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A Turkish 3-center study evaluation of serum folic acid and vitamin B12 levels in Alzheimer disease

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Background/aim: Alzheimer disease, a common proteopathy of advanced age, is characterized by cortical atrophy, neuron degeneration, neuronal loss, and accumulation of extracellular amyloid β plaques. We aimed to investigate serum vitamin B12 and folic acid levels in Alzheimer disease and other dementia patients, as a potential screening test to detect presymptomatic Alzheimer disease in Turkish patients.

Materials and methods: We evaluated folic acid and vitamin B12 levels in Alzheimer disease patients as well as in other dementia and geriatric patients from Ankara, Dokuz Eylül, and Çukurova university hospitals; 290 female and male geriatric subjects were enrolled. Vitamin B12 and folic acid levels were measured using Roche E170 and Beckman Coulter DXI 800 immunoassays (chemiluminescence) according to the manufacturers' guideline in all centers.

Results: We evaluated the results of folic acid and vitamin B12 in Alzheimer disease, other dementias and geriatric patients. No significant difference between the groups regarding the routine control of biochemical parameters was observed.

Conclusion: Currently, serum folic and vitamin B12 levels are not diagnostically reliable tests for screening presymptomatic Alzheimer disease. However, the results may statistically be significant if we increase the sample size.

Key words: Alzheimer disease, vitamin B12, folic acid

1. Introduction

Almost a century ago, Alzheimer disease was first described by German psychiatrist and neuropathologist Alois Alzheimer and named after him (1). Alzheimer disease is the most common form of dementia of the human central nervous system. This protein misfolding disease (proteopathy) accounts for an estimated 60%–80% of cases of dementia (2,3). Alzheimer disease is a very severe public-health problem, especially in view of increased life span. In fact, approximately 50% of people over age 85 suffer from it. The global prevalence of dementia has been estimated to double every 20 years and consequently, by 2040, as many as 24 million new diagnoses are on the horizon, which is an enormously challenging number (4). Following in the footsteps of global trends in increasing human longevity, the elderly population ratio projects an incremental pattern in Turkey as well. It is estimated that the average life expectancy is 71.8 for men and 76.8 for women as of 2010 (5).

Alzheimer disease is multifactorial brain impairment and the causes of the degenerative brain function remain unknown (6). Through the interplay of underlying genetics and biochemical derangements, in addition to environmental factors, Alzheimer disease has some features that distinguish this disease from the other neurodegenerative disease types such as neuronal loss, accumulation of extracellular (amyloid β containing) plaques, and accumulation of intracellular (tau) neurofibrillary tangles, which are the dominant traits of this disease. Beside these features, cortical atrophy and

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neuron degeneration are also commonly seen as the disease progresses and dementia sets in. In the very etiology of tangles and senile plaques accumulation, oxidative stress has a triggering effect in emergence of disease-related pathologies (7–10). Amyloid β (Aβ) plaques and tangles are characteristic major markers of Alzheimer disease and, in addition to these properties, tau phosphorylation is one of the most important fundamental factors in disease progression (11,12). In fact, it was discovered that female patients with psychotic Alzheimer disease, but not males, have significantly higher levels of phosphorylated tau in the frontal cortex. Male sex, but not female, is associated with the presence of α-synuclein pathology, which supports a sex dissociation of pathology in Alzheimer patients with psychotic disease (13).

Various neurochemical as well as serum biomarkers are proposed in Alzheimer disease as indicators to assess the risk or presence of the disease. For this end, biochemical clinical laboratory blood tests may have critical and potential roles in some aspects such as identifying co-morbidity and screening complications of the patients to provide appropriate care. These tests may also be important for evaluation of the other potential risk factors such as identifying and discovering associated correlations (14). In fact, in clinical medicine, biomarkers provide reliable and objective measures to assess disease progression as well as being indispensable for early detection.

For a better and coherent understanding of complex disease pathologies in Alzheimer disease, which may in turn be translated into patient care, instead of fixation on an isolated system, a multifactorial, holistic approach that includes the exploration of enzyme deficiencies is essential (12,14). In fact, research investigating the analysis of Aβ (1-42), total tau, and phospho-tau-181 in cerebrospinal fluid allows a reliable, sensitive, and specific diagnosis of Alzheimer disease but not of other forms of dementia (14).

Aging begins at a molecular level. In our previous study, we concluded that the enzymes isolated from young animals (15) and old animals present variations in kinetic behavior as a result of the natural aging process (16). Studies in recent years have shown the importance of progression of brain aging is influenced by a complex interaction of genetic and environmental factors (17). It is also established that brain activities, correlated with brain gray matter structures, undergo changes through aging. It is also known that there is an important reduction in the volume and size of the cortex (18,19). Once again, the potential effect of nutrition has become a trending subject in scientific and public interest in reversing the detrimental cognitive/motor decline. There are debates that foods that contain specific vitamins, trace minerals, and lipids can affect the risk of cognitive decline and dementia, especially in frail elderly people at risk of deficiencies (20). Furthermore, adequate dietary nutrition intake thorough a healthy and balanced diet has an undisputed role in healthy aging as its essentiality has been confirmed by various researchers (21,22).

Vitamin B12 is a complex organometallic cofactor that has crucial roles associated with 3 subfamilies of enzymes: the adenosylcobalamin-dependent isomerases, the methylcobalamin-dependent methyltransferases, and the dehalogenases (23). Folic acid, on the other hand, is essential for numerous bodily functions. Folic acid is not biologically active on its own; its biological importance is due to tetrahydrofolate and other folic acid derivatives. The human body needs folic acid in DNA and RNA synthesis, as well as in DNA methylation and the reactions that require single, activated carbon transfer. The metabolisms of folic acid and vitamin B12 intersect during the transfer of the methyl group from 5-methyltetrahydrofolate to homocysteine catalyzed by B12-dependent methionine synthase. Regeneration of tetrahydrofolate via this reaction makes it available for synthesis of nucleotide precursors (24). Due to its indispensable roles in normal human metabolism, folic acid fortification of breakfast cereals, a popular consumer product in Western diet, was introduced in many countries to prevent neural tube defect occurrence.

Folate and/or vitamin B12 deficiency may have additive effects in many diseases including various types of dementia (25).

In order to investigate a potential correlation between B12 and folic acid deficiency and Alzheimer disease, we analyzed the serum B12 and folic acid results of Alzheimer disease patients and geriatric patients from various cities in Turkey. We selected these cities from different geographic regions of Turkey; Ankara is in the Central Anatolia region, İzmir is in the Aegean region, and Adana is in the Mediterranean region. These 3 geographic regions are quite distinct from one other in terms of their populations' diverging dietary habits and lifestyles, and even climate conditions.

Through this study, we aim to determine a simple and cost-effective method that may be beneficial in early diagnosis by recognizing the warning signs of Alzheimer disease and screening to identify individuals who have disease but do not yet have symptoms.

2. Materials and methods

Subject enrolment lasted for 1 year (2013–2014), at Ankara, Çukurova, Dokuz Eylül university hospitals' neurology clinics. Patients' medical records for serum vitamin B12 and folic acid were examined retrospectively for a correlation between serum levels and cognitive decline documented with a clinical diagnosis of dementia. Patients with clinically documented malignancies, drug and/or alcohol abuse, and ongoing vitamin replacement therapies were excluded from the study.

2.1. Biochemical measurements and sampling

In all 3 centers, biochemical measurements were made according to these rules: all patients' blood samples were collected after a 10–12-h fasting period. Venous blood samples, which were drawn between 0900 and 1100, were stored at 4 °C. Vitamin B12 and folic acid levels were measured using Roche E170 and Beckman Coulter DXI 800 immunoassays (chemiluminescence) according to manufacturers' guidelines.

2.2. Statistical analysis

We evaluated our results as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to check correlations between variables and the normal distribution and homogeneity among subgroups was analyzed with the Levine test. In subgroup comparisons, as the parametric test assumptions were met, one way ANOVA was used.

Since there was a variation in the average age among subgroups in 2-group comparisons, Tamhane's post hoc test was adopted. Finally, in discerning the sex variations, we chose the chi-square test.

P-values < 0.05 were considered statistically significant for all analyses.

3. Results

In this retrospective study 290 female and male geriatric subjects were enrolled. We aimed to identify Alzheimer disease during preclinical, asymptomatic phases before the onset of cognitive decline. Patients' data were collected from 3 different centers and were evaluated for serum folic acid and B12 vitamin retrospectively. From Adana center (Çukurova University Hospital, Neurology Clinic), 94 subjects were recruited, of which 49 were female and 45 male. Patients with a previous history of vitamin replacement therapy were excluded.

Subjects were further divided and assigned to 3 subgroups according to their symptoms (Figures 1 and 2). While the first subgroup included subjects with mixed-type Alzheimer disease (no: 38) (vascular type + Alzheimer disease), the second subgroup included subjects with Alzheimer disease (n: 37). Finally the third subgroup included subjects suffering from vascular-type dementia (n: 19). In the first subgroup, the average age of the subjects was 83.26 ± 6.38 (75–104), and vitamin B12 (Figure 1) and folate levels (Figure 2) were 222.79 ± 117.67 (41–506) and 4.58 ± 2.88 (1–10), respectively. In the second subgroup, the average age of the subjects was 76.57 ± 7.6 (55–89), and while the serum vitamin B12 levels were 244.08 ± 105.52 (75–524), serum folate levels were 6.15 ± 4.73 (1–26). Finally, in the third subgroup, the average age among the subjects was 65.58 ± 13.97 (40–91), and serum vitamin B12 and folate levels were 184.58 ± 96.46 (58–443) and 4.42 ± 1.80 (1–8), respectively. In the third subgroup, although not statistically significant, both vitamin B12 and folic acid levels were lower compared to the other groups (Figures 1 and 2).

When the 94 subjects from the Adana center were divided into 2 groups according to their age, as above 65 and below 65, in the 82 subjects 65 or older, the average

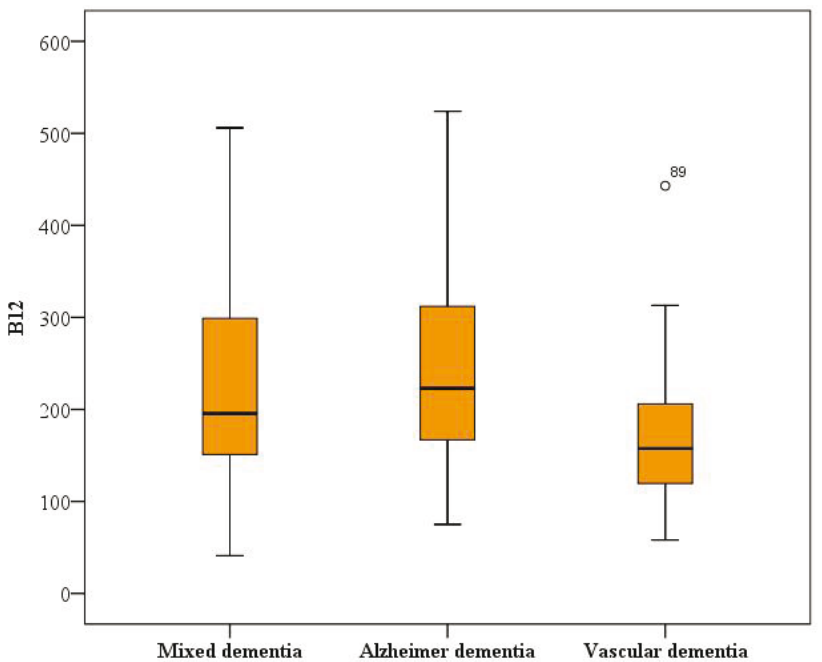

Figure 1. Results of serum vitamin B12 in patients with Alzheimer disease and other dementias from the Mediterranean region, Adana, Çukurova University Hospital.

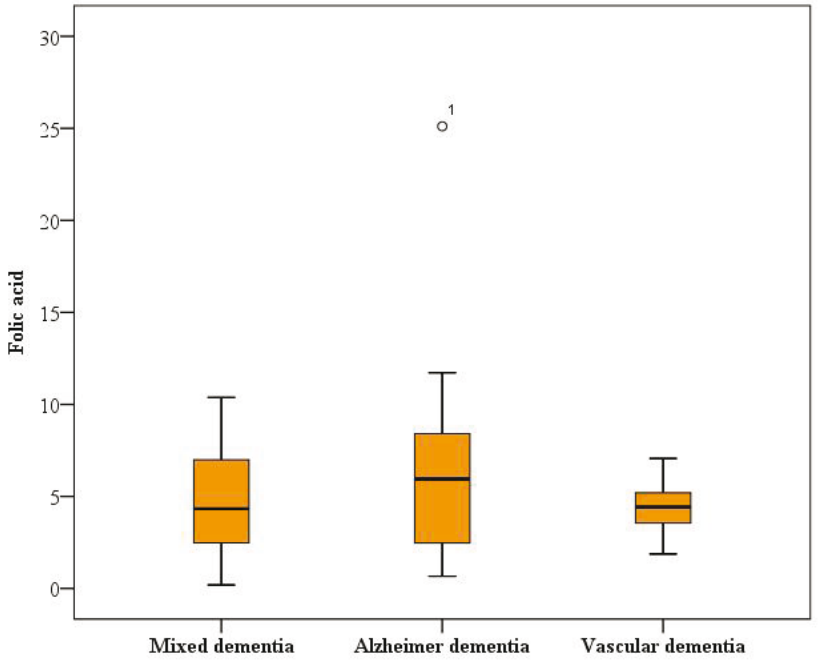

Figure 2. Results of serum folic acid in patients with Alzheimer disease and other dementias from the Mediterranean region, Adana, Çukurova University Hospital.

vitamin B12 level was 222.83 ± 103.53 and the average folate level was 4.58 ± 2.50. In the 12 subjects younger than 65, the average vitamin B12 level was 223.54 ± 111.51 and the average folate level was 5.25 ± 3.78. No meaningful variation in vitamin B12 or folate levels was observed between the groups (Figures 1 and 2).

In the İzmir center (Dokuz Eylül University Hospital, Neurology Clinic), 119 subjects were recruited, of which 73 were female and 46 male. The subjects were further divided and assigned to 4 subgroups according to their clinically observable symptoms. While the first subgroup included subjects with Alzheimer disease (n: 37), the second subgroup included subjects with frontotemporal dementia (n: 7). The third subgroup included subjects suffering from early-onset dementia (n: 22) and the fourth subgroup consisted of normal geriatric subjects.

In the first subgroup, the average age of the subjects was 76.54 ± 7.96 (66–95), and vitamin B12 and folate levels were 469.59 ± 237.08 (155–1000) and 8.04 ± 5.44 (1.8–25), respectively. In the second subgroup, the average age of the subjects was 78.42 ± 5.56 (69–84), and while the serum vitamin B12 levels were 601.57 ± 388.89 (167–1244), serum folate levels were 5.36 ± 1.63 (3.2–7.5). In the third subgroup, the average age among the subjects was 60.36 ± 3.37 (54–65), and serum vitamin B12 and folate levels were 561.86 ± 258.20 (214–1307) and 6.37 ± 1.91 (2.4–10.7), respectively. Finally, in the normal geriatric subjects, the average age was 78.18 ± 7.09 (66–93) and while vitamin B12 levels were 474.35 ± 320.59 (123–2000), folate levels were 6.85 ± 3.61 (2.4–25) (Figures 3 and 4).

Although there was a statistically significant difference ($P < 0.001$) between the groups in terms of average age, no statistically significant difference was observed for vitamin B12 (0.29) or folate (P: 0.50).

Even though there was no statistically significant difference in the second and the third subgroups, while folic acid levels were noted as lower, vitamin B12 levels were found to be higher, compared to the other groups (Figure 3).

From the Ankara center (Ankara University Hospital, Geriatric Clinic), 122 subjects were recruited (61 Alzheimer disease patients and 61 normal geriatric patients). Patients with a previous history of vitamin replacement therapy were excluded. The 122 subjects from the Ankara center were divided into 2 subgroups: while the first subgroup included patients with Alzheimer disease, normal geriatric subjects were assigned to the second subgroup (Figures 5 and 6). Serum folic acid values of 4.6–18.7 ng/mL and serum vitamin B12 values of 191–663 pg/mL are considered to be in the normal reference range and according to these reference values, in our study, we identified 5 Alzheimer patients with below-normal folic acid levels.

However, in the second subgroup with normal geriatric subjects, only 3 subjects had normal reference range folic acid levels. Only 3 subjects with Alzheimer disease had

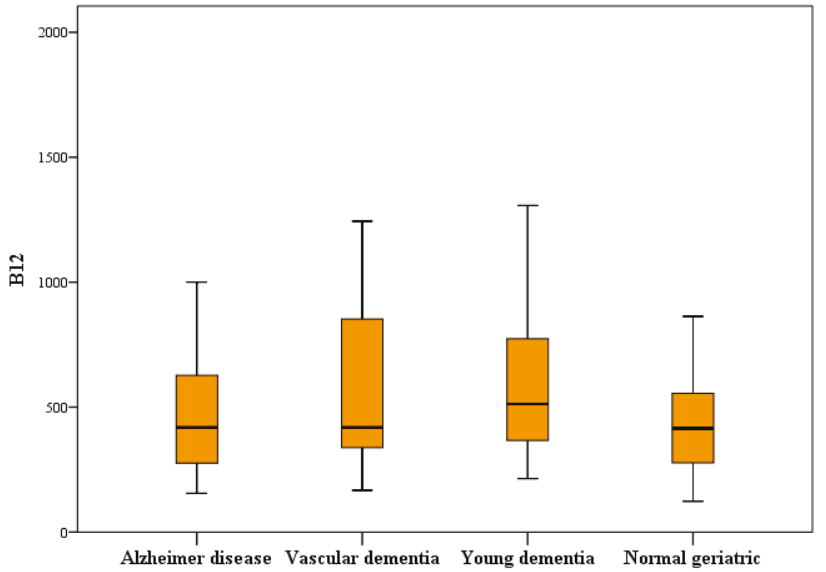

Figure 3. Results of serum vitamin B12 in patients with Alzheimer disease and other dementias from the Aegean region, İzmir, Dokuz Eylül University Hospital.

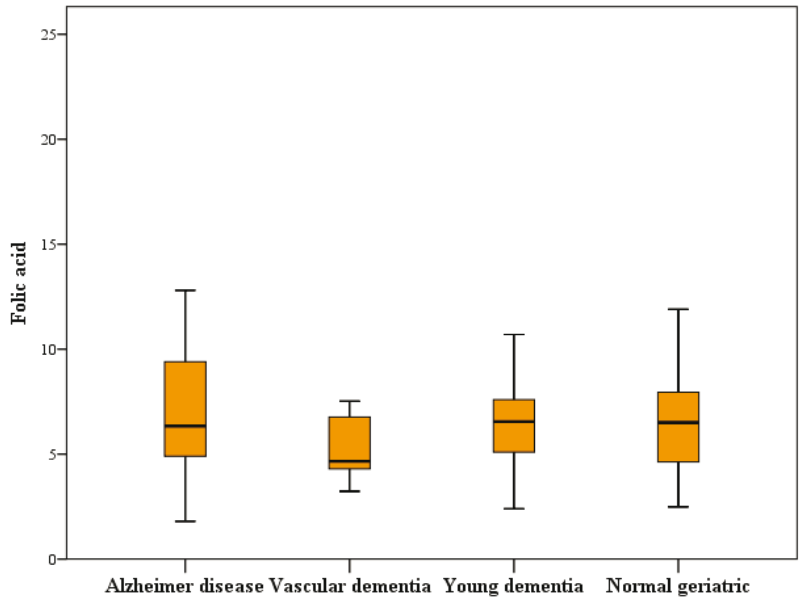

Figure 4. Results of serum folic acid in patients with Alzheimer disease and other dementias from the Aegean region, İzmir city, Dokuz Eylül University Hospital.

below-normal vitamin B12 levels and, interestingly, 20 subjects with higher than normal vitamin B12 levels were identified. In addition to this unexpected result, vitamin B12 levels were completely in the reference range in all of the geriatric patients from the second subgroup.

4. Discussion

It is accepted that the major cause of Alzheimer disease is old age. However, it is a multifactorial irreversible neurodegenerative disease among individuals over the age of 85 and its pathophysiologic mechanism is not

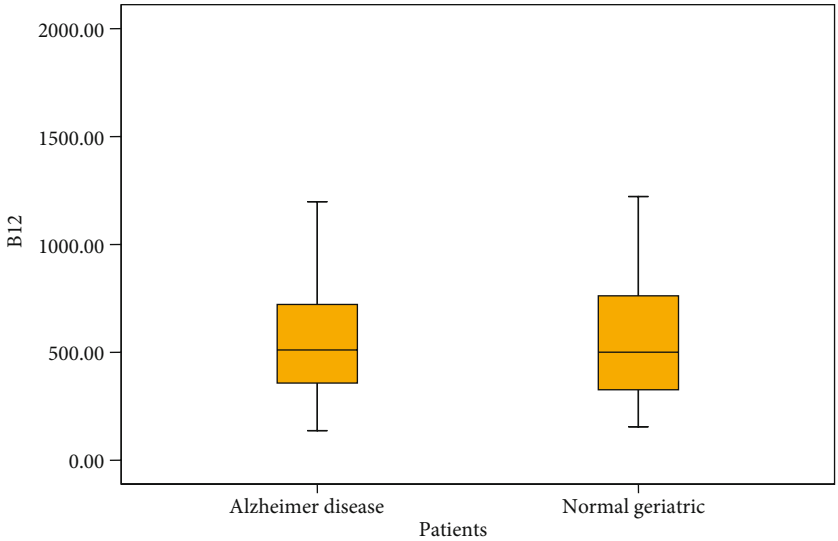

Figure 5. Results of serum vitamin B12 in normal geriatric patients from the Central Anatolia region, Ankara, Ankara University Hospital.

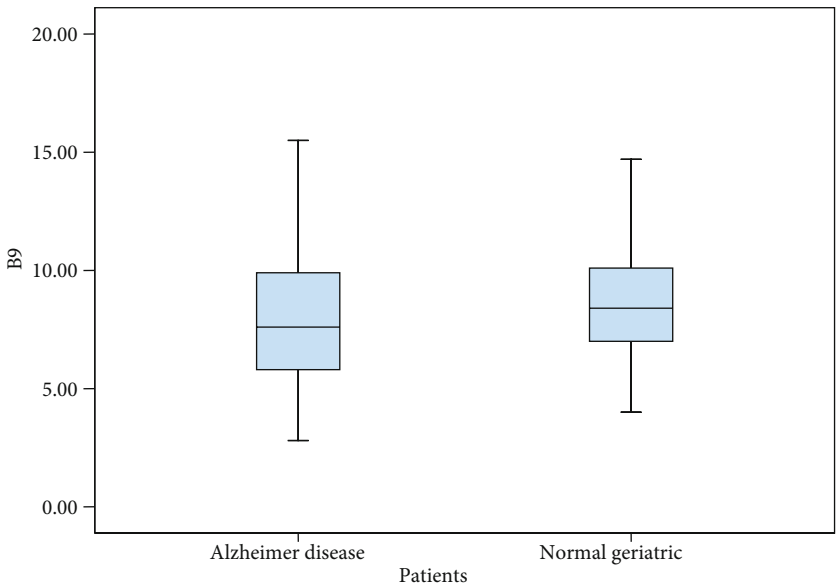

Figure 6. Results of serum folic acid in normal geriatric patients from the Central Anatolia region, Ankara, Ankara University Hospital.

fully understood (26). In a previous study, researchers tried to address the question of whether it is possible to prevent atrophy of key brain regions related to cognitive decline and Alzheimer disease. One approach is to modify nongenetic risk factors, for instance by lowering elevated plasma homocysteine levels using vitamin B derivatives. In an initial, randomized controlled study in elderly subjects with increased dementia risk (mild cognitive impairment according to 2004 Petersen criteria), researchers showed that high-dose B-vitamin treatment (folic acid 0.8 mg, vitamin B6 20 mg, vitamin B12 0.5 mg) slowed shrinkage of the whole brain volume over 2 years. In fact, it was demonstrated that B-vitamin supplementation can indeed slow the atrophy of specific brain regions that are key components of the Alzheimer disease process and are associated with cognitive decline (27).

Alzheimer disease affects patients in several ways and we have identified the Alzheimer disease patients according to the general symptoms such as memory loss that interferes with the quality of daily life, for example

confusion with time or place and especially in problems with finding the correct expression in both oral and written communication. Misplacing things and losing the ability to sustain vital daily activities are other common traits of this neurodegenerative disease. Accordingly, personality changes are widely observed that affect the patients' professional and social lives.

Progression of the disease shows variation in Alzheimer patients. While in some patients emergence of symptoms presents a gradual pattern with decline in cognitive and motor abilities, in others it may present a more rapid and severe onset. Unaffected family members could sometimes fall short in recognizing the failing brain functions and the patients could feel isolated.

Metabolism of homocysteine is innately related to metabolism of vitamin B12 and folic acid, and the deficiencies of these 2 crucial vitamin products could result in high homocysteine levels, which in turn increase the susceptibility for cerebrovascular diseases (22,28). Nonetheless, due to insufficient subject numbers enrolled in the study, higher than normal vitamin B12 level results and, more importantly, homocysteine levels were not included in the scope of this study, and so it is not possible to derive direct conclusions.

Intake of sufficient vitamins and other nutritional factors is crucial in healthy aging. In fact, aging as a molecular process begins with the alterations of biochemical processes that synthesize biologically important macromolecules. In addition to this subtle yet highly significant remodeling, excessive production of reactive oxygen species (ROS) may lead to cellular injury through base breaks in DNA, and nonspecific modifications and disruption of proteins, phospholipids, and nucleic acids. In fact, accumulations of these detrimental effects are considered to be the prime suspect behind many clinically challenging diseases, including Alzheimer disease.

Changes in kinetic behavior of enzymes as well as the clinically symptomatic enzyme deficiencies have additive effects in molecular aging and are highly relevant in pathologies. As the trends in clinical medicine shift to multifactorial evaluations in discerning human diseases, many are now correlated with the altered enzymatic activities. In this study, we analyzed vitamin B12 and folic acid levels in dementia patients including Alzheimer and normal geriatric patients.

5. Conclusion

Alzheimer disease is a complex, multifaceted disease that has genetic, molecular, cellular, enzymatic, nutritional, organ, and system levels of implications. With the improvements in modern medical sciences, average human life has increased exponentially. However, it is now an established fact that many diseases, including but not limited to cancer and neurodegenerative ones, in fact have a high prevalence among the elderly population. Many of the random and deleterious biochemical alterations that are prevalent in advanced age could induce progressive damage at cellular level and these driving factors make leeway for Alzheimer disease and other dementias.

We are trying to find a biomarker in the patients with Alzheimer disease that is to be used as an indicator of a precise presymptomatic biological state or condition before the onset of dementia as a screening test and for the differential diagnosis of neurodegenerative diseases. Our secondary aim is to learn the differences between Alzheimer disease and geriatric patient populations' laboratory results for vitamin B12 and folic acid.

In our opinion, although fast and cost-effective, biochemical routine works such as blood biomarkers, for the time being, are not reliable for identification of presymptomatic Alzheimer disease. However, ultrasensitive analytical methods have potency to provide physicians with an opinion about the status of the patient. With our current understanding, Alzheimer disease has no cure and therefore is still a challenging disease with both sociological as well as clinical implications and we are confident that biomarker discovery as a rapidly expanding field of research will soon come up with a solution for screening patients at high risk for neurodegenerative diseases even before they become symptomatic or severely deficient in both cognitive as well as motor functions.

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