district Tharparkar of Sindh province. **Methods:** A community based descriptive cross-country sectional study was conducted during 2016 with 200 apparently healthy males and females either lactovegetarian or non-vegetarian aged between 15-55 years. Venous blood samples were collected for CBC and serum folic acid levels. Samples were labelled properly, cold chain was maintained and samples were then transferred to Dow diagnostic research laboratory. Sample size was collected by using Open EPI software and data was analysed by using SPSS version 16.0. **Results:** The mean age of participants with SD was 30.5± 8.36 and 30.13± 9.22 for lactovegetarian and non-vegetarian group respectively. Prevalence of decrease haemoglobin 22(22%) and serum folic acid 23(23%) was higher among non-vegetarians. Serum folic acid levels (p=0.002) and haemoglobin levels (p=0.01) were significantly low in non-vegetarian group. However erythrocyte count (p=0.01), total leukocyte count (p=0.01), neutrophil count (p=0.001), lymphocyte count (p=0.00) and monocyte count (p=0.002) was significantly low in lacto-vegetarian group. **Conclusions:** Our study concluded increased prevalence of decrease haemoglobin and serum folic acid levels among non-vegetarian population. However, erythrocyte count, total leukocyte count, neutrophil count, lymphocyte count and monocyte count was high among non-vegetarian group as compared to lactovegetarians.

Keywords: Folic Acid, Haemoglobin, Vegetarians, Erythrocyte Count, Total Leukocyte Count, Haematocrit, Platelet Count.

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1. AP, Department Of Pathology, Sindh Medical College, Jinnah Sindh Medical University
2. Student MBBS Final; SMC Karachi.
3. Lecturer, Pathology, SMC, JSMU Karachi
4. Lecturer, Department Of Pathology, Sindh Medical College, Jinnah Sindh Medical University
5. Lecturer, Pathology, SMC, JSMU Karachi
6. Lecturer, Pathology, Liaquat College of Medicine and Dentistry. Karachi

Corresponding author: Dr. Suresh Kumar Langhani, Assistant Professor, Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University

Email: mukhi_suresh@yahoo.com

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INTRODUCTION

Folate also called as vitamin B9 is naturally occurring water-soluble B-complex vitamin. The synthetic form of folate called folic acid has increased bioavailability than folate and is available in the form of supplements^{1, 2}. Daily recommended nutrient intake of folic acid is 400mcg³. Fruits, green leafy vegetables, whole grain, cereals, liver, yeast, beans, peas and lentils are some of the important natural sources of folic acid whereas in developed countries across the world some foods are also fortified with it^{1,2, 4}. Biologically active form of folic acid, tetrahydrofolic acid is necessary for synthesis of purines, pyrimidine, nucleoproteins and maintenance of erythropoiesis^{1,5}.

Inadequate intake, increased demand, excessive heating during cooking, malabsorption, alcoholism, use of some drugs like anticonvulsant and congenital deficiency of

enzymes required for folate metabolism are some of the important causes of folic acid insufficiency^{1, 6}. Deficiency of folic acid leads to macrocytic anaemia, neuropathy, cognitive impairment, neuropsychiatric disorders, cardiovascular diseases (due to increase in homocysteine levels) and obstetric complications like pre-eclampsia, spontaneous abortion, placenta abruption, neural tube defect in foetus and preterm delivery^{1,7}. Therefore, World Health Organization recommended 400µg of folic acid throughout pregnancy⁸. High prevalence of folic acid deficiency was reported among older adults in Ireland. However, in Europe, individuals had sufficient levels of serum folate⁹. In India, 33.33% of participants with folic acid deficiency had macrocytic anaemia¹⁰. Recent study in Iran reported higher prevalence of folic acid deficiency in females as compared to males¹¹.

Previous study conducted in Pakistan during 2009 reported that 39.7% of the participants had folic acid deficiency. However, it was conducted in context of only non-vegetarian population¹². District Tharparkar, inhabited by Hindus and Muslims is located in province of Sindh, Pakistan. Lack of education, knowledge, basic health facilities, different dietary patterns according to religious beliefs of two different populations, unavailability of clean drinking water and poor socioeconomic conditions are some of the major risk factors contributing to deficiency of micronutrients such as vitamin B12 and folic acid. Therefore, the current study was designed to identify the prevalence of folic acid deficiency and to demonstrate the haematological parameters among lactovegetarian and non-vegetarian population of district Tharparkar. To the best of my knowledge, no such study has been conducted until now in Pakistan focussing rural population belonging to two different groups i.e. lactovegetarian and non-vegetarian.

METHODS

A descriptive cross-sectional study was conducted between June 2016 to November 2016 with a total of 200 participants comprising of 100 lactovegetarians and 100 non-vegetarians from village Chachro, district Tharparkar, of Sindh province. Ethical approval was obtained from ethical review committee of Dow University of Health Sciences (IRB-305/DUHS-11). Apparently, healthy males and females aged between 15-55 years either lactovegetarian or vegetarian, residents of village Chachro were included in our study. All non-consented participants, aged less than 15 years or more than 55 years, participants with history of blood transfusion, any vitamin intake in last 3 months, persistent diarrhoea, recent worm infestation and pregnant women were excluded from our study. A non-probability convenient sampling technique was used to enrol the participants. Inhabitants of Chachro, district Tharparkar were requested to join open gathering in local meeting place called Autaq by making announcement in mosque. Participants were selected by taking short personal history/interview to fulfil the inclusion and exclusion criteria carefully. After explaining the participants about the importance of project in local language, informed consent was obtained in their local language i.e. Sindhi. Before sample collection, site of venepuncture (cubital fossa) was cleaned with 70% Ethanol. For complete blood count, 7cc blood was withdrawn from cubital vein and was collected in sterile purple top tube containing EDTA. For serum folic acid levels, 5cc blood was collected in sterile yellow top tube containing silicone gel. Both the test tubes were then labelled properly. After 20-25 minute, blood in the silicone gel was centrifuged at the rate of 1500 rpm for 6 minutes to separate the serum. All the samples were placed in two ice boxes. Samples for Complete

Blood Count and folic acid were kept at 4 degree centigrade and at 0 degree centigrade respectively. Cold chain was maintained in this way and samples were then transferred to Dow Diagnostic Research Laboratory within 8-10 hours. Lab code was given to each sample and was handed over to haematology and biochemistry departments for analysis. For complete blood count automated cell counter TAC- alpha (5parts) was used and for serum folic acid, COBUS E411 analyser was used. Sample size was calculated by using Open EPI software with confidence interval of 95% (z score=1.96), 84.7%¹³ as prevalence from previous study and 5% allowable error of known prevalence, the sample size obtained was 200. Data was analysed by using SPSS version 16.0. A P-value of less than 0.05 was considered significant. Descriptive statistics were used to determine mean and standard deviation for numerical variables. Categorical variables were expressed in frequency and percentages.

RESULTS

A total of 200 participants comprising of 100 lactovegetarians and 100 non-vegetarians were included in our study. Majority of the participants in lactovegetarian 68(68%) and in non-vegetarian 79(79%) group were males. Ratio of male to female in lactovegetarian and non-vegetarian group was 68:32 and 79:21 respectively. Mean age with standard deviation was 30.5± 8.36 and 30.13± 9.22 for lactovegetarian and non-vegetarian group respectively. Prevalence of folic acid deficiency in lactovegetarian group was 7% whereas in non-vegetarian group was 23%. Decreased haemoglobin concentration was reported in 9(9%) lactovegetarians and 22(22%) non-vegetarians (**Table 1**). On direct questioning, weakness and fatigue 54(54%) was reported as most common symptoms of anaemia in lactovegetarians and dyspnoea in non-vegetarians 39(39%) followed by dyspnoea (**Figure-1**). Serum folic acid levels (p=0.002) and haemoglobin levels (p=0.01) were significantly low in non-vegetarian group as compared to lactovegetarian group. However erythrocyte count (p=0.01), total leukocyte count (p=0.01), neutrophil count (p=0.001), lymphocyte count (p=0.00) and monocyte count (p=0.002) was significantly low in lactovegetarian group as compared to non-vegetarian group (**Table-02**). Significant difference in mean value was observed between two groups for serum folic acid (P<0.01), total leukocyte count (P=0.01), erythrocyte count (0.00) and Platelet count (P=0.020) using independent sample t test (**Table-03**).

DISCUSSION

Due to lack of knowledge and awareness among public, importance of micronutrients such as folate, vitamin B12, iron etc. is usually overlooked.

Table 1: Serum folic acid and haemoglobin levels in lactovegetarian (N=100) and non-vegetarian (N=100) individuals according to gender.

Variables:	Folic acid deficient (≤2.6ng/ml)		Folic acid sufficient (>2.6ng/ml)		Haemoglobin deficient (<130g/l for male and <120g/l for female)		Haemoglobin sufficient (>130g/l for male and <120g/l for female)	
	Lacto-vegetarian N (%)	Non-vegetarian N (%)	Lacto-vegetarian N (%)	Non-vegetarian N (%)	Lacto-vegetarian N (%)	Non-vegetarian N (%)	Lacto-vegetarian N (%)	Non-vegetarian N (%)
Male	4(5.88)	21(26.5)	64(94.1)	58(73.4)	4(5.88)	12(15.2)	64(94.1)	67(98.5)
Female	3(9.4)	2(9.5)	29(90.6)	19(90.5)	5(15.65)	10(47.6)	27(84.3)	11(52.3)
Total	7(7.0)	23(23.0)	93(93.0)	77(77.0)	9(9.0)	22(22.0)	91(91.0)	78(78.0)

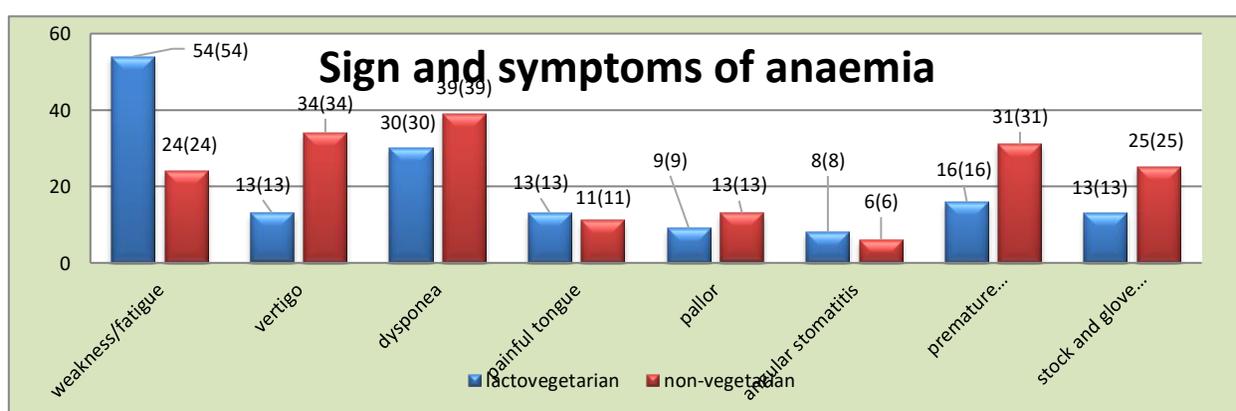
Table 2: Association of serum folic acid levels and haematological parameters with lactovegetarian (n=100) and non-vegetarian (n=100) population using Chi-square test.

Variable:	Lacto-vegetarian N (%):	Non-vegetarian N (%):	p-value
Folic acid deficiency	7(7)	23(23)	0.002
Decrease haemoglobin	9(9)	22(22)	0.01
Decrease erythrocyte count	36(36)	20(20)	0.01
Decrease haematocrit	19(19)	23(23)	0.48
Leukopenia	26(26)	12(12)	0.01
Decrease MCV	30(30)	26(26)	0.52
Decrease MCH	6(6)	7(7)	0.77
Neutropenia	40(40)	18(18)	0.001
Lymphocytopenia	61(61)	34(34)	0.00
Monocytopenia	11(11)	3(3)	0.002
Eosinopenia	5(5)	8(8)	0.38
Thrombocytopenia	4(4)	7(7)	0.35

Table 3: Association of mean values of serum folic acid and haematological parameters with lacto-vegetarians and non-vegetarians.

Variable	Lactovegetarian Mean ± SD	Non-vegetarian Mean ± SD	p-value
Folic acid	6.72±2.70	4.41±2.79	<0.01
Haematocrit	40.42±4.45	40.48±5.33	0.923
RBC count	4.33±0.53	4.62±0.53	0.00
TLC	6.09±1.62	6.71±1.82	0.01
Platelet	2.39±62.90	263±81.99	0.020

Figure1: Distribution of sign and symptoms of anaemia N (%).



In order to save the population from nutritional deficiency anaemia studies are required in different areas of Pakistan to detect clinical and subclinical cases of folate deficiency. The exact frequency of folic acid deficiency is not known however, countries that do not have food fortification programmes have high proportion of folic acid deficiency¹⁴. Majority of the participants in lactovegetarian 68(68%) and non-vegetarian 79(79%) group were males. This is because females especially in rural areas mostly

like to do house chores and prefer to stay at home. They are least interested in attending surveys, screening camps and research programs due their religious beliefs, customs, values and traditions. Our study mainly focussed on age group ranging from 15-55 years in order to limit the consequences of increasing age on folic acid levels. Nevertheless, no significant association was found between age and folic acid levels between two groups in our study. Hence, we can say that folic acid deficiency in our study

population could be due to low socio-economic status, lack of knowledge regarding sources and importance of folic acid. Our study reported that prevalence of folic acid in lactovegetarian group was 7(7%), whereas in non-vegetarian group was 23(23%). Only 4(5.88%) out of 68 males and 3(9.4%) out of 32 females in lactovegetarian group and 21(26.5%) out of 79 males and 2(9.5%) out of 21 females in non-vegetarian group had folic acid deficiency. In contrast to this, study in Thailand reported higher prevalence of folic acid deficiency among females in non-vegetarian group¹⁵. In our study only 4(5.88%) males and 5(15.65%) females in vegetarian group and 12(15.2%) males and 10(47.6%) females in non-vegetarian group had anaemia. However, study conducted in India reported higher prevalence of anaemia in non-vegetarian females¹⁰. Therefore, in general, according to our findings folic acid and haemoglobin deficiency was more prevalent in males as compared to females if we look at total number of males and females. However, percentage wise, haemoglobin deficiency was more prevalent in females. It could be due to decrease number of total participating females as compared to males. So, it would be better to consider equal number of males and females in further studies to get more accurate prevalence among two genders. Our study reported significantly low levels ($p=0.002$) of serum folic acid in non-vegetarian group as compared to lactovegetarians. Our findings are in contrast to study conducted in Spain where significantly higher levels ($P=0.019$) of homocysteine were reported in lactovegetarians which is usually elevated in folic acid deficiency¹⁶. Similarly, study done by Gilsing et al in 2010 reported higher folate concentrations among vegetarians as compared to meat eaters¹⁷. Hence, further studies should be done to look into the cause of increased prevalence of folic acid deficiency in non-vegetarian group. Although apparently healthy individuals were enrolled in our study, but on direct questioning and clinical examination, fatigue and weakness were reported as the most common symptom of anaemia experienced by the participants. It was in accordance with study conducted by Ankita Dhanuka et al¹⁰. Another study in India reported that angular stomatitis was present in 57% of the participants whereas in our study only 14(0.07%) participants had complained of angular stomatitis¹⁸. MCV is the sensitive index in diagnosis of macrocytic anaemia. However, in our study no significant association was found between serum folic acid levels and MCV between both groups. Other studies indicated that raised MCV is usually associated with Vitamin B12 deficiency in lactovegetarian as well as non-vegetarian group but normal MCV levels does not exclude macrocytic anaemia. Other causes of raised MCV include alcohol consumption, hypothyroidism, liver diseases, pregnancy and reticulocytosis. We collected data after excluding all these factors by taking short history of participants^{19, 20}. In our study, 6(6%) lactovegetarians and 7(7%) non-vegetarians had

laboratory evidence of leukopenia. No significant correlation was found between folic acid and total leukocyte count ($P>0.05$). Different studies reported that the effect of vitamin B12 and folic acid deficiency on White Blood Cell lineage occurs late in the disease²¹. In our study mean values of total leukocyte count (6.71 ± 1.82), platelet count (263 ± 81.99) and erythrocyte count (4.62 ± 0.53) were significantly higher in non-vegetarian subjects with P value of 0.01, 0.020 and 0.00 respectively, whereas mean value of haematocrit (40.48 ± 5.33) was not significantly higher ($p>0.05$). Our findings related to leukocyte count, total, erythrocyte count and platelet count were in accordance with study of India and UK^{22, 23}. The results may not be generalized over entire population of Sindh due to small sample size. Folic acid deficiency can be confirmed with a normal serum vitamin B12 level, methyl malonic acid (MMA) level and elevated homocysteine level, which were not performed due to financial problem.

CONCLUSION

Our study concluded that non-vegetarian population seems to be more prone to folic acid and haemoglobin deficiency. Hence they should take folic acid supplements and increase the intake of green leafy vegetables, meat, fish, eggs, beans and pulses to prevent the consequences of folic acid and haemoglobin deficiency.

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Ethical Approval/ Disclosure: Ethical review committee of Dow University of Health Sciences approved this study (IRB-305/DUHS-11). ROCHE Pvt Pakistan funded the project.

Patient Consent: Written informed consent was acquired from participants in their local language i.e. Sindhi before data collection

Authors' Contribution: SK conceived the idea, did literature review and design study, AK did literature review and manuscript writing, VK did literature review and final approval, ZI did statistics, critical review and editing, FZ did literature review and statistics, SK did literature review and data collection.

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