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Original Article

Public Health Section

Micronutrient Deficiency and Burden of Anaemia: A Population-based Study from Haryana, India

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ABSTRACT

Introduction: Anemia is a state where there is reduction in the number of red blood cells or haemoglobin levels (a major component in red blood cells), resulting in the lowering of overall oxygen-carrying capacity of the blood. In addition to this, nutritional anemia is caused due to deficiency of micronutrients such as folate and vitamin B12 that are vital for the development of red blood cells.

Aim: To assess the role of micronutrient (i.e. folate and vitamin B12) deficiency in the causation of anaemia.

Material and Methods: Cross-sectional study on apparently healthy individuals of either sex aging 30-65 years from nine villages of Palwal district of Haryana, Northern India was conducted 2012 month of September to November, 2013 February to April, July to November 2014. Complete Blood Count (CBC) was done using Sysmex-KX 21 (Transasia) and homocysteine, vitamin B12 and folate levels were measured by chemilumniscence using Immulite-1000 (Siemens-Diagnostic-Products, and Flanders, NJ, USA). Anaemia status was categorised into macrocytic, normocytic and microcytic anemia, based on Haemoglobin (Hb) and Mean Corpuscular Volume (MCV) levels.

Results: Prevalence of anaemia, folate deficiency and vitamin B12 deficiency was found to be 51.9%, 36.9% and 45.5% respectively. Anaemia was found to be highest in the age group 41-50 years. Individuals with macrocytic, microcytic and normocytic anaemia showed high prevalence of micronutrient deficiency i.e. vitamin B12 and folate.

Conclusions: Majority of anaemic in an apparently healthy population indicates a forthcoming economic burden in the community. Micronutrient deficiencies along with hyperhomocysteinemia among anaemics indicate a plausible co-existence of iron and vitamin B12 deficiency in this cohort. The study hints towards the need for awareness regarding dietary sources of micronutrients along with their supplementation among vegetarian populations.

Keywords: Folate, Lacto-vegetarian population, Public health, Vitamin B12

INTRODUCTION

Anaemia is a condition characterized by reductions in haemoglobin concentration, red-cell count, or packed-cell volume, and the subsequent impairment in meeting the oxygen demands of tissues [1]. In country like India which has a large population size along with vast socio-cultural diversity and cultural barriers towards some food items, people are more prone towards nutritional anaemia which is generally asymptomatic in nature [2]. According to World Health Organization, around 2 billion people, amounting to over 30% of the world's population are anaemic. Globally, incidence of anaemia is highest in South Asia, Central and West Africa, and among the South East Asian countries, India has the highest prevalence of the anaemia, where iron deficiency is the most common cause of anaemia [3].

In India, dietary supplementation of iron is practiced and prescribed as a governmental policy since late 1970s, especially to pregnant mothers and neonates. In spite of reduction in the prevalence of anaemia it has increased from 50% to 59 % [4]. Studies conducted in this respect hints towards the fact that iron deficiency could not be the sole cause for the high incidence of anaemia in the subcontinent, and therefore other nutritional and environmental variables need to be taken into account [5]. This has also been demonstrated in a study by Rousham et al., 2013 which shows no significant decrease in anaemia in a group of school children who were given weekly iron supplementation. They further suggested that the deficiency of other micronutrients (vitamin B12 and folate) could be one of the reasons for ineffectiveness of the supplementation [6]. Other studies conducted in eastern India have also showed 50% population deficient in vitamin B12 and 11% deficient in folate [7] where there was significant role played by both the micronutrients in the causation of anaemia. Majority of population in India resides in rural areas [8] and therefore such populations are more likely to be prone to nutritional deficiencies, specifically among lacto vegetarians owing to their dietary habits and lower socio-economic status. So an attempt has been made in the present study to explore the role played by micronutrient (Vitamin B12 & folate) deficiency in the causation of anaemia and its subtypes in the selected lacto-vegetarian adult population (30-65 years), who are

prone to physical and mental impairments that undoubtedly may be aggravated with the co-existence of anaemia.

MATERIALS AND METHODS

The present cross-sectional study was carried out as a part of research project sponsored by Department of Biotechnology (DBT), Government of India. The study was approved by the Departmental Ethical committee, Department of Anthropology, University of Delhi. Participants for the present study were recruited after taking prior informed written consent.

The study population consisted of total 583 apparently individuals of either sex in the age group 30-65 years from a single endogamous population i.e. Jat community, across nine different villages of Palwal District, Haryana during 2012-15.

Apparently healthy individuals within the age group of 30-65 from Jat community.

Individuals unrelated up to first cousin were included in the study. Non-Jat individuals, individuals suffering from any illness, infections or disorders, pregnant and lactating mothers were excluded from the study.

The presently studied community is majorly a lacto-vegetarian community owing to the cultural and religious practices.

Five milliliter fasting blood samples was collected by standard venipuncture into evacuated tubes with and without EDTA (2.5 mL each) and were transported on ice to the Molecular Anthropology Laboratory, Department of Anthropology, University of Delhi for processing within two hours of collection. Complete blood count was done on whole blood using Sysmex KX-21 haematology blood analyzer. Plasma and serum were separated from evacuated tubes with EDTA and without EDTA respectively and stored at -80°C until further analysis. Total serum vitamin B12, folate and plasma homocysteine levels were measured by chemilumniscence (Immulite1000, Siemens-Diagnostic-Products, and Flanders, NJ, USA). Anaemia among men was defined as a haemoglobin concentration <11.0 g/dL and for women <12.0 g/dL [9]. Low serum folate, low vitamin B12 and high homocysteine was defined as concentration <3 ng/mL, <220 pg/mL, >15 µmol/lit respectively. On the basis of Mean Corpuscular Volume individuals were categorised into microcytic (MCV ≤80 fL), macrocytic (MCV >99 fL) and normocytic (MCV ≥81 fL and ≤99 fL) [7].

Statistical analysis was done using SPSS (version 16.0, IBM Corporation, Somers, NY). p value <0.05 was considered significant.

RESULTS

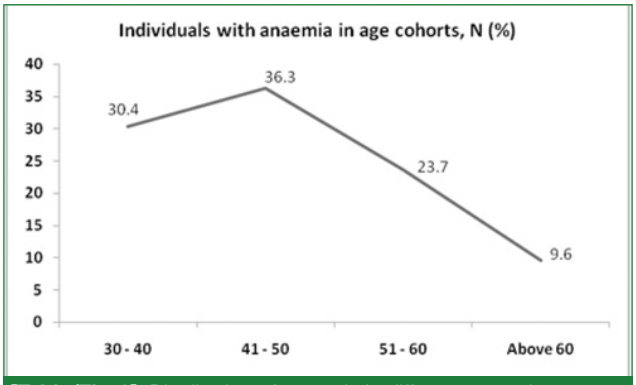
A total of 583 adults in the age group 30-65 years were recruited for the present study of which 170 (29.15%) were males and 413 (70.84%) were females[Table/Fig-1].

	Overall population N (%)	Males N (%)	Females (%)	χ^2 (p value)*
Anaemics	303 (51.97%)	83 (48.8%)	220 (53.3%)	0.95 (0.329)
Non-anaemics	280 (48.03%)	87 (51.2%)	193 (46.7%)	
Total	583	170	413	

[Table/Fig-1]: Sex-wise distribution of anaemia among the studied population.
* p value ≤ 0.05 considered significant

The median concentration of haemoglobin (Hb) was 12.10 g/dL in the present study. Though the median values of haemoglobin concentration falls within the normal range the individuals falling under the anaemic category were 51.9% (~ 52%) and its incidence among females was found to be relatively higher (53.3%) as compared to males (48.8%), though the difference was not found to be statistically significant [Table/Fig-2].

Anaemia was found to be highest in the age-group 41-50 years and a declining trend was observed with respect to



[Table/Fig-2]: Distribution of anaemia in different age cohorts.

		Total (n -583)	Anaemic (n -303)	Non-anaemic (n -280)	χ^2 (p value)*
Vitamin B12	Deficient	270 (46.3%)	138 (45.5%)	132 (47.1%)	0.15 (0.69)
	Normal	313 (53.7%)	165 (54.5%)	148 (52.9%)	
Folate	Deficient	215(36.9%)	111(36.6%)	104(37.1%)	0.01 (0.89)
	Normal	368(63.1%)	192 (63.4%)	176(62.9%)	
Homocysteine	High	453 (77.7%)	228 (75.2%)	225 (80.4%)	2.19 (0.13)
	Normal	130(22.2%)	75 (24.8%)	55 (19.6%)	

[Table/Fig-3]: Distribution of micronutrient levels and homocysteine among anaemic and non-anaemic individuals.
* p value ≤ 0.05 considered significant

	MCV (fL)			Total (n=583)
	Normocytosis (n-436)	Microcytosis (n -105)	Macrocytosis (n -42)	
Anaemics	220 (44.7%)	60 (57.1%)	23 (54.8%)	303 (51.9%)
Vitamin B12 deficient	103 (52.8%)	18 (30%)	17 (73.9%)	138 (49.6%)
Folate deficient	78 (40%)	22 (36.7%)	11 (47.8%)	111 (39.9%)
Both deficient	35 (17.9%)	7 (11.7%)	10 (43.5%)	52 (18.7%)
Both normal	74(33.63%)	27(45%)	5(21.15%)	106(18.18%)

[Table/Fig-4]: Distribution of anaemic individuals and micronutrient deficiency on the basis of mean corpuscular volume (MCV).

age. However, the prevalence of anaemia in the productive population was quite high (>65%) [Table/Fig-3].

Vitamin B12 deficiency was found to be higher among the anaemic individuals as compared to that of normal ones, though the difference among them was not found to be statistically significant. Folate deficiency was almost equally distributed among anaemic and non-anaemic groups. Also, homocysteine levels among both the groups were higher which was a general trend of the present population, may be because of vegetarian diet [Table/Fig-3].A similar trend was also observed in both sexes with no statistical significance. Since no difference was seen between the anaemic and non-anaemic group, the deficiency of micronutrients was further explored on the basis of MCV among the anaemic individuals [Table/Fig-4].

Anaemia was found to be highest in the microcytic group (57.1%) followed by macrocytic (54.8%) and normocytic group (44.7%). Vitamin B12 deficiency was found to be highest in individuals with macrocytic anaemia (73.9%) as vitamin B12 deficiency is an established cause for macrocytic anaemia. Strikingly, quite high frequency of vitamin B12 deficiency was observed among individuals with normocytic anaemia (52.8%) and microcytic anaemia (30%)[Table/Fig-4].

DISCUSSION

Anaemia is a major public health concern in the developing countries. High incidence of anaemia ~ 52% in the studied cohort is well above the reported frequency for the state among the adults[4]. However, the incidence of anaemia in the present study is lower than reported by Kaur and Kochar, 2009 (88.7%) and this could be possibly due to the inclusion criteria of the studied subjects (only females) in a wider older age group (40-80 years)[10]. It was also found to be lower than that reported by Sukla et al., 2013 (65%) in North Indian population, though the recruited individuals were in the age group 3years to ≥60 years [7]. The presently studied subjects belong to Jat community residing in rural areas of district Palwal of Haryana, north India. Consumption of milk, milk products (curd, buttermilk) and vegetarian diet is a reflection of their psycho-cultural food identity and they refrain from consumption of egg and meat. Vegetarian diet is a expected to lead to poor iron bioavailability [11,12]. Thus, the prevalence of iron deficiency is expected to be much higher in the present population due to strict vegetarian diet which is mirrored in high prevalence of anaemia in the studied cohort.

A substantial high prevalence of anaemia (66.7%) is seen in the productive population of the studied cohort (30-50 years). Individuals in the age-group 30-50 years constitute majority of the productive population group [13] and such high prevalence of anaemia in this age-group may affect the economy. Presence of anaemia may lead to fatigue, decreased ability to work, increased risk of mortality and may also lead to decline in cognition [14]. This hints towards a burden of anaemia induced morbidity and co-occurrence of reasons other than iron deficiency for anaemia in the studied population. However, a decline in the prevalence of anaemia with age may however be attributed to lower number of older individuals in the studied cohort.

Further, the deficiency of micronutrients was seen in the studied cohort. Low vitamin B12 was quite frequent (46.3%) in the overall studied cohort. Micronutrient malnutrition is a blazing situation in developing countries [15] and vitamin B12 is a micronutrient with poor bioavailability in typical Indian vegetarian diet [12]. Folate deficiency was also found to be prevalent in 36.3% of the studied cohort. Consumption of potato, eggplant and cabbage was found to be higher in the studied population owing to easy and inexpensive availability. A major dietary shift in the eating behaviour of the population from green leafy (iron and fibre rich) vegetables grown in field to carbohydrate rich vegetables has occurred in last two decades as claimed by the elderly members of the community. Thus, the consumption of dietary folate in the population is low and could be one of the reasons for low folate. Also, majorly cash crops such as wheat, pearl millet, mustard etc are grown in this geographical area and horticulture practices are rare in this area. This may be another reason for micronutrient deficiencies in the present population as also stated by Shetty, 2002 [16]. Vitamin B12 and folate deficiency leads to an increase in homocysteine levels [17]. Prevalence of high homocysteine in the studied cohort may thus be because of vitamin B12 deficiency and folate deficiency. No difference in the prevalence of vitamin B12 deficiency, folate deficiency and hyperhomocysteinemia between anaemic and normal individuals suggests that the population in general follows this trend.

The frequency of anaemic individuals with microcytosis was highest (57.1%) followed by anaemic individuals with

normocytosis and macrocytosis.This strongly suggests that causes other than iron deficiency might be contributing to high incidence of anaemia in the study population. Mal-absorption of micronutrients could also be one of the reasons that need further exploration. Smoking (both in form of beedi and hookah), consumption of tea and untreated water is present in the population that may affect iron absorption. A high prevalence of vitamin B12 deficiency in individuals with normocytic and microcytic anaemia clearly indicates that individuals with vitamin B12 deficiency otherwise expected to have macrocytic anaemia, if also have folate deficiency leads to normocytic or microcytic anaemia, as also seen in studies conducted by Ahmed et al., 2008 [18]. This hints towards a dual burden of folate deficiency and vitamin B12 deficiency in the studied cohort. Though the consumption of milk (considered to be vitamin B12 rich) is high in the studied population but the milk is heated on low fire for a prolonged duration (almost 4-5 hours) before consumption. This destroys the vitamin B12 content of the milk and therefore dietary intake is low. A study conducted by Ganji and Kafai, 2007 also found that the proportion of subjects with low serum vitamin B12 and without macrocytosis were significantly higher in post-fortification era suggesting that the folate supplementation may have led to correction of vitamin B12 induced macrocytosis but failed to address the iron-deficiency anaemia [19]. Therefore, the deficiency of both the micronutrients i.e. vitamin B12 and folate need to be addressed as reduction of anaemia with folate alone can mask concomitant vitamin B12 deficiency and may lead to other problems such as neurological problems [20]. Deficiency of vitamin B 12 along with folate is a compounding factor in the etiology of anaemia. Estimation of serum vitamin B 12 levels could possibly help in the prognosis of anaemia.

Despite the efforts in reducing anaemia nationwide, a high prevalence of anaemia among the adults in the rural North Indian population practicing vegetarianism indicates a need for considering vitamin B12 supplementation in addition to folate supplementation. So, may be the ineffectiveness of folate supplementation in reduction of anaemia is rather due to high prevalence of vitamin B12 deficiency across population groups in India, specifically in vegetarian populations. As nutritional anaemia is very common in developing countries like India, where there is huge diversity in eating patterns and dietary and cultural habits pose constraints on particular food items containing essential micronutrients. In such a situation, consumption of indigenous foods like wild plant foliage, vegetables and fruits can be an important mode of dietary diversification [21]. Along with this, one need to consider another fact that supplementation cannot be the only way out, as synthetic forms of the micronutrients are hard to metabolise and absorb and would also be an addition to the burden on the economy of developing nations. Therefore, to reduce this burden of both anaemia and supplementation on the economy, there is a need for awareness in the communities about the available resources of folate, iron and vitamin B12 in the natural form in the diet, as the prevalence of anaemia in almost 50% of the population is considered severe [9].

LIMITATIONS

One of the limitations of the present study is unequal number of individuals in either sex to retrieve further insights into the prevalence of anemia in each age-cohort.

CONCLUSION

The present study revealed anaemia to be a major health problem among apparently asymptomatic adult males and females residing in rural areas of Haryana, North India. Micronutrient deficiencies (both vitamin B12 and folate) were also higher among individuals with anaemia indicating the dual burden of their simultaneous co-existence among them. Strikingly, vitamin B12 deficiency was higher among both normocytic anaemic and microcytic anaemic individuals, hinting towards a need for the correction of both folate and vitamin B12 in order to reduce anaemia. Thus, there is a need for awareness programmes in the communities regarding the dietary food reserves of micronutrients. Also, both vitamin B12 and folate supplementation need to be considered to reduce the burden of anaemia, specifically among vegetarian populations in India.

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Disclosure statement

None of the authors report conflicts of interest. The authors are solely responsible for the content and writing of the paper.

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