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Vitamin B_{12} and Folate Deficiencies and Hyperhomocysteinemia in Elderly

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The object of this study was to evaluate the levels of homocysteine (Hcy) and to evaluate the status of vitamin B_{12} and folate in elderly males living in the Riyadh city. Notably 34.1 and 88.6% of subjects had vitamin B_{12} and folate intakes below the DRI, respectively. Mean serum vitamin B_{12} was 298.8±114 pmol L^{-1} Moreover, low serum vitamin B_{12} was observed in 5.6% of sample subjects (<148 pmol L^{-1}) and 23.3% had marginal vitamin B_{12} deficiency (148-221 pmol L^{-1}). Mean serum folate was 8.6±2.3 ng mL^{-1}. No one of the study subjects had a serum folate below 3 ng mL^{-1}. However, 11.4% of subjects had marginal folate deficiency, with serum folate between 3-6 ng mL^{-1}. Mean serum Hcy level was 12.3 ± 3.5 μmol L^{-1} and was inversely correlated with serum vitamin B_{12}. Nearly, fifteen percent (14.8%) of elderly people had HHcy (Hcy>15 μmol L^{-1}). Serum Hcy levels were increased significantly with age, in contrast serum vitamin B_{12} levels were decreased significantly with age. In conclusion, low serum levels of vitamin B_{12} and HHcy exist in older Saudi. Serum Hcy level in elderly Saudi is markedly increased with age and is attributable more to cobalamin deficiency than to folate deficiency. The vitamin B_{12} and folate status of elderly People (≥ 60 years) should be regularly controlled and a general supplementation with vitamin B_{12} and folate should be considered.

Key words: Vitamin B_{12}, vitamin B_{12} deficiency, folate, folate deficiency, elderly, homocysteine, hyperhomocysteinemia

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INTRODUCTION

Several epidemiologic studies have demonstrated that elevated blood Hcy is associated with an increased risk of cardiovascular disease, stroke, neural tube defects and (Herrmann, 2006; Herrmann *et al*., 2006). HHcy is associated with old age, male sex and blood creatinine concentrations (Blom *et al*., 1998; Jacques *et al*., 2001). Low intakes and decreased absorption of the B-vitamins are the most common causes of HHcy, which is very prevalent in elderly people (Herrmann *et al*., 2006). Vitamin B_{12} and folate deficiency is the most common cause of HHcy (Herrmann, 2006) Folate and vitamin B_{12} are cofactors in the metabolic process of Hcy (García *et al*., 2002). The most common causes of vitamin B12 and folate deficiency are inadequate nutrition or malabsorption (Nygard *et al*., 1998; Nilsson-Ehle, 1998) Moreover; ageing of the intestinal mucosa might cause a lower degree of absorption and reabsorption (Nilsson-Ehle, 1998; Haller, 1999). Vitamin B_{12} deficiency is a recognized problem among elderly, although vitamin B_{12} status among differing ethnic groups remains unclear. Hcy levels increase significantly with age and total HHcy prevalence has been reported to be higher in the elderly than in other age groups (Herrmann, 2006; Herrmann *et al*., 2006). However, the prevalence of HHcy is still unknown in Saudi elderly. In addition, there are no enough data about the status of vitamin B_{12} and folate among Saudi elderly males Therefore, the purpose of this study was to evaluate the levels of tHcy and to evaluate the status of vitamin B_{12} in folate among elderly males living in the Riyadh city as part of an ongoing prospective study on diet and disease carried out in an institutionalized Saudi elderly population.

MATERIALS AND METHODS

Eighty eight elderly males were randomly chosen from elderly people attended to prance Salamn Social Center in Riyadh city. All subjects of this study were health in age ≥ 60 years. Blood samples and dietary data were collected in early 2006. Subjects accepted the participation in this study by freely signing a written informed consent. Three day food records were obtained from each subject. Food-processor software was used, to calculate daily nutrient intakes. The folate and vitamin B_{12} intakes were compared with Dietary Reference intake (Dietary Reference Intake, 1998). From each subject, a blood samples were drawn by venipuncture after a 12 h fast and collected in separated tubes for serum and plasma. Vitamin B_{12}, folate and Hcy levels were measured in all subjects. Serum folate and vitamin B_{12} were measured by electrochemiluminescence immunoassay and serum Hcy was determined by a fluorescence polarization immunoassay (Baker *et al*., 1989). The cut-off values for abnormal concentrations were as follows: deficient vitamin B_{12}, <148 pmol L^{-1} (200 pg mL^{-1}); marginal vitamin B_{12}, 148-221 pmol L^{-1} (200-300 pg mL^{-1}); (Gilfix *et al*., 1997; Snow, 1999; Miller *et al*., 2003), Hcy <15 μmol L^{-1}, 15-30 μmol L^{-1}, >30 μmol L^{-1} (Araki and Sako, 1987; Bottigleri, 1996; Joosten, 2001; Ariogul *et al*., 2005). Serum folate levels less than 3 ng mL^{-1} have been considered folate deficiency (Gilfix *et al*., 1997; Snow, 1999; Miller *et al*., 2003 Ariogul *et al*., 2005; Henriquez *et al*., 2004; Chen *et al*., 2005a) whereas, serum folate between 3-6 ng mL^{-1} (7-14 nmol L^{-1}) have been considered marginal folate deficiency (Henriquez *et al*., 2004; Chen *et al*., 2005a). The statistical analysis included means; standard deviations, were analyzed by SSPS version 10. Pearson correlation coefficients were calculated for continuous normalized variables

RESULTS

The mean age was 65±4.7 years, meanwhile the mean body weight was 81±13.2 kg. Most of the study population was in overweight (body mass index = BMI > 25), with overall sample mean of 28.8±3.4 kg m^{-2}. The analysis of the food intake showed that the mean intake of vitamin B_{12} was 3.5±1.5 μg day^{-1} (Table 1). Notably 34.1% of subjects had vitamin B_{12} intake below the DRI. In contrast, the mean intake of folate was 249±98 μg day^{-1}. Nearly ninety (88.6%) of sample study had folate intake below the DRI. Serum Hcy, Vitamin B_{12} and folate levels are shown in Table 2. Serum vitamin B_{12} ranged between 70.7 and 510.2 pg mL^{-1} with an overall sample mean of 298.8±114 pmol L^{-1}. Moreover, low vitamin B_{12} was observed in 5.6 % of sample subjects (<148 pmol L^{-1}) and 23.3% had marginal vitamin B_{12} deficiency (148-221 pmol L^{-1}). Serum folate ranged between 3.9 and 13 ng mL^{-1} with an overall sample mean of 8.6±2.3 ng mL^{-1}. No one of the study subjects had a serum folate below 3 ng mL^{-1} (7 nmol L^{-1}). However, 11.4.% of subjects had marginal folate deficiency, with serum folate between 3-6 ng mL^{-1} (7-14 nmol L^{-1}). In contrast, serum Hcy ranged between 6.6 and 22.2 μmol L^{-1} with an overall sample mean of 12.3±3.5 μmol L^{-1}. Nearly, fifteen percent (14.8%) of elderly people had HHcy

Table 1: Dietary intake of vitamin B12 and folate (Mean±SD)

Nutrients	Dietary intake	DRI	DRI (%)
Vitamin B_{12} (μg day^{-1})	3.5±1.5	2.4	34.1
Folate (μg day^{-1})	249±98	400	88.6

DRI are for age 60 and older

Table 2: Vitamin B_{12}, folate and homocysteine levels

Parameters	Reference values	Mean (±SD)	% of subjects
Homocysteine (μmol L^{-1})	<15	12.3±3.5	85.2
	15-30		14.8
	>30		0.0
Folate (ng mL^{-1})	<3	8.6±2.3	0.0
	3-6		11.4
	>6		88.6
Vitamin B_{12} (pmol L^{-1})	<148	298.8±114	5.6
	148-221		23.9
	>221		70.4

Table 3: Correlation between variables

	Age		Homocysteine	
Parameters	r	p-value	r	p-value
Serum vitamin B_{12}	-0.48	<0.01	-0.62	<0.01
Serum folate	NS		NS	
Serum Homocysteine	0.54	<0.01	-	-
Age	-	-	0.54	<0.01

n = 88

(Hcy>15 μmol L^{-1}). Furthermore, data presented in Table 3 indicted that the negative correlation between Vitamin B_{12} and age was statistically significant (r = -0.48; p<0.01) In contast, the positive correlation between Hcy levels and age (r = 0.54; p<0.01) was statistically significant. In addition, the negative correlation between Vitamin B_{12} and Hcy levels was statistically significant (r = -0.62; p<0.01).

DISCUSSION

The main outcome of is study was that serum Hcy concentrations were inversely associated with vitamin B_{12} levels, which is consistent with many other studies (Refsum *et al*., 2001; Flood *et al*., 2006; Chambers *et al*., 2000; Miller *et al*., 2006). Moreover, HHcy was associated with increased age; in contrast vitamin B_{12} levels were inversely associated with age which is also consistent with many other studies (Flood *et al*., 2006; Chambers *et al*., 2000; Miller *et al*., 2006). HHcy is common among elderly people and elevates with the increasing age (Ariogul *et al*., 2005). Mean serum Hcy concentration observed in this study was 12.3±3.5 μmol L^{-1}, a value that is slightly higher than the overall average of 11.9 and 10.8 μmol L^{-1} that were observed in Bangladesh (Gamble *et al*., 2005) and Indian Asian men (Chambers *et al*., 2000), respectively, but lower than the average of 13.3 μmol L^{-1} (Chen *et al*., 2005b). Hcy levels elevate significantly with age and total HHcy prevalence has been demonstrated to be higher in the elderly than in other age groups (Boushey *et al*., 1995; Moustapha and Robinson, 1998; Kannel, 1997). It was shown in this study Hcy concentrations were increased significantly with age. Nearly, fifteen percent (14.8%) of elderly people in this study had HHcy (Hcy>15 μmol L^{-1}). Similarly, Miller *et al*. (2006) found that 17.0% of an elderly (age = 60 years had Hcy higher than 13 μmol L^{-1}. In addition, Chen *et al*. (2005b) found that the overall prevalence of HHcy (Hcy>15 mumol L^{-1}) was 23.4% for elderly males and 11.2% for elderly females. Chambers *et al*. (2002) observed that, among healthy male subjects, plasma Hcy concentrations were 6% higher in Indian Asian men residing in the United Kingdom than in their white European counterparts. Refsum *et al*. (2001) reported that 77% had plasma tHcy concentrations >15 μmol L^{-1}, In that population, 38% of whom were vegetarian and 47% had serum cobalamin concentrations <150 pmol L^{-1}. Mean plasma tHcy concentrations in this study were higher than in others (Gamble *et al*., 2005; Chambers *et al*., 2000), probably because of the high percentage of subjects with an inadequate vitamin B_{12} and folate status (both intake and serum values) (Table 1 and 2). It has been shown that HHcy is elevated in plasma of patients with deficiency of vitamin B_{12} or folate (Stabler *et al*., 1988; Welch and Loscalzo, 1998) Moreover, epidemiological studies observe a prevalence of cobalamin deficiency of around 20% (between 5 and 60%) in the general population. HHcy in this study appears largely attributed to cobalamin defficency. The prevalence of cobalamin deficiency in elderly depends on the definition of cobalamin deficiency and on age used in the study. Vitamin B_{12} deficiency in Turkish elderly is estimated to affect 10-30% of adults aged > 65 year (Krasinski *et al*., 1986; Hurwitz *et al*., 1997). Recently, Flood *et al*. (2006) found that 22.9% of older Australians had low serum B12 (< 185 pmol L^{-1}). Low serum levels of vitamin B12 and elevated serum Hcy are relatively frequent in older Australians. In Bangladesh, 11% of elderly (age > or = 60 year) was found to have cobalamin concentrations below 150 pmol L^{-1} (Gamble *et al*., 2005). Recently, low serum vitamin B12 (< 148 pmol L^{-1}) was observed in 5.6% of an elderly (age > or = 60 years (Miller *et al*., 2006). In this study, low serum vitamin B_{12} (< 148 pmol L^{-1}) was observed in 5.6% of sample subjects and 23.9% had marginal vitamin B_{12} deficiency (148-221 pmol L^{-1}) (Table 2). Notably, most of subjects in this study who had vitamin B_{12} deficiency were older than 65 year. Moreover, serum vitamin B_{12} levels were decreased significantly with age (r = 0.48; p<0.01). The low serum vitamin B_{12} observed in this study appears largely attributed to sub-optimal vitamin B_{12} intake found in 34.1% of study sample or to malabsorption of vitamin B_{12}. Low serum vitamin B_{12} has been attributed to impair vitamin B-12 absorption. The main cause of impaired vitamin B-12 absorption is atrophic gastritis, which is estimated to affect 10-30% of elderly aged > 65 year in the United States (Krasinski *et al*., 1986; Hurwitz *et al*., 1997) Food-cobalamin malabsorption has only been reported as a significant cause of vitamin B_{12} deficiency among elderly people and is characterized by the inability to release vitamin B_{12} from food or a deficiency of intestinal vitamin B_{12} transport proteins or both (Andres *et al*., 2004).

Low plasma folate concentrations were less common (Allen, 2004). Mean serum folate concentration observed in this study was 8.6±2.3 ng mL^{-1}, a value that is slightly higher than the overall average of 8.2 ng mL^{-1} that were observed in elderly subjects (Henriquez *et al*., 2004) but lower than the average of 10.1 ng mL^{-1} that were observed in elderly males (Chen *et al*., 2005a). None of the study subjects had a serum folate below 3 ng mL^{-1} (7 nmol L^{-1}) however, 11.4% of subjects had marginal folate deficiency, with plasma folate between 3-6 ng mL^{-1} (7-14 nmol L^{-1}). Marginal folate deficiency observed in this study could be attributed to sub-optimal folate intake found in 88.6% of study subjects In agreement with this result, Chen *et al.* (2005a), found that no one of the study subjects had a serum folate below 7 nmol L^{-1} (3 ng mL^{-1}). However, 18.6% of males and 12.1% of females had marginal folate deficiency, with serum folate between 7-14 nmol L^{-1} (3-6 ng mL^{-1}). This suggests that elderly males have a poorer folate status in the Taiwanese population (Chen *et al*., 2005a). Similarly, Flood *et al.* (2006) found that only 2.5 of older Australians had low serum folate (< 6.8 nmol L^{-1}). Moreover, Henriquez *et al.* (2004) found that Mean serum folate was 8.2 ng mL^{-1}. Only one individual had serum folate below 3 ng mL^{-1} and 21.7% showed moderate deficits (3-6 ng mL^{-1}). Marginal folate deficiency observed in this study could be attributed to sub-optimal folate intake found in 88.6% of study sample (88.6% of subjects in this study had intake of folate below DRI). In addition, in New Jersey Kemp *et al.* (2002) found that 94.4 of elderly males and 93.6 of elderly females had intakes below the reference range. In comparison to the DRIs, the mean dietary intakes of elderly were particularly low for folate, the recently increased RDAs for folate may in part explain this observation (Food and Nutrition Board 1998, 2000). Despite intakes below the DRIs for considerable percentages of subjects, no one of subject had low serum concentrations of folate. Thus, the below DRI intakes of many subjects did not result in low serum concentrations in most cases, consistent with the DRI as a value that is sufficient for 97.5% of healthy people.

HHcy is markedly pronounced among elderly males and is markedly increased with age. The negative correlation between Vitamin B_{12} and Hcy levels was statistically significant (r = 0.62; p<0.01) meanwhile, no correlation was found between serum folate and Hcy levels. Therefore, HHcy appears largely attributed to cobalamin deficiency than to folat deficiency. This finding is in consisting to reports on Asian Indians residing in the United Kingdom, where hyperhomocysteinemia is more likely due to cobalamin deficiency (Chambers *et al*., 2000).

In summary, low serum levels of vitamin B12 and elevated serum Hcy were observed in elderly Saudi. Hcy level is markedly increased with age and is attributable more to cobalamin deficiency than to folate deficiency. Appropriate strategy should be considered to reduce the prevalence of low serum vitamin B12 and elevated Hcy in older Saudi. To prevent such situation and improve the quality of life of elderly people, in Saudi as in many other countries the vitamin B_{12} and folate status of elderly People (≥60 years) should be regularly controlled and a general supplementation with vitamin B_{12} and folate should be considered, different strategies for increasing vitamin B_{12} and folate intake should be applied, among them food fortification and individual diet supplementation. However, further studies are necessary in order to confirm these findings. In future studies we well evaluate the levels of Hcy in elderly who older than 70 year and its relation with other factors.

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