Plasma Vitamin B-12 Levels and Risk of Alzheimer's Disease: A Case-Control Study

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Abstract

Introduction: Vitamin B-12 deficiency is a frequent condition in the elderly population. High homocysteine levels, which can contribute to arterial damage and blood clots in blood vessels, usually indicate a deficiency in vitamin B-12. Different studies have shown an association of raised total homocysteine with incident Alzheimer's disease. This study aimed to evaluate the association between vitamin B-12 levels and the risk of Alzheimer's disease (AD). Methods: A case-control study with a sample size of 90 was conducted at Tertiary hospital, Kathmandu. The participants who visited the psychiatric outpatient department from 2019 onward at Tertiary hospital, Kathmandu, were recruited. The Mini-Mental State Examination (MMSE) was administered to the participants by a trained medical doctor. The medical doctor used the MMSE scores to classify the participants into two groups: the healthy control group and the AD group. Results: The AD group had higher percentages of hypertension (20.9%), diabetes (13.6%), smoking habit (27.3%), vitamin B-12 deficiency (22.7%), and alcohol consumption (13.8%) relative to the control group. Among these features, a significant association was found between alcohol and vitamin B-12 status and between systolic blood pressure and MMSE score. Conclusion: This study concluded that there is an association between low levels of vitamin B-12 and the risk of AD. Further studies are needed to determine the cause-effect.

Introduction

The WHO mentioned Alzheimer's disease (AD) as the most common cause of dementia (Parry & Weiyuan, 2011). Dementia is a generalized decline of intellect, memory, and personality without impairment of consciousness, leading to functional impairment. Dementia affects about 25 million people worldwide, with the numbers set to increase dramatically as life expectancy increases further. AD is considered the biggest killer among the growing elderly population (Maharaj, 2017). Dementia has many causes among older patients; the majority of cases are caused by Alzheimer's disease (55%), vascular dementia (20%), and Lewy body dementia (15%) (Geddes et al., 2012).

The risk of vitamin B-12 deficiency among the elderly increased because of the high prevalence of atrophic gastritis-associated foodcobalamin (vitamin B-12) malabsorption and the increasing prevalence of pernicious anemia with advancing age (Wong, 2015). Deficiency of the B vitamins (folate, vitamin B-6, and vitamin B-12) may play a role in the pathogenesis of cognitive impairment in the elderly through hyper-homocysteinemia (Parnetti et al., 1997). High homocysteine levels, which can contribute to arterial damage and blood

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clots in blood vessels, usually indicate a deficiency in vitamin B-12 (Seshadri et al., 2002).

In the developed countries, 6% of those aged 60 years and above are vitamin B-12 deficient (plasma vitamin B-12, 148 pmol/L). Deficiency is much more common, starting in early life and persisting across the life span, and the prevalence of deficiency is increasing with age in developing countries (Allen, 2009). In addition, vitamin B-12 deficiency is a frequent condition in the elderly population, often overlooked in clinical practice (Goebels & Soyka, 2000).

Since the past few years, the number of people with AD has been increasing among the elderly population in Nepal (Baral et al., 2020). The causes of AD are poorly understood (Carpenter et al., 2011). There is no study regarding plasma vitamin B-12 levels in elderly people and their association with AD in our country. Though similar studies are done in the Western world, the study will help to know the association in our part of the world.

Methods

A case-control study approved by the institutional authorities was conducted to evaluate the association of plasma vitamin B-12 levels with AD in Nepalese elderly individuals. The participants who visited the psychiatric outpatient department from 2019 onward at XXX hospital, Kathmandu, with age > 60 years were recruited. First, the participants were interviewed by trained medical officers, applying a structured questionnaire which was designed to obtain the following information regarding general characteristics: age (in years), sex, education, diet habitus, type of family, smoking status, alcohol use, and medical history. The patient's height and weight were measured, and their body mass index (BMI) (kg/m^2) was calculated. Second, blood samples were collected from all participants. The samples were drawn by venipuncture in a Gel/Clot activator tube at the outpatient department and then taken to a specialized lab (Samyak Diagnostic Pvt. Ltd., Lalitpur) where it was centrifuged for 10 minutes at 3500 rpm. Serum was used for the analysis of vitamin B-12 levels. All specimens were collected and analyzed within the same day. The concentrations of vitamin B-12 were determined in the Advia Centaur XP instrument based on CLIA technology. This is a competitive immunoassay using direct (CLIA) chemiluminescent technology. The normal value for vitamin B-12 is 239–931 pg/mL (Siemens Healthcare Diagnostics Inc, 2013). Finally, a trained medical doctor used the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) to measure cognitive functions and determine the risk of AD.

Analysis

Data are expressed as the frequency, mean, and *SD*. The MMSE scores were used to classify participants into 2 groups: (1) healthy control group and (2) AD group. Baseline values were compared among AD and control groups using independent student t-test and stratified analysis using the MantelHaenszel chi-square test. All analyses were performed using "Statistical Packages for the Social Science" IBM statistics version 20.

Results

The demographic, clinical, and biochemical characteristics of the healthy and AD groups are shown in Table 1. In terms of their demographic, though mean age was more in the control group, the analysis showed statistical significance with relation to age. More females with 60.9% and well economic status group with 97.8%, were in the control group whereas

Table 1. Demographic, Clinical, and Biochemical Characteristics of Case and Control Group.

Profile	Case (*n* = 44)	Control (*n* = 46)
Demographics		
Above 80	2 (4.5)	14 (30.43)
Age 71–80	11 (25.0)	13 (28.26)
60–70	31 (70.45)	19 (41.31)
Female	22 (50.0)	28 (60.9)
Illiterate	21 (47.7)	18 (39.1)
Economic status (enough for 12 months)	35 (79.5)	45 (97.8)
Clinical features		
Hypertension	9 (20.9)	5 (10.9)
Diabetes	6 (13.6)	1 (2.2)
Smokers	12 (27.3)	7 (15.2)
Alcohol consumers	14 (13.8)	6 (13.0)
Diet habit (vegetarian)	6 (13.6)	10 (21.7)
Biochemical measures		
Vitamin B-12 (deficient)	10 (22.7)	3 (6.5)

more illiterate with 47.7%, were in the AD group. In terms of clinical features, compared to the control group, there were more hypertensive people, 20.9%; diabetics, 13.6%; smokers, 27.3%; vitamin B-12 deficient, 22.7%; and alcohol consumers, 13.8% in the AD group.

Table 2: Study findings showed a significant association between vitamin B-12 deficiency and Alzheimer's disease after adjusting for age ($p < .01$) and an association was seen among males than females with vitamin B-12 deficiency. Vegetarians with vitamin B-12 deficiency were also found to be significantly associated with Alzheimer's disease when compared with non-vegetarians. Non-alcoholics and

nonsmokers with vitamin B-12 deficiency were also significantly associated with Alzheimer's disease compared with alcoholics and smokers, respectively.

Mean BMI is more among the AD group (23.54 + 3.00) compared to the control group. The statistically significant association found in relation to age and systolic blood pressure between the two groups is shown in Table 2.

Discussion

The objective of this study was to investigate the association between levels of vitamin B-12 and the risk of AD. In the

Table 2. Association Between Different Variables and Case and Control groups.

Variable			Case	Control	OR (CI 95%)	p-Value
Age	> 65	Vit B-12 Deficient	7 (77.8)	2 (22.2)	7.43 (1.38–39.90)	.01
		Vit B-12 Non-deficient	16 (32.0)	34 (68.0)		
	≤ 65	Vit B-12 Deficient	3 (75.0)	1 (25.0)	1.5 (0.13–16.54)	.73
		Vit B-12 Non-deficient	18 (66.7)	9 (33.3)		
Gender	Male	Vit B-12 Deficient	5 (100.0)	0 (0.0)	—	.031
		Vit B-12 Non-deficient	17 (48.6)	18 (51.4)		
	Female	Vit B-12 Deficient	5 (62.5)	3 (37.5)	2.45 (0.51–11.64)	.250
		Vit B-12 Non-deficient	17 (40.5)	25 (59.5)		
Smoking	Yes	Vit B-12 Deficient	1 (100.0)	0 (0.0)	—	.43
		Vit B-12 Non-deficient	11 (61.1)	7 (38.9)		
	No	Vit B-12 Deficient	9 (75.0)	3 (25.0)	4.69 (1.14–19.18)	.02
		Vit B-12 Non-deficient	23 (39.0)	36 (61.0)		
Diet	Vegetarian	Vit B-12 Deficient	2 (100.0)	0 (0.0)	—	.05
		Vit B-12 Non-deficient	4 (28.0)	10 (71.4)		
	Non-Vegetarian	Vit B-12 Deficient	8 (72.7)	3 (27.3)	2.93 (0.71–12.08)	.12
		Vit B-12 Non-deficient	30 (47.6)	33 (52.4)		
Alcohol	Yes	Vit B-12 Deficient	1 (100.0)	0 (0.0)	—	.50
		Vit B-12 Non-deficient	13 (68.4)	6 (31.6)		
	No	Vit B-12 Deficient	9 (75.0)	3 (25.0)	5.28 (1.28–21.69)	.01
		Vit B-12 Non-deficient	21 (36.2)	37 (63.8)		

Variable	Case (Mean ± SD)	Control (Mean ± SD)	p-value
Age	67.73 ± 6.67	73.67 ± 9.09	.01
BMI (kg/m^2)	23.54 ± 3.00	22.67 ± 2.78	.16
Systolic BP	125.9 ± 12.63	119.76 ± 12.34	.03
Pulse	77.14 ± 9.01	76.74 ± 5.65	.80

present study, we identified that a low serum level of vitamin B-12 is significantly associated with the risk of AD. In our study, there were more female and more MMSE scores in the control group, which is similar to Refsum and Smith's study (Refsum & Smith, 2003), and Ma et al.'s study (Ma et al., 2017). In our study, more smokers were in the AD group than the control group, which is inconsistent with Ma et al.'s study but similar to Refsum and Smith's study.

In the present study, we identified that AD is significantly associated with a low serum level of vitamin B-12. This is consistent with the study findings by Tucker et al. (Tucker et al., 2005), whereas it is inconsistent with the findings of the Kungsholmen population-based study (Abyad, 2002; Smith et al., 2008), the Bronx Longitudinal Aging Study (Goebels & Soyka, 2000), and a case-control study by Ma et al.(2017).

A study done by Refsum and Smith (2003) found that the holotranscobalamin level was more strongly associated with Alzheimer's disease and with cognitive function scores in controls. A similar finding in elderly people has already been reported (Bjorksten et al., 2001).

There are limitations to our study. Since this is a case-control study, we can prove causality. Our result could not apply to the general population as we did not use random samples from the general elderly population. Despite the above limitations, based on the findings of this study, it can be concluded that the low levels of vitamin B-12 may be associated with the risk of AD in elderly people. The future longitudinal studies need to plan in these areas to show the evidence for the relationships of holotranscobalamine and cognition.

Conclusion

This study concluded that there is an association between low levels of vitamin B-12 levels and Alzheimer's disease, though not proof as a causal factor. Different factors play a role in the causation of Alzheimer's disease. If we could identify the particular causal factor, this disease could be prevented in advance.

Recommendation

Subjects with Alzheimer's disease often have impaired cobalamine status, which can be corrected by treatment with vitamin B-12. Hence, based on our study findings, which showed association but not proof of causal relationship, large, randomized controlled trials are required to get solid evidence.

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