Homocysteine-related alterations of ^{18}F-FDG brain pattern in metabolic diseases

Zita Képes[1] MD,
Csaba Aranyi[1] Msc,
Attila Forgács[2] PhD,
Ferenc Nagy[2] PhD,
Kornél Kukuts[2] Bsc,
Regina Esze[3] MD,
Sándor Somodi[4] MD, PhD,
Miklós Káplár[3] MD, PhD,
József Varga[1] PhD,
Miklós Emri[1] PhD,
Ildikó Garai[1,2] MD, PhD

1.Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
2. Scanomed Ltd. Nuclear Medicine Centers Debrecen, Debrecen, Hungary
3.Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
4. Department of Emergency Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Keywords: Diabetes - Obesity - ^{18}F-FDG PET/CT - Homocysteine - Neurodegeneration

Corresponding author:
Zita Képes MD
Nagyerdei krt. 98, Debrecen, Hungary, 4032
Tel: 06-70-364-6025
kepes.zita@med.unideb.hu, zitakepes@gmail.com

Received:
27 August 2021
Accepted revised:
3 December 2021

Abstract
Since hyperhomocysteinaemia (HHcys) is implicated as a risk factor for the development of neurodegeneration, and is associated with the development of metabolic diseases, we aimed at analysing the effect of homocysteine (Hcys) on regional fluorine-18-fluorodeoxyglucose (^{18}F-FDG) brain metabolismin 51 controlled type 2 diabetic and in 48 non-DM obese participants. Plasma Hcys levels were measured by an immunoassay. Homocysteine-related ^{18}F-FDG regional brain metabolism was evaluated applying ^{18}F-FDG positron emission tomography/computed tomography (PET/CT) using magnetic resonance imaging (MRI)-based brain template for statistical parametric mapping (SPM) analysis. Homocysteine-related decreased ^{18}F-FDG uptake was shown in the right middle temporal gyrus in the whole population. Diabetics with Hcys above the reference limit expressed decreased glucose metabolismin the left calcarine cortex compared to the obese with Hhcys. Regional metabolic alterations evoked on the basis of HHcys draw attention to the potential risk of neurodegeneration ca-used by metabolic disturbances.

Hell J Nucl Med 2021; 24(3): 222-227 *Published online: 28 December 2021*

Introduction

Taking the increasing prevalence and incidence of dementia and neurodegeneration into account, effective solutions are required to combat this global epidemic [1]. While nowadays approximately 50 million people are reported to live with dementia worldwide, by 2050 this number is estimated to reach 152 million [1].

Given that neurodegenerative disorders and cognitive deterioration represent a major and escalating public health problem worldwide and concomitantly deteriorating quality of life as well as reduced life expectancy, the necessity for the introduction of sensitive diagnostic modalities that excel in the early detection of subtle dementia-related brain changes even before clinical signs are actually present is highlighted [2]. Better understanding the pathophysiology of neurodegeneration with the application of sensitive imaging techniques may provide opportunity to search for novel preventive approaches and treatment options that could possibly prevent the occurrence or delay the progression of cognitive decline.

Positron emission tomography (PET) imaging is considered to be a sensitive imaging modality for the investigation of characteristic cerebral metabolic patterns induced by neurodegenerative disorders [3]. Previous PET studies reported regional hypometabolism in the temporal and parietal cortex in Alzheimer's disease in addition to the generalized decrease in global cerebral metabolism [4]. Metabolic reduction in the region of the precuneus, posterior cingulate cortex, lateral parietotemporal and frontal brain regions was also depicted [4]. Several other studies applying fluorine-18-fluorodeoxyglucose (^{18}F-FDG) PET technique confirmed hypometabolism in the precuneus in people with Alzheimer's disease compared to a healthy control group [5-7].

Further, the relationship between metabolic diseases, including type 2 diabetes mellitus (T2DM) and obesity, and neurodegeneration is also frequently investigated [8, 9]. According to literature data mild cognitive impairment (MCI) could be present in severe obesity [8]. Recent research also confirmed that both T2DM and obesity mean a 50% increase in the development of neurodegenerative disorders [10].

Another significant aspect is that elevated plasma homocysteine (Hcys) level is regarded as a risk factor for the onset of neurological disturbances, such as dementia and neurodegeneration [11]. Further, recent meta-analysis of prospective cohort studies also supported the association between HHcys and increased risk for the appearance of cognitive decline [12].

Hyperhomocysteinaemiais also associated with metabolic diseases [13]. Relationship has been depicted between HHcys and both T2DM and central obesity [14,15]. In a Chinese meta-analysis strong relationship was found between the Hcys level and obesity [16].

Based on the existing research findings related to the association between HHcys and both metabolic disturbances and cognitive impairment, we hypothesize that alterations in Hcys levels influence ^{18}F-FDG brain metabolic pattern of individuals with metabolic disturbances. In our study we examined Hcys-associated regional ^{18}F-FDG brain metabolism in patients with T2DM and non-DM obese subjects. In addition, we also investigated the frequency of different genotypes of MTHFR C677T polymorphism (rs1801133) in study participants involved.

Subjects, Material and Methods

Study participants

In this prospective study, we involved 51 patients with controlled T2DM, and 48 age-matched non-DM obese subjects. Patients were selected from the Department of Internal Medicine of the University of Debrecen as well as from a private general medical praxis (Miskolc, Hungary). Inclusion criteria were as follows: age between 18 and 70 years, manifest obesity (BMI >30kg/m^2) or controlled T2DM, and no history of mental or brain disorders. The exclusion criteria were: gravidity, breastfeeding, acute or chronic inflammatory disease, ongoing steroid treatment, retinoid intake, history of malignant diseases, changes in therapy in the previous six months, use of anticoagulant treatment, and brain injury or cerebrovascular event in medical history. Before enrolment, subjects were given detailed information concerning the aims of the study and the examinations as well. Informed consent was collected from all patients based on an ethical approval (OGYEI/2829-4/2017).

Laboratory assays

Homocysteine levels were measured from K3-EDTA anticoagulated plasma samples. The samples were transported immediately on ice at 2°C-8°C to the Department of Laboratory Medicine, University of Debrecen. Sample preparation with the measurement of Hcys concentration was performed within 30 minutes after sampling. Homocysteine levels were determined by chemiluminescent microparticle immunoassay (CMIA) on an Architect-i1000SR® analyser (Abbott, Wiesbaden, Germany, threshold value: 12.6μmol/L).

Additionally, serum glucose levels (reference range: 3.6-6.0 mmol/L) were determined from serum samples spectrophotometrically (Roche Diagnostics), and HbA1c levels were measured by high-performance liquid chromatography (BioRad, Hercules, CA, USA) from K3-EDTA anticoagulated whole blood samples (reference range: 4.2%-6.1%).

^{18}F-FDG PET/CT

All participants had ^{18}F-FDG PET/computed tomography (CT) examinations to evaluate brain metabolism applying AnyScan PET/CT (Mediso, Hungary). Acquisition started 45 minutes (±5 minutes) post injection (MEDRAD Intego, Bayer) of 3.5MBq/Bw ^{18}F-FDG. The parameters of static PET acquisition were the following: 10min/FOV, with voxel size of 2x2x2mm, and matrix size of 160x160x76, while low-dose CT parameters were: 120kV and 100mAs. Additional brain T1-weighted 3D MR images (Achieva 3.0T (TX)-DS, Philips) were obtained with a voxel size of 0.5x0.5x1mm and matrix size of 480x480x175 for brain mapping.

Image processing, brain mapping

Initially, T1 weighted MR images of the patients were brain-segmented using Freesurfer 5.3, and we transformed brain volume to the MNI152-space (2x2x2mm voxel size) using the ANTS linear and non-linear image registration to-ols [17, 18]. The ^{18}F-FDG PET images of the subjects were registered to their T1-weighted MR images using the FLIRT linear registration software [19]. Their spatially normalized ^{18}F-FDG PET images were used to create study-specific brain template. It was used for spatial normalisation of patients' ^{18}F-FDG images applying FNIRT software of the FSL package [20]. Before statistical analyses we smoothed the spatially normalised images with a 16mm FWHM 3D isotropic Gaussian-kernel, and thresholded them at eighty per cent of average brain voxel values.

Methylenetetrahydrofolate reductase (MTHFR) enzyme genotyping

Since literature data pointed out association between C677T polymorphism (rs1801133) of methylenetetrahydrofolate reductase (MTHFR) enzyme - the key enzyme in Hcys metabolism - and metabolic diseases, MTHFR genotyping of the study patients was also performed [21]. Methylenetetrahydrofolate reductase genotyping was performed in all participants. DNA was extracted from peripheral blood samples obtained into K3-EDTA Vacutainer tubes (Becton Dickinson, San Jose, CA, USA), using QiaAmp DNA Blood Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's recommendations. Genotyping was carried out with the use of the LightCycler 480 Real-Time PCR Instrument (Roche Diagnostics, Mannheim, Germany) with the application of hybridisation probes based on melting point analysis. LightCycler technology combines rapid-cycle polymerase chain reaction with real-time fluorescent monitoring and melting curve analysis [22].

Statistical analyses

Data normality was tested using Shapiro-Wilk test. For voxel wise group comparison Statistical Parametric Mapping (SPM) methods were used [23]. For thresholding of the statistical images, we used family-wise error correction (FWE) method ($P<0.05$), and we considered region clusters only with an extent of at least 40 voxels (0.32cm^3, $T=4.01$).

Results

Laboratory findings

Thirty three percent (33%) of the diabetic patients (17) and 25% of the non-DM obese participants (12) had their Hcys levels above the reference limit (shown in Table 1). There was no remarkable difference between the proportion of elevated Hcys levels between the two study groups. Serum glucose and HbA1c levels were obviously significantly higher in the diabetic group compared to the non-DM obese individuals due to the presence of diabetes mellitus itself.

Table 1. *Genotype distribution of methylenetetrahydrofolate reductase gene C677T polymorphism of all and HHcys participants.*

Participants	All		Hhcys (Hcys>12.6 μmol/L)	
	TT/CT	CC	TT/CT	CC
Type 2 diabetic	28	23	12	5
Non-DM obese	26	22	8	4

Hcys, homocysteine; HHcys, hyperhomocysteinaemia; DM, diabetes mellitus

Brain metabolism

We examined the association of increased Hcys values and regional ^{18}F-FDG brain metabolism.

Statistical parametric mapping analysis showed that in the region of the right middle temporal gyrus (rMTG) decreased uptake of ^{18}F-FDG was present in participants with Hcys levels above the reference limit compared to those who had their Hcys levels in the normal range, irrespective of the metabolic disease present (demonstrated in Figure1A). SPM also showed reduced tracer uptake in the left calcarine cortex in those diabetics whose Hcys levels exceeded the reference limit compared to the obesewho had their Hcys levels above 12.6μmol/L (shown in Figure1B). The main statistical characteristics of the mentioned brain regions are shown in Table 2.

MTHFR genotyping

The genotype distribution of patients is demonstrated in Table 1. There was no significant difference between the genotype distribution of the study groups (Fisher's exact test: P>0.05). Interestingly, no significant difference was shown between the frequency of elevated Hcys values in case of different genotypes, although elevated Hcys levels were slightly more frequent in subjects with the presence of T allelein both groups (see Table 1). However, this elevated frequency was not statistically significant (Fisher's exact test: P>0.1).

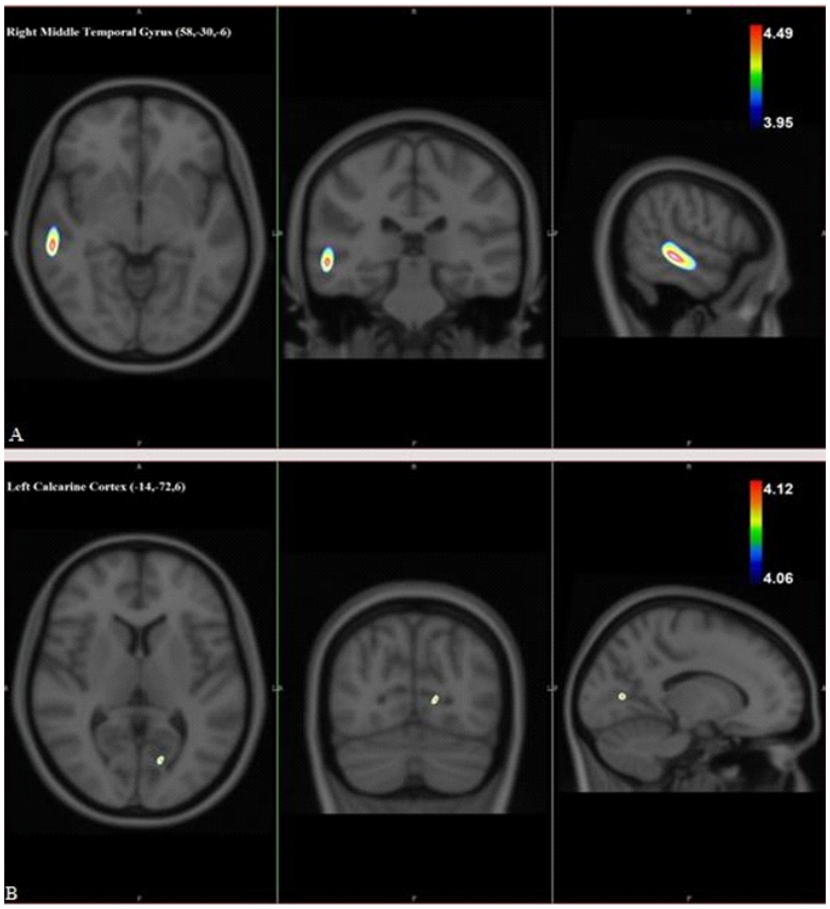

Figure 1. SPM maps showing reduced ^{18}F-FDG uptake A) in all study participants with homocysteine (Hcys) levels above the reference limit (12.6μmol/L) in the right middle temporal gyrus; B) In type 2 diabetic patients with Hcys levels above the cut-off value compared to the non-DM obese patients with Hcys above 12.6μmol/L in the left calcarine cortex. SPM maps were thresholded at FWE-corrected P<0.05 at the cluster level.

Table 2. *Results of SPM analysis and statistical characteristics of right middle temporal gyrus, left calcarine cortex and superior frontal gyrus.*

Region	Subpopulation	Groups	pFWE (peak)	volume (cluster, cm^3)	pFWE (cluster)
Right middle temporal gyrus	All participants	Hcys above and below 12.6μmol/L	0.009	2.58	0.017
Left calcarine cortex	Type 2 diabetic participants	Hcys above and below 12.6μmol/L	0.042	0.14	0.041

Hcys, homocysteine; FWE, Family-Wise Error; SPM, statistical parametric mapping

Discussion

Given the overwhelming burden that neurodegenerative disorders force upon societies, the need for the reduction of their prevalence becomes relevant [24]. Therefore, clinical implementation of diagnostic tools that are capable to provide timely diagnostic assessment of dementia-induced cerebral changes is warranted.

Association between metabolic diseases and cognitive deterioration is frequently investigated. According to the results of the Rotterdam study with 6370 elderly involved, T2DM was reported to represent a two-fold risk for the development of Alzheimer's disease [25]. Connection between obesity and dementia was also strengthened in an Italian study carried out with the enrolment of people suffering from Metabolic Syndrome (MetS) and MCI [26].

Accumulating literature data examined the connection between HHcys and both cognitive decline and metabolic diseases. Previous study strengthens that HHcys is a potential risk factor and biomarker for several cardiovascular and neurological diseases including cognitive impairment [27, 28]. The aforementioned research findings inspired us to evaluate the impact of HHcys on brain metabolism in metabolic diseases applying ^{18}F-FDG PET/CT, that sensitively indicates subtle alterations in regional cerebral metabolism. The investigation of occurrence of elevated Hcys levels in T2DM and obesity was our focus of interest as well. No remarkable difference was found regarding the rate of appearance of increased Hcys levels between the two study groups. Although, based on available literature data, no previous studies have compared the Hcys levels of T2DM and obese patients, some findings exist regarding Hcys values in each group separately. In one study, type 2 diabetic patients were featured with a higher mean value of total Hcys levels (10.5μmol/L; P=0.02) compared to a healthy control group (7.7μmol/L) [29]. In a meta-analysis including 14 studies with 710 obese and 607 healthy subjects involved, non-obese patients were depicted with significantly lower concentrations of Hcys than the obese participants, regardless of nutritional status, insulin resistance (IR), dietary habits, history of medicine, previous anamnesis or genetic characteristics (P<0.05) [30]. Owing to the lack of normal controls in our study, we could not support the aforementioned findings.

We detected decreased metabolism in the rMTG in participants with elevated Hcys levels regardless of the type of metabolic disease present. Interestingly, in those diabetics who had increased Hcys values the left calcarine cortex was also registered with reduced ^{18}F-FDG uptake. To our best knowledge no previous studies have investigated Hcys-related ^{18}F-FDG metabolism in metabolic diseases. Although no PET studies have been published with similar results to ours, some other neuroimaging research report about the regions of these two brain areas. In an MR-^{18}F-FDG and ^{18}F-florbetaben PET study conducted by H. Jeong et al. (2020), medial temporal atrophy was shown to be associated with the prediction of Alzheimer's disease [31]. A resting state functional MRI study pointed out perfusion deficit in the region of the calcarine sulcus bilaterally in patients with dementia [32]. Further, neuroimaging studies experienced grey matter atrophy in the left calcarine sulcus in elderly characterised by vitamin D deficiency [33].

Another aspect is that previous studies applying PET examinations investigated brain metabolic alterations in metabolic disturbances [34, 35]. A recent ^{18}F-FDG PET study reported decreased regional brain glucose metabolism in the ventral prefrontal, cingulate, temporal, insular, posteromedial cortices and in the cerebellum associated with higher Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), a parameter characterising metabolic diseases [36]. Although metabolic diseases may be featured with IR, our results could not be compared to the outcome of the mentioned research which might possibly be explained by the fact that neither the severity of IR was defined in our work, nor we took the effect of IR on cerebral glucose metabolism into account. In another study, decreased glucose uptake of the frontotemporal brain areas was also found in T2DM even after controlling for vascular risk factors [35]. Further, obese individuals expressed higher fasting metabolism in the parietal somatosensory cortical regions compared to lean people [34]. Our study could not support this result since we did not involve slim patients in our study. In addition, regional metabolic alterations were not evaluated in the detailed study, which could also explain why our results are not in line with the mentioned one.

Considering our results, Hcys seems to influence ^{18}F-FDG brain metabolism. Given that the rMTG is considered to be a dementia-associated brain region, we suppose that Hcys-dependent metabolic alteration in this region may project the appearance of future cognitive decline in patients with metabolic diseases [37]. This finding may draw the attention to the clinical importance of the measurement of Hcys levels in long-term prediction of cognitive deterioration in metabolic disturbances. The underlying mechanism behind decreased ^{18}F-FDG uptake in the left calcarine cortex in diabeticsis not exactly known. It may be related to the mechanism of microvascular damage associated with diabetes.

Finally, our result did not show significant difference between the genotype distribution of the two study groups and the appearance of elevated Hcys values in case of different genotypes, although Hcys levels above the reference limit were slightly more frequent in subjects with the presence of T allele (in both patient groups separately, and in all cases of our study irrespective of the metabolic disease). The available findings considering the association between T2 DM/obesity and MTHFRC677T polymorphism are inconclusive. In a meta-analysis of 29 epidemiologic studies, Al-Rubeaan K. et al. (2013) reported a significant association bet ween the MTHFRC677T polymorphism and T2DM in the Chinese Han population [38]. The same group also found an association between T2DM and MTHFR polymorphism in the Arabian population, however they did not confirm this connection among the Caucasians [38]. Although previous studies have not drawn definitive conclusion concerning the relationship between the onset, development, or the progression of T2DM or other types of metabolic disturbances and MTHFRC677T genetic polymorphism, higher frequency of T allelein patients with MetS-related HHcys may suggest some associations. Future studies with greater sample size are required to further examine the relationship between metabolic diseases and the detailed genetic alteration.

In conclusion, we detected regional metabolic alterations related to HHcys in T2DM and obesity, which may be in connection with microvascular alterations induced by metabolic diseases. Fluorine-18-FDG PET/CT seems to be a sensitive diagnostic tool showing alterations in brain metabolism in both T2DM and obesity. It may have importance in the follow-up of patients with metabolic disturbances, since biological markers derived from brain imaging may precede the development of dysmetabolism-related brain changes even before the appearance of clinical symptoms, and could be useful early indicators of the appearance of neurological disorders.

Limitations

Since we did not involve a group of healthy volunteers and because of the limited number of recruited individuals, the relationship between the brain metabolic impairments and the metabolic diseases themselves could not be examined in more detail. Furthermore, medically controlled diabetic patients were enrolled in this study under different types of medications (e.g. antidiabetics with different mechanisms of action, antihypertensive and lipid-lowering drugs, or antidiuretics), and we did not examine the separate effects of the mentioned drugs on our results.

Bibliography

1. Patterson C. The state of the art of dementia research: New frontiers. *World Alzheimer* Rep 2018.
2. Daly A, Walsh D.Dementia-a major public health problem: the role of in-patient psychiatric facilities. *Irish J Med Sci* (1971) 2019; 188: 641-7.
3. Zukotynski K, Kuo PH, Mikulis D et al. PET/CT of Dementia. *Am J Roentgenol* 2018; 211: 246-59.
4. Friedland RP, Budinger TF, Ganz E et al. Regional Cerebral Metabolic Alterations in Dementia of the Alzheimer Type. *J Comput Assist Tomogr* 1983; 7: 590-8.
5. Minoshima S, Giordani B, Berent S et al. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann Neurol* 1997; 42: 85-94.
6. Huda A, Kartamihardja AS, Darmawan B et al. Metabolic Activity Value in the Posterior Cingulate Cortex Using F-18 Fluorodeoxyglucose Positron Emission Tomography Brain to Predict the Severity of Alzheimer's. *World J Nucl Med* 2017; 16: 108-13.
7. Yokoi T, Watanabe H, Yamaguchi H et al. Involvement of the Precuneus/Posterior Cingulate Cortex Is Significant for the Development of Alzheimer's Disease: A PET (THK5351, PiB) and Resting fMRI Study. *Front Aging Neurosci* 2018; 10: 304.
8. Rochette AD, Spitznagel MB, Strain G et al. Mild cognitive impairment is prevalent in persons with severe obesity. *Obesity* 2016; 24: 1427-9.
9. Baker LD, Cross DJ, Minoshima S et al. Insulin Resistance and Alzheimer-like Reductions in Regional Cerebral Glucose Metabolism for Cognitively Normal Adults With Prediabetes or Early Type 2 Diabetes. *Arch Neurol* 2011; 68: 51-7.
10. Shalev D, Arbuckle MR. Metabolism and Memory: Obesity, Diabetes, and Dementia. *Biol Psychiatry* 2017; 82: e81-3.
11. Tinelli C, Di Pino A, Ficulle E et al. Hyperhomocysteinemia as a Risk Factor and Potential Nutraceutical Target for Certain Pathologies. *Front Nutr* 2019; 6: 49.
12. Nie T, Lu T, Xie L et al. Hyperhomocysteinemia and Risk of Cognitive Decline: A Meta-Analysis of Prospective Cohort Studies. *Eur Neurol* 2014; 72: 241-8.
13. Wang C, Wu Q, Zhang L et al. Elevated total plasma homocysteine levels are associated with type 2 diabetes in women with hypertension. *Asia Pac J Clin Nutr* 2015; 24: 683-91.
14. Zhi X, Yang B, Fan S et al. Additive Interaction of MTHFR C677T and MTRR A66G Polymorphisms with Being Overweight/Obesity on the Risk of Type 2 Diabetes. *Int J Environ Res Public Health* 2016; 13: 1243.
15. Vayá A, Rivera L, Hernández-Mijares A et al. Homocysteine levels in morbidly obese patients. Its association with waist circumference and insulin resistance. *Clin Hemorheol Microcirc* 2012; 52: 49-56.
16. Fu L, Li Y, Luo D et al. Plausible relationship between homocysteine and obesity risk via MTHFR gene: a meta-analysis of 38,317 individuals implementing Mendelian randomization. *Diabetes, Metab Syndr Obes Targets Ther* 2019; 12: 1201-12.
17. Avants B, Epstein C, Grossman M, Gee J. Symmetric diffeomorphic image registration with cross-correlation: Evaluating automated labeling of elderly and neurodegenerative brain. *Med Image Anal* 2008; 12: 26-41.
18. Dale AM, Fischl B, Sereno MI. Cortical Surface-Based Analysis. *Neuroimage* 1999; 9: 179-94.
19. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Med Image Anal* 2001; 5: 143-56.
20. Jenkinson M, Beckmann CF, Behrens TEJ et al. FSL. *Neuroimage* 2012; 62: 782-90.
21. Wang J, Xu L, Xia H et al. Association of MTHFR C677T gene polymorphism with metabolic syndrome in a Chinese population: a case-control study. *J Int Med Res* 2018; 46: 2658-69.

22. Lyon E, Wittwer CT. LightCycler Technology in Molecular Diagnostics. *J Mol Diagnostics* 2009; 11: 93-101.
23. Ashburner J. SPM: A history. *Neuroimage* 2012; 62: 791-800.
24. Ricci G. Social Aspects of Dementia Prevention from a Worldwide to National Perspective: A Review on the International Situation and the Example of Italy. *Behav Neurol* 2019; 2019: 1-11.
25. Ott A, Stolk RP, van Harskamp F et al. Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology* 1999; 53: 1937-42.
26. Solfrizzi V, Scafato E, Capurso C et al. Metabolic syndrome, mild cognitive impairment, and progression to dementia. The Italian Longitudinal Study on Aging. *Neurobiol Aging* 2011; 32: 1932-41.
27. Battezzati A, Bertoli S, San Romerio A, Testolin G. Body composition: An important determinant of homocysteine and methionine concentrations in healthy individuals. *Nutr Metab Cardiovasc Dis* 2007; 17: 525-34.
28. Brustolin S, Giugliani R, Félix TM. Genetics of homocysteine metabolism and associated disorders. *Brazilian J Med Biol Res* 2010; 43: 1-7.
29. Ebesunun MO, Obajobi EO. Elevated plasma homocysteine in type 2 diabetes mellitus: a risk factor for cardiovascular diseases. *Pan Afr Med J* 2012; 12: 48.
30. Wang J, You D, Wang H et al. Association between homocysteine and obesity: A meta-analysis. *J Evid Based Med* 2021; 14(3): 208-17.
31. Jeong HE, Shin DH, Lee DC. Medial Temporal Atrophy Alone is Insufficient to Predict Underlying Alzheimer's Disease Pathology. *Korean J Fam Med* 2020; 41: 352-8.
32. Yan S, Qi Z, An Y et al. Detecting perfusion deficit in Alzheimer's disease and mild cognitive impairment patients by resting-state fMRI. *J Magn Reson Imaging* 2019; 49: 1099-104.
33. Sultan S. Neuroimaging changes associated with vitamin D Deficiency - a narrative review. *Nutr Neurosci* 2021; 28: 1-9.
34. Wang G-J, Volkow ND, Felder C et al. Enhanced resting activity of the oral somatosensory cortex in obese subjects. *Neuroreport* 2002; 13: 1151-5.
35. García-Casares N, Berthier ML, Jorge RE et al. Structural and Functional Brain Changes in Middle-Aged Type 2 Diabetic Patients: A Cross-Sectional Study. *J Alzheimer's Dis* 2014 40: 375-86.
36. Willette AA, Bendlin BB, Starks EJ et al. Association of Insulin Resistance With Cerebral Glucose Uptake in Late Middle-Aged Adults at Risk for Alzheimer Disease. *JAMA Neurol* 2015; 72: 1013-20.
37. Scheltens P, Leys D, Barkhof F et al. Atrophy of medial temporal lobes on MRI in 'probable' Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. *J Neurol Neurosurg Psychiatry* 1992; 55: 967-72.
38. Al-Rubeaan K, Siddiqui K, Saeb ATM et al. ACE I/D and MTHFR C677T polymorphisms are significantly associated with type 2 diabetes in Arab ethnicity: A meta-analysis. *Gene* 2013; 520: 166-77.