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Original Article

Internal Medicine Section

Association of Vitamin B12 Deficiency and Use of Reverse Osmosis Processed Water for Drinking: A Cross-Sectional Study from Western India

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ABSTRACT

Introduction: Prevalence of Vitamin B12 deficiency has increased in community in recent time. Possibility is raised for new and yet unidentified factors being associated with this increased prevalence. One of these factors frequently questioned is use of Reverse Osmosis (RO) processed water for drinking.

Aim: We aimed to study association of use of RO processed water for drinking with Vitamin B12 deficiency.

Materials and Methods: This cross-sectional study was done at tertiary care centre of Western India. Total 250 participants were recruited after excluding those participants with known factors responsible for Vitamin B12 deficiency. Information about gender, type of diet, milk intake and duration, dairy product intake, use of RO water and Vitamin B12 level was collected.

Results: Total 70 (28%) participants out of 250 were having Vitamin B12 deficiency. Forty (50.6%) of 79 participants using RO water were Vitamin B12 deficient against 30 (17.5%) of 171 using other sources. Logistic regression analysis showed independent association between use of RO water and Vitamin B12 deficiency. Although association of male gender, milk quantity of less than 100 ml per day and duration of RO water intake with occurrence of Vitamin B12 deficiency was found statistically significant in univariate analysis, logistic regression analysis did not show significant association.

Conclusion: Use of RO processed drinking water was associated with Vitamin B12 deficiency. This being cross-sectional study, further longitudinal studies with large sample size and taking confounding factors into consideration, are required to establish this association.

Keywords: Dairy products, Demineralised water, Milk intake, Micronutrients, Vegetarian diet

INTRODUCTION

Importance of Vitamin B12 in the production of DNA and RNA is been well established. Deficiency of Vitamin B12 causes macrocytic anaemia, peripheral neuropathy, dementia, weight loss, neural tube birth defects and various other adverse health effects. Recently increased prevalence of Vitamin B12 deficiency is been identified in various studies across different parts of India [1-3]. Easy availability of diagnostic facility to identify B12 deficient individuals, who were otherwise unidentified earlier as well as increased awareness among public causing voluntary testing of B12 levels may be some of the reasons for recently increased prevalence. Pure vegetarian diet lacking in milk or dairy product is one of the most commonly identified risk factors for development of Vitamin B12 deficiency, yet there is possibility that some newly developing but unidentified risk factors to be responsible for its recently increasing prevalence.

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 B12 deficiency. One of the frequently questioned such factors is use of drinking water processed by Reverse Osmosis (RO) technology. With population growth and exploitation of available water sources causing scarcity of safe drinking water, mainly in developing countries, public based RO plants and home based RO units are being increasingly used. RO system very effectively removes dissolved solids as well as many microorganisms making water palatable and relatively safe to use [4-6]. With increased use of RO processed demineralised water, many adverse health effects are also been increasing identified. Increased cardiovascular morbidity and mortality is now been well established due to use of demineralised water low in calcium and magnesium [7-9]. Recent studies also suggest association of drinking soft water with higher risk of fractures in children, neurodegenerative diseases, gastric and duodenal ulcers, chronic gastritis and goiter. Several complications are been recognized in newborns and infants, including low birth weight babies, pre-term births, jaundice, anaemia, fractures and growth disorders. Study in rats has shown increased occurrence of anaemia in rats fed with demineralised water [8]. Looking at various adverse health effects of drinking demineralised water, there is possibility that it may also be one of the newly associated factors for increasing incidence and prevalence of Vitamin B12 deficiency. Yet, it is not been proven so far by any scientific studies to the best of our knowledge.

AIM

We aimed to study association between Vitamin B12 deficiency and use of RO processed drinking water.

MATERIALS AND METHODS

This cross sectional hospital based study was done in a tertiary care teaching hospital of Western India from May 2013 to April 2014. The study was approved by the Institutional Ethics Committee. Individuals of age >18 years, who came to the hospital either for routine health check-up as a part of institutional preventive health check-up program, for OPD consultation or as indoor patient and who either voluntarily asked for or were advised by their treating consultant for testing of Vitamin B12 level, were considered for this study. Two hundred and fifty participants were selected. Written consent was obtained from all study participants. Privacy and confidentiality of study participants were maintained throughout the study. All participants fulfilling inclusion criteria were enrolled

in the study irrespective of their diet being either pure vegetarian or mix and their intake of milk and dairy products. Individuals who have taken parenteral Vitamin B12 within last two years or oral Vitamin B12 medications within last six months were excluded. Individuals with other known etiological factors for B12 deficiency namely use of H2 receptor blocker drugs or proton pump inhibitor drugs for more than 3 months; patients diagnosed with pernicious anaemia, atrophic gastritis, post-gastrectomy, ileal malabsorption, Crohn's disease, ileal resection, diagnosed tapeworm infestation and patients on metformin therapy were also excluded. Primary outcome was to find out prevalence of Vitamin B12 deficiency and its association with use of RO processed drinking water. Secondary outcome was to find out association of Vitamin B12 deficiency with gender, milk quantity, milk intake duration and frequency of dairy product intake.

Information of participant's age, sex, type of diet as pure vegetarian (never taken any of the animal products in their diet other than milk or dairy products) or mixed (taking vegetarian as well as animal products in their diet) was collected. Information was collected about milk intake quantity (< 100 ml/day or ≥ 100 ml/day) and milk intake duration (≤ 5 years or > 5 years). This included intake of milk (boiled or unboiled) without its use in any other form like in tea, coffee, milk shake or any other milk containing beverage. As per clinical observations, an average Indian adult takes 100 ml of milk per day in his/her diet. Body store of Vitamin B12 is sufficient to supply it for three to four years even if its supply is completely cut off in the diet. Hence, cut off limit of 100 ml and five years respectively were considered in our study. Information was also collected about dairy product intake (never, less than daily or daily), use of RO processed Vs other sources of drinking water and its duration (≤ 5 years or > 5 years). Vitamin B12 value was recorded. Sample for Vitamin B12 was obtained irrespective of time of the day or participant's fasting or non-fasting state. Vitamin B12 was assessed by electrochemiluminescence immuno assay. A cut off value of less than 156 pmol/L of Vitamin B12 was taken as suggestive of Vitamin B12 deficiency.

STATISTICAL ANALYSIS

The demographic information of the study participants was presented using descriptive statistics, frequency and proportions. Univariate analysis was performed with B12 levels as dependent variable and other associated factors as independent variables using chi square test. Fisher's exact test was considered for cases where the expected cell frequency was less than 5. Binary Logistic regression analysis was also done to observe the final association between independent variables and vitamin B12 deficiency present or absent.

RESULTS

Mean age of study participants was 48.5 years. Of 250 participants, 140 (56%) were males and 110 (44%) were females. Total 70(28%) participants out of 250 were having Vitamin B12 deficiency. Median age of participants with Vitamin B12 deficiency was 48, while it was 49.5 for non-deficient group. Twenty (18.2%) females and 50 (35.7%) males were deficient of Vitamin B12. This gender difference was statistically significant (p-value 0.002). One hundred ninety seven (78.8%) participants were vegetarian and 53 (21.2%) were taking mixed diet. Sixty nine (35%) of vegetarians were having Vitamin B12 deficiency against in one (1.9%) participant taking mixed diet. This was found statistically significant (p <0.05).

Seventy nine (31.6%) participants were using RO processed drinking water and 171 (68.4%) were using other sources of drinking water. Most importantly 40 (50.6%) participants using RO water were Vitamin B12 deficient against 30 (17.5%) of using other sources of water for drinking [Table/Fig-1]. This was statistically significant (p<0.001). In relation to duration of RO processed water intake, 40 participants were using it for five or less than five years and 39 were using for more than five years. Seventeen (42.5%) of participants taking RO water for five years or less had Vitamin B12 deficiency against 23 (59%) in those taking RO water for more than five years. This was statistically (p <0.05) significant.

We found more number of participants having B12 deficiency among participants who were taking milk <100 ml than persons who were regularly taking ≥100 ml. Total 57 (49.5%) out of 115 of participants with <100 ml milk intake were deficient against only 13 (12.5%) out of 135 taking milk ≥100 ml were B12 deficient. The correlation was statistically (p <0.05) significant when duration of milk intake was tested for association with B12 deficiency, correlation for duration was also statistically (p<0.001) significant [Table/Fig-2].

Regarding dairy products also we found that B12 deficiency was more prevalent in persons who are not consuming dairy products. More than half of participants were B12 deficient in this group. Forty seven (53.4%) out of 88 were B12 deficient against 21 (23.3%) of dairy products taking less than daily and two (2.8%) of taking dairy products daily. It was statistically (p <0.001) significant [Table/Fig-3].

Logistic regression analysis was done with Vitamin B12 deficiency as dependent variable. Independent variables included in Model were Sex, Diet, Milk intake duration, Dairy product intake and Water type [Table/Fig-4].

Hence with logistic regression analysis vegetarian diet, milk intake duration > 5 years, absence of dairy products in the diet and RO water consumption were found independent associated factors for Vitamin B12 deficiency.

DISCUSSION

Most important finding of this study was independent association of use of RO processed drinking water and existence of Vitamin B12 deficiency. RO system removes most of the minerals from the water. As mentioned above, use of low mineral water is been associated with various health hazards and is been well cautioned by WHO [7,8]. Most of these adverse effects are shown to be occurring because of low calcium or magnesium in RO processed water. Importance of role of other micronutrients in drinking water

Water type	Vitamin B12 Deficiency		Total	p-value
	No	Yes		
RO	39(49.4%)	40(50.6%)	79	p <0.001
All others	141(82.5%	30(17.5%)	171	
Total	180	70	250	

[Table/Fig-1]: Study of association of use of RO water with Vitamin B12 deficiency.

Milk intake Duration	Vitamin B12 Deficiency		Total	p-value
	No	Yes		
Not taking	58(50.4%)	57(49.6%)	115	p-value <0.001
≤ 5 years	3(75.0%)	1(25.0%)	4	
>5 years	119(90.8%0	12(9.2%)	131	
Total	180	70	250	

[Table/Fig-2]: Study of association of milk intake duration with Vitamin B12 deficiency.

Dairy product intake	Vitamin B12 Deficiency		Total	p-value
	No	Yes		
Never	41(46.6%)	47(53.4%)	88	p-value <0.001
Less than daily	69(76.7%)	21(23.3%)	90	
Daily	70(97.2%)	2(2.8%)	72	
Total	180	70	250	

[Table/Fig-3]: Study of association of dairy product intake with Vitamin B12 deficiency.

Variable	Odds Ratio	Confidence Interval of variable	p-value
Sex			
Female(reference)			
Male	2.256	0.938-5.260	0.060
Diet			
Mix(reference)			
Veg	13.022	1.251-135.545	0.032
Milk Intake duration			
Never (reference)			
Taking ≤ 5 years	0.514	0.044-6.012	0.596
Taking for > 5 years	0.157	0.069-0.359	<0.0001
Dairy product Intake			
Never(reference)			
Occasional	0.290	0.131-0.640	0.002
Daily	0.047	0.09-00.247	<0.0001
Water Type			
Other water type(reference)			
RO water	2.647	1.253-5.590	.011

[Table/Fig-4]: Logistic regression analysis.

is also been studied [4] and has shown possible association with various health hazards of drinking water low in these microelements [8]. Positive association between Vitamin B12 deficiency and use of RO processed drinking water found in our study suggests possibility of some similar yet unidentified mechanism responsible for development of Vitamin B12 deficiency in individuals drinking RO processed water. One of the possible mechanisms is removal of the cobalt by RO system. Cobalt is essential component of Vitamin B12; its removal from the drinking water may be one of the reasons to develop Vitamin B12 deficiency. Like other micronutrients, drinking water may not be a major source of daily cobalt intake. But in modern diet many times it is not adequate in supplying minerals and micronutrients in adequate quantity and an individual may have meagre store or borderline deficiency of these elements. In such situation, even the small quantity available in drinking water may play vital role in preventing their deficiency and subsequent consequences [8]. Second possible mechanism for this association may be reduced absorption of Vitamin B12 available in diet, due to low mineralized water causing chronic atrophic gastritis. Third possible mechanism may be RO system removing those microorganisms responsible for endogenous production of Vitamin B12 directly or indirectly. Although there are many unpublished literatures and clinical observations suggesting possibility, we could not find any scientific study mentioning positive or negative association between RO processed drinking water and Vitamin B12 deficiency.

Prevalence of Vitamin B12 deficiency was 28% in our study. Our study included participants coming to tertiary care centre and who had voluntarily asked for or were advised by their treating consultants about Vitamin B12 level. This increases the chances of recruitment of those participants who are more likely to be Vitamin B12 deficient. Hence, our study population is not representative of actual local community. Studies from other regions of India have shown different prevalence rate from 43% to 81% [1-3]. The difference is mainly because of different study setting, study populations, different dietary habits of regional population and different cut off value used to define Vitamin B12 deficiency.

Other independent associated factors in our study were vegetarian diet, milk intake duration > 5 years and absence of dairy products in the diet. Vegetarian diet without milk or dairy products is well known risk factor to develop Vitamin B12 deficiency. We included participants with vegetarian diet in our study because majority of population takes vegetarian diet in this western part of India and it was not possible to recruit required sample size within limited period of study; nevertheless studies done on participants taking mix diet may give more clear association with other possible risk factors. Milk and dairy products remain important sources of Vitamin B12 supply especially in vegetarian diet. Our study showed protective effect of intake of milk of more than five years as well as dairy product intake against development of Vitamin B12 deficiency.

In univariate analysis, positive association was observed between Vitamin B12 deficiency and male gender, milk quantity of <100 ml/day and duration of RO processed water use of > five years; in logistic regression analysis it was found non-significant. Nevertheless, studies with large sample size and exclusively focusing on each of these factors separately may give us better clarity about their association with Vitamin B12 deficiency. In study by Arora S in North Indian population, women were more prone to develop Vitamin B12 deficiency compared to men [1]. In another study done at Finland, males were more at risk compared to females [10]. Association of Vitamin B12 deficiency with gender shown in other studies may be because of sample population selected for the study, local cultural factors and difference in dietary habits between males and females. Importance of milk in diet is been well established protective factor but quantity of milk is not been studied to the best of our knowledge. Scientific basis to define minimum quantity of milk required to prevent Vitamin B12 deficiency in individuals may be helpful in counselling patients during consultation as well as sometimes forming policy applicable to large size population. Logically if RO water is associated with Vitamin B12 deficiency, longer the duration of RO water consumption more likely is the possibility of developing Vitamin B12 deficiency.

LIMITATION

There were certain limitations also with our study. First, this being cross-sectional study, cause-effect relationship is not known. Second, details of type of RO unit, amount of demineralisation done by the unit and its technical information were not collected. Third, information about use of RO water was collected only verbally from participants. Fourth, this study being done at tertiary care centre, sample was not true representative of actual community. Moreover, participants who were either voluntarily asking for or who were advised Vitamin B12 level by their treating clinicians were selected; this may incur selection bias to some extent. More sensitive as well as specific methods like measurement of Methylmelonic Acid, holo-Transcobalamin and homocysteine levels were not used for assessing Vitamin B12 deficiency. Nevertheless, this study shows at least some possibility of another hazard of using RO water. It also gives the basis for the researchers for doing further longitudinal studies to assess the relationship between uses of RO processed drinking water and Vitamin B12 deficiency.

CONCLUSION

Prevalence of Vitamin B12 deficiency has increased in recent time. Use of RO processed drinking water has also increased significantly in recent time. Various studies have shown adverse health effects of drinking demineralised water processed by RO system. In our cross-sectional study, we found positive association between drinking RO processed water and Vitamin B12 deficiency. Although association of male gender and milk quantity of less than 100 ml per day for developing Vitamin B12 deficiency were not established statistically, positive trend was observed. Further longitudinal researches, with large sample size and taking various confounding factors into consideration, are required to study their association with recently increasing prevalence of Vitamin B12 deficiency.

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