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VITAMIN B_{12} DEFICIENCY AMONG A MENDELIAN POPULATION OF NORTH INDIA

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ABSTRACT

Prevalence of vitamin B_{12} deficiency has increased in community in recent time. The present study was done to assess vitamin B_{12} status with respect to age and sex. Vitamin B_{12} assays were performed on 1512 individuals from a North Indian Lacto vegetarian community and further validated on a familial sample of 79 individuals. Individuals were considered to be deficient, if vitamin B_{12} levels were <220 pg mL^{-1}. Median levels of vitamin B_{12} was below the normal range i.e. <220 pg mL^{-1}. Significant differences in median levels between males and females were observed (Mann-Whitney $p<0.01$). The results revealed no significant differences in females (χ^2 value = 37.82, $p<0.01$). Similar results among the families possibly indicate multigenerational dietary inadequacy along with the changes in lifestyle apart from vegetarian diet.

Key words: Culture, dietary habits, gender, lifestyle, vegetarianism, vitamin B_{12} deficiency

INTRODUCTION

Vitamin B_{12} is an essential water-soluble vitamin, with its key role in the normal functioning of brain and nervous system, in DNA synthesis and in the formation of blood (Solomon, 2007; Roddie, 2009). The deficiency of this vitamin was first described in 1849 and was considered to have a fatal outcome in term of homocystinuria until 1926 when a diet of liver, high in vitamin B_{12}, was shown to slow down the disease process (O'Leary, 2010). Vitamin B_{12} deficiency can lead to a wide spectrum of hematologic and neuropsychiatric disorders that can cause hyperhomocysteinemia and impaired immune system (Ciaffoni, 1992; Pradeep, 2009). The diagnosis of vitamin B_{12} deficiency has become more complicated with the classification of a "sub-clinical" deficiency category, characterized by serum vitamin B_{12} concentrations that were once considered adequate. Recent studies have suggested some disease implications associated with sub-clinical B_{12} deficiency (O'Leary, 2010), which develop most commonly due to malabsorption or dietary inadequacy. The rates of sub-clinical deficiency of vitamin B_{12} causes megaloblastic anemia, leads to elevated levels of homocysteine and methylmalonic acid and is associated with cognitive and neuropsychiatric disorders (Remacha, 1991; Pradeep, 2009). Early diagnosis and prompt treatment of patients in early stage of disorder may often produce an improvement in their condition (Erickson, 2008; Mohammad, 2010).

Vitamin B_{12} levels reportedly decreases with age (Gonzalez-Gross, 2007) which means that deficiency risk increases in parallel with age (Wolters, 2003; Loikas, 2007; Favrat, 2011). However, at younger ages a higher risk of developing vitamin B_{12} deficiency has been observed in

vegetarians (Palacios, 2003; Herrmann, 2013), individuals with gastrointestinal disease (Battat, 2014), depression (Durand, 2002), high alcohol consumption (Halsted, 2013) and among the individuals suffering from renal insufficiency (Battat, 2014). The screening and identification of individuals with subtle vitamin B_{12} deficiency and the potential benefit of vitamin B_{12} fortification are largely debated (Hvas, 2006; Allen, 2008; McLean, 2008; Iqtidar, 2012,). This may possibly be due to the absence of studies predicting the prevalence of deficiency among various ethnic groups.

South Asians, being from developing countries, have long been identified as an at-risk ethnic group for vitamin B_{12} deficiency (Gupta, 2004). Non-vegetarians in developing world also have marginal amounts of vitamin B_{12} (Wokes, 1955). People in India due to their vast heterogeneity with respect to religious, ethnic and socioeconomic practices have enormous diversity in their life style and dietary habits. Since vegetarianism has widely been practiced for several millennia in India, so large population of country is at the risk of having low vitamin B_{12} status throughout life (Antony, 2003). Thus, one may presume (in contrast to the literary evidence) that vitamin B_{12} deficiency may or may not be an effect of age or sex. Therefore, the present study was aimed to understand the age- and sex-wise status of vitamin B_{12} deficiency in a Lacto vegetarian community from a North Indian state, which has not been reported till date.

MATERIALS AND METHODS

Recruitment of the subjects

The present study was approved by the Departmental Ethical Committee, Department of Anthropology vide communication: Anth/2010/455/1 dated 29.4.2010. The study was conducted in the years 2011-2013 in two phases involving a population based cross-sectional study and a family based cross- sectional study.

Population based cross-sectional study

The participants were recruited from 15 villages of 2 blocks (Hodal and Hathin) of Palwal district of Haryana, India. These villages were inhabited by Jat community; a predominant community of Haryana. Individuals of community were mostly engaged in agriculture and practiced vegetarianism, especially milk and dairy products were more frequently consumed by the participants. Individuals (1647) of either sex, in the age group of 30-70 years, were included in the study conducted in 2011-2103. Vitamin B_{12} was available in 1512 individuals. Apparently healthy participants were included in the study whereas individuals on medication and supplementation were excluded. The participants included 455 males and 1057 females. Females were more as compared to males due to the inclusion of individuals un-related up to first cousin as the study was a genetic study. Participants were recruited through door to door household survey. Ethnicity of participants was confirmed through genealogies. Data on their demography (age and sex) and lifestyle habits (dietary habit, smoking and alcohol consumption) were collected from the participants after taking prior informal written consent.

Family based cross-sectional study

To validate the results of vitamin B_{12} deficiency a cross-sectional study was planned in which 11 families belonging to the same community, having individuals up to 3rd generation were recruited. The recruited individuals from the families were in a wide range of age groups including 8-19 years adolescents, young adults 20-30 years, 31-60 years older adults and elderly >60 years. This study was conducted after the cross-sectional study was completed and the families whose members were not included in previous phase of study were included in this phase. A total of 79 individuals of either sex in above mentioned groups were recruited. Families having any of its individual on medication and supplementation along with chronic gastritis were excluded. Informal written consent was obtained from each participant, if minor parental consent was taken, including the

consent of head of the family. The study was approved by the Departmental Ethical Committee of Department of Anthropology of the institution.

Estimation of serum vitamin B_{12} concentration

Fasting blood sample of 3 mL was collected by a trained technician from the participants. Blood samples were collected in non-EDTA coated vials which were transported from collection site to the Department of Anthropology, under refrigeration conditions within 3-4 h on the same day. Blood was centrifuged at 3000 rpm for 5 min. The clear serum was stored at -70°C until analysis. Serum vitamin B_{12} concentrations were assessed using commercial kits (Siemens Medical Solutions Diagnostics; Los Angeles, USA) on fully automated enzyme amplified chemiluminescence immuno-assay based Immulite 1000 analyzer.

Statistical analysis

The normality of distribution of vitamin B_{12} level was checked. Also, median levels were taken into consideration for the analysis of age-related changes. The median levels of vitamin B_{12} according to sex- and age-group in overall were compared using Kruskal Wallis (Goodman, 1959) whereas differences in median levels between males and females were compared using Mann-Whitney (Kasuya, 2001). Vitamin B_{12} deficient subjects and controls were compared using χ^2-test. Statistical significance was taken as P value <0.05. All statistical analyses were performed using Statistical Package for Social Sciences version 16.0 SPSS Inc, Chicago, IL, USA 2007.

RESULTS AND DISCUSSION

The mean age of the individuals recruited, irrespective of sex, for present study was 48.11±9.96 and 51.4±9.61 and 46.66±9.76 years for males and females, respectively (Table 1). The differences between males and females were significant with respect to age, where females were younger than males. Median inter-quartile range (IQR) of serum vitamin B_{12} levels of studied group were 204 pg mL^{-1} (150-308). Age-wise distribution of median levels of vitamin B_{12} were not found varying within the population. Similar kind of trend was observed with respect to the distribution of median levels of vitamin B_{12} among males. But there seemed to be a decreasing trend of vitamin B_{12} with increasing age among females. However, the observed differences in median levels of vitamin B_{12} with respect to age the cohort were not statistically significant (χ^2 value 0.674, p = 0.879). On comparing the median levels of vitamin B_{12} between male and female significant differences were observed (p<0.05), where females have higher levels of vitamin B_{12} as compared to males. Further analysis was done to see the prevalence of vitamin B_{12} deficiency. The reference cut off 220 pg mL^{-1} significant differences were observed (p<0.01), where females have higher levels of vitamin B_{12} as compared to males. Further analysis was done to see the prevalence of vitamin B_{12} deficiency. The reference cut off 220 pg mL^{-1} (Sukla, 2016) was taken for deficiency state. More than half of the

Table 1: Age- and sex-wise distribution of median levels of vitamin B_{12} among the studied north Indian population

Age groups (years)	Overall median pg mL^{-1} (IQR)	Kruskall Wallis p-value	Males median pg mL^{-1} (IQR)	Kruskall Wallis p-value	Females median pg mL^{-1} (IQR)	Kruskall Wallis p-value	Mann-Whitney
30-40	207 (154-315)		168 (150-216)		216 (160-331)		
41-50	204 (150-314)	0.197	170 (150-250)	3.5	217 (158-333)	0.67	
51-60	203 (150-298)	P = 0.98	192 (150-267)	P = 0.32	209 (157-322)	P = 0.88	P<0.001
>60	205 (153-295)		189 (153-248)		220 (155-346)		
Total	204 (150-308)		177 (150-248)		216 (158-965)		

IQR – Inter-quartile range

Table 2: Age- and sex-wise distribution of individuals (No.) with vitamin B_{12} deficiency among the studied north Indian population

Age (years)	Overall*		χ^2	Males*#			χ^2	Females*#			χ^2
	Normal	Deficient	p-value	Normal	Deficient	Total	p-value	Normal	Deficient	Total	p-value
30-40	204 (45.2)	247 (54.8)		17 (23.9) [**11.3**]	54 (76.1) [**17.8**]	71 [**15.6**]		187 (49.2) [**36.0**]	193 (50.8) [**35.9**]	380 [**36.0**]	
41-50	241 (45.0)	295 (55.0)	1.09 (0.81)	54 (32.3) [**35.8**]	111 (67.27) [**36.5**]	165 [**36.3**]	5.32 (0.26)	187 (50.4) [**36.0**]	184 (49.6) [**34.3**]	371 [**35.1**]	1.65 (0.80)
51-60	140 (42.0)	193 (58.0)		49 (36.6) [**32.5**]	85 (63.43) [**28.0**]	134 [**29.5**]		91 (45.8) [**17.5**]	108 (54.2) [**20.1**]	199 [**18.8**]	
>60	86 (44.8)	106 (55.2)		31 (36.5) [**20.4**]	54 (63.52) [**17.7**]	85 [**18.6**]		55 (51.4) [**10.5**]	52 (48.6) [**9.7**]	107 [**10.1**]	
Total	671 (44.4)	841 (55.6)	37.82 (0.01)	151 (33.3)	304 (66.7)	455		520 (49.2)	537 (50.8)	1057	

*The values in small bracket show the percent normal and deficient individuals amongst the population in a particular age group.

#The bold values shown in long brackets within a column show the percent distribution of normal and deficient individuals among male and females across the age group.

individuals were found deficient in this population. Age-wise distribution of deficient individuals revealed no significant difference with respect to age which means that vitamin B_{12} deficiency does not increase with age. Age-wise distribution of vitamin B_{12} deficiency among males and females showed similar results (Table 2). Vitamin B_{12} deficiency was significantly high in males as compared to females (χ^2 value 37.82, $p<0.001$). The frequency of deficiency across age groups among both males and females was consistent.

To validate the observed results showing no relation between age and vitamin B_{12} deficiency, vitamin B_{12} levels in 11 families were analyzed. In these families there were adolescents (8-19 years), young adults (20-30 years), adults (31-60 years) and elderly (>60 years). The individuals were divided into different age categories to understand the trends of deficiency with age through familial data. The median (IQR) levels of vitamin B_{12} were 200 (150-314) pg mL^{-1} among the individuals of families, whereas 165 (150-243) and 289.5 (165-453) pg mL^{-1} among males and females, respectively. The differences in median vitamin B_{12} levels were significant (χ^2 value 30.52, <0.001) between male and female, whereas no significant differences with respect to the age were observed (Table 3).

The major limitation of the study is more number of females as compared to the males and absence of detailed nutritional profiling of participants. The results on family data based on median levels were validated using the distribution of deficient individuals in selected age categories of 8-19 (adolescents) and 20-30 (young adults) [Table 4]. Similar results were observed showing that there was no such trend of vitamin B_{12} deficiency which is increasing with advancing age in overall

Table 3: Age- and sex-wise distribution of median levels of vitamin B_{12} among north Indian families

Age group (N)*	Median (IQR) vitamin B_{12} levels (pg mL^{-1})			Mann Whitney (p-value)
	Overall	Males	Females	
8-19 (21)	205 (152-329)	177 (150-245)	369 (185-369)	
20-30 (11)	225 (150-281)	183 (150-242)	269 (168-530)	
31-60 (29)	210 (153-335)	160 (150-220)	291 (165-417)	30.52
>60 (19)	211 (150-369)	175 (155-408)	175 (155-397)	(<0.01)
Total	200 (150-314)	165 (150-243)	289 (165-453)	
Kruskal Wallis (p-value)	0.23 (0.97)	1.25 (0.74)	2.12 (0.55)	

*N denotes the number of individuals in a particular age group

Table 4: Age- and sex-wise distribution of individuals (No.) with vitamin B_{12} deficiency among the north Indian family members

Age in years	Overall		Males		Females	
	Normal	Deficient	Normal	Deficient	Normal	Deficient
8-19	9 (42.9)*	12 (57.1)	4 (28.6)	10 (71.4)	5 (71.4)	2 (28.6)
20-30	6 (54.5)	5 (45.5)	3 (42.9)	4 (57.1)	3 (75.0)	1 (25.0)
31-60	13 (44.8)	16 (55.2)	4 (26.7)	11 (73.3)	9 (64.3)	5 (35.7)
>60	9 (50.0)	9 (50.0)	5 (55.5)	4 (44.4)	4 (44.5)	5 (55.5)
χ^2 value	0.9		0.26		0.68	

*The values in brackets show the percentage of normal and deficient individuals amongst the population in a particular age group.

population and among males and females independently. In fact, vitamin B_{12} deficiency was relatively higher in younger age groups. Similar trend of distribution of deficient individuals was observed among males. Among females there seems to be an increasing trend of deficiency with advancing age. However, this so-called observed vitamin B_{12} deficiency trend with age was statistically insignificant ($p < 0.05$).

Vitamin B_{12} is involved in myriad biochemical reactions and may prevent many chronic diseases (Lucock, 2004). The deficiency state has a very wide mark of presentation and can aggravate hematological and neuropsychiatric symptoms (Remacha, 1991; Pradeep, 2009). In early stage, the deficiency might confront with slender and subtle cognitive impairment, but later on it could be irreversible even after optimizing the vitamin levels. Hence, early identification of vitamin B_{12} deficiency becomes essential for precluding permanent damage. Dietary vitamin b_{12} deficiency is a severe problem in Indian subcontinent (Stabler, 2004; Vogiatzoglou, 2009; Arora, 2014), which was observed in the present study also. The median levels of vitamin B_{12} was found to be 204 pg mL^{-1} which was far below the normal reference range used in the present study, much lower than the levels reported by Arora *et al.* (2011) where the individuals of urban north India were studied.

In present study vitamin B_{12} deficiency was observed in 66.7% men and 50.5% women suggesting that risk of developing B_{12} deficiency is affected by gender. This is in concordance to a study conducted in Finnish elderly population where male gender was observed to increase the probability for vitamin B_{12} deficiency (Loikas, 2007). In another study conducted on South Asian patients the risk appeared to be similar for men and women (Gupta, 2007).

Both males and females were found severely deficient with respect to vitamin B_{12}, possibly due to their vegetarian diet (Antony, 2003) while on comparing males and females, contrastingly females had higher serum vitamin B_{12} levels as well as the prevalence of deficiency was low among females. The presently studied population is a rural population where the prevalence of smoking is very high (unpublished data). It is a common lifestyle among both males and females, whereas males also consume alcohol and have less physical activity being engaged in private jobs. In contrast to this, the females were engaged in household chores along with cattle rearing and other agricultural tasks making them physically active. These contrasting features in terms of alcohol, smoking (Khaled, 1986) and less physical activity (Cravo, 1996; Van den Berg, 2002) among males may make them more prone to develop vitamin B_{12} deficiency.

In general, the study revealed that vitamin B_{12} deficiency is more common in Indians as compared to the populations of other countries (Bindra, 1987, Vogiatzoglou, 2009). Among western populations vitamin B_{12} deficiency is more common and as high as 35% in people older than 65 years to that reported in lower age groups (Matthews, 1992). However, whether its younger or older age group the deficiency reported in western countries seems to be lower than that reported in Indian context (Penninx 2000; Ray, 2000; Loikas, 2007). This differential distribution of vitamin B_{12} deficiency in various world population is usually attributed to the dietary pattern, where western populations widely follow non-vegetarian dietary regimen. However, in present study, apart from

diet, other important factors like geographical variation (Bindra, 1987), different life style (Papandreou, 2006) racial and ethnic differences (Bindra, 1987), genetic factors (Feng, 2009), normal absorption of vitamin B_{12} (Roddie, 2009), and the imbalance in vitamin B_{12} and folic acid might be playing an important role. While confirming the results in the family it was observed that young adults and adolescents were equally affected, which is more than the reported earlier (Pawlak, 2014). Among adolescents the deficiency was lower than reported by Bhardwaj *et al.* (2013). Hence, it was indicated that vitamin B_{12} is not affected by age in the studied population which was not found in previous reported studies (Roehl, 2008; Feng, 2009).

The observed higher frequency of vitamin B_{12} deficiency in general in the studied population especially among males may not be attributed exclusively to dietary insufficiency. But along with life style changes, changes have kept on occurring in people's dietary habits and type of drinking water over period of time; there is a possibility that some of these changes may be associated with recently increasing vitamin B_{12} deficiency. Earlier the individuals of present population were consuming well water which has efficient micro-biota which can synthesize vitamin B_{12}. With the population growth and exploitation of available water sources causing scarcity of safe drinking water, public based reverse osmosis plants and home-based reverse osmosis units are being increasingly used (Gupta, 2016). Reverse Osmosis system removes dissolved solids as well as many microorganisms making water palatable and relatively safe to use. These mentioned factors could be playing more important role apart from vegetarian diet in the present population. Though, gender has an important influence on vitamin B_{12} deficiency while age does not seem to effect B_{12} levels which is indicative of some sort of genetic predisposition for developing deficiency in the studied population.

Conclusions: Vitamin B_{12} deficiency was found to be a major public health concern which is affecting all individuals irrespective of age and sex.

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