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DIFFERENCE IN APOLIPOPROTEIN E GENOTYPE DISTRIBUTION BETWEEN DENTATE AND EDENTULOUS ELDERLY PATIENTS WITH ALZHEIMER DISEASE

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Association between dementia and tooth loss has been shown although the nature of that association is not clear. It has also been shown that risk of dementia was increased in apolipoprotein E4 allele carriers. The objective of this study was to determine the frequency of APOE alleles and their association with the dental status in elderly demented patients. Dental status of 67 patients with dementia was recorded. DNA was isolated from buccal swabs and genotyping was done by PCR-RFLP. The majority of participants had E3/E4 genotype (55.2%) and these heterozygotes were significantly more frequent than any other genotype ($p<0.001$). There was no significant association between dental status and genotype. However, partial edentulousness with very few teeth in both jaws (1-9 teeth) was significantly more frequent among demented patients with E3/E4 genotype ($p=0.021$). Patients with Alzheimer disease most frequently had E3/E4 genotype and had very few or no teeth.

Key words: Alzheimer disease, apolipoprotein E polymorphism, teeth number, dental status.

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INTRODUCTION

Apolipoprotein (ApoE) is a protein that is well known for its key roles in the transport of cholesterol and other lipids in blood circulation and central nervous system (SHORE and SHORE, 1973; MAHLEY and RALL, 2000). ApoE includes three common isoforms (ApoE2, ApoE3, ApoE4), coded by three codominant alleles (e2, e3, e4). Hence, six phenotypes commonly exist in the general population, three homozygous (ApoE2/2, ApoE3/3, and ApoE4/4) and three heterozygous (ApoE2/3, ApoE2/4, and ApoE3/4). ApoE2 and ApoE4 differ from ApoE3 by a single amino acid substitution. While ApoE3 contains cysteine at position 112 and arginine at position 158, the arginine is substituted by a cysteine in ApoE2 carriers and the cysteine is substituted by an arginine in ApoE4 carriers (SHORE and SHORE, 1973). ApoE4 (Arg at 112 and 158), has generated remarkable interest since it emerged as a major genetic risk factor for development of late onset Alzheimer disease (AD) (LAHIRI *et al*., 2004; SUNDSTROM *et al*., 2007; TILVIS *et al*., 1998; ROSICH-ESTRAGO *et al*., 2004; FRIKKE-SCHMIDT *et al*., 2001; BENNETT *et al*., 2003; LITTLE *et al.,* 2009). ApoE4 is also associated with moderately increased risk of cerebrovascular disease and stroke (MCCARRON *et al*., 1999; ZHU *et al.,* 2000).

Association was also found between edentulousness and the presence of apoE4 (BERGDAHL *et al.,* 2008). Independently, several studies have established a link between tooth loss and risk of dementia (SHIMAZAKI *et al.,* 2001; SPARKS STEIN *et al.,* 2007; KIM *et al*., 2007). Tooth loss is in most cases caused by local factors such as dental caries and periodontal disease. In both conditions genetic factors appear to be implicated (SHULER, 2001; MICHALOWICZ *et al*., 2000; MUCCI *et al*., 2005; JOSHIPURA *et al*., 2003).

However, data on both dental status and apoE genotype in AD patients are very scarce, i.e., there are no evidence about simultaneous impact of teeth loss and presence of apoE4 on dementia onset. This fact prompted us to evaluate the frequency of apolipoprotein (APOE) alleles and genotypes in demented patients and determine whether APOE type 4 allele was associated with dental status in Serbian population.

MATERIALS AND METHODS

Subjects

Patients with dementia were recruited at The Institute of Neurology, University Hospital Centre and at Geriatric Centre in Belgrade. Inclusion criteria were diagnosis of stable form of Alzheimer disease and no significant changes in dental status over the past 5 years (no multiple extractions or new fixed dentures). Alzheimer disease was previously diagnosed by neurologists in all the cases. Interview was conducted and written consent was obtained from patients relatives. The study was approved by the Ethical committee of the School of Dental Medicine, University of Belgrade.

General patients' characteristics were recorded: sex, age group (early old age: 65-74 years; middle old age: 75-84 years and very old age: 85 years and more), education (basic elementary school – 4 grades; elementary school – 8 grades; high school; faculty – 2 years; faculty – 4 to 6 years;) chronic diseases (none; 1 or 2; and 3 or more diseases beside dementia) and the period that elapsed from the moment of dementia being diagnosed (up to 3 years; more than 3 years). Swabs of buccal mucosa were taken for DNA extraction and patient's genotyping. Dental examination was carried out in order to determine basic dental status (edentulous, partially edentulous or had a full dental arch in both jaws) and the number of natural teeth. Groups of

natural teeth were: 0, 1-9, 10-19 and 20 and more. Fixed prosthetic dentures were considered equivalent to natural teeth.

APOE genotyping

Genomic DNA was isolated from buccal swabs using commercial kit for isolation (PureLink TM Genomic DNA Mini Kit, Invitrogen) according to the manufacturer's recommendations. Isolated DNA was stored at -20°C until further analysis. A polymerase chain reaction (PCR) was performed using 200 ng of genomic DNA as template in 25 ml reaction mixture containing 20 pmol of PCR primers APOE-A (5′-TCCAAGGAGCTGCAGGCGGCGCA-3′) and APOE-B (5′-ACAGAATTCGCCCCGGCCTGGTACACTGCCA-3′), 1U of Taq DNA polymerase (Gibco BRL, Gaithersburg, MD, USA), 1.0 mM $MgCl_2$ 75 mM Tris-HCL (pH 9.0), 20 mM $(NH_4)_2SO_4$ and 10% dimethyl sulphide.PCR amplification consisted of 35 cycles of 30 s at 95, 45 s at 63°, and 45 s at 72°C. PCR products (270 bp) were digested using 5 U of HhaI (Thermo Fisher Scientific, Inc.). After digestion, products were separated on a 10% polyacrilamide gel and visualised on a UV transilluminator after ethidium bromide staining. The fragment lengths were as follows: for ε2 allele: 91 bp, 83 bp and 38 bp; for ε3 allele: 91 bp, 48 bp, 38 bp and 35 bp; for ε4 allele: 72 bp, 48 bp, 38 bp and 35 bp.

Statistical analysis

For statistical analysis, investigated parameters were presented as nominal values (sex, marital status, education, age group, duration of dementia, genotype, and dental status). The significance of difference, for 95% confidential interval, was evaluated using non-parametric tests: hi-square test and Mann-Whithney (for two) or Kruskal-Wallis (for several) independent samples test.

RESULTS

There were 67 participants in the study, aged 65 and more. Most of them were females (71.6%), in the early old age (47.8%), with diagnosed dementia for 3 years or less (61.2%), and had only elementary education (8 years of elementary school; 34.4%). Regarding general health condition, most of respondents had up to two diagnosed chronical diseases (besides Alzheimer disease) - 59.7%, mostly cardiovascular diseases or diabetes mellitus type 2.

The frequency of APOE alleles was the following 2.2% (E2), 69.4% (E3) and 28.4 (E4). The majority of patients had E4 allele in the heterozygous genotype combination E3/E4 (Figure 1). E3/E4 genotype was significantly more frequent compared to other genotypes individually ($p<0.001$).

Regarding dental status, edentulous patients were most frequent (37.3%), Figure 2. There was a highly significant difference between different dental statuses ($p< 0.001$). Partial edentulousness in both jaws was significantly more frequent comparing to other forms of dental status ($p=0.038$).

There was no significant association between dental status and genotype ($p=0.336$). Among patients with E4 allele, totally edentulous patients were not significantly more frequent ($p=0.157$) compared to other dental statuses, whilst partial edentulousness in both jaws was significantly more frequent among demented patients with E3/E4 genotype compared to other dental statuses ($p=0.021$).

Regarding the number of teeth, the majority of patients had none (37.3%) or very few teeth (1-9) 25.4% (figure 3). Genotype and number of teeth did not show statistically significant association (p=0.302).

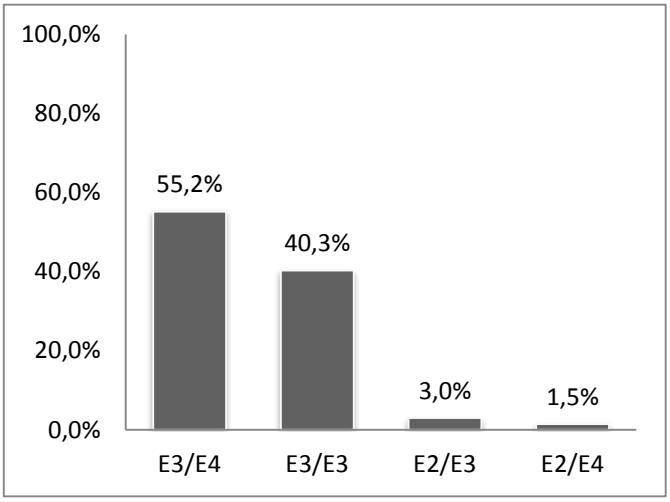

Figure 1. Distribution of apoE genotypes in demented patients.

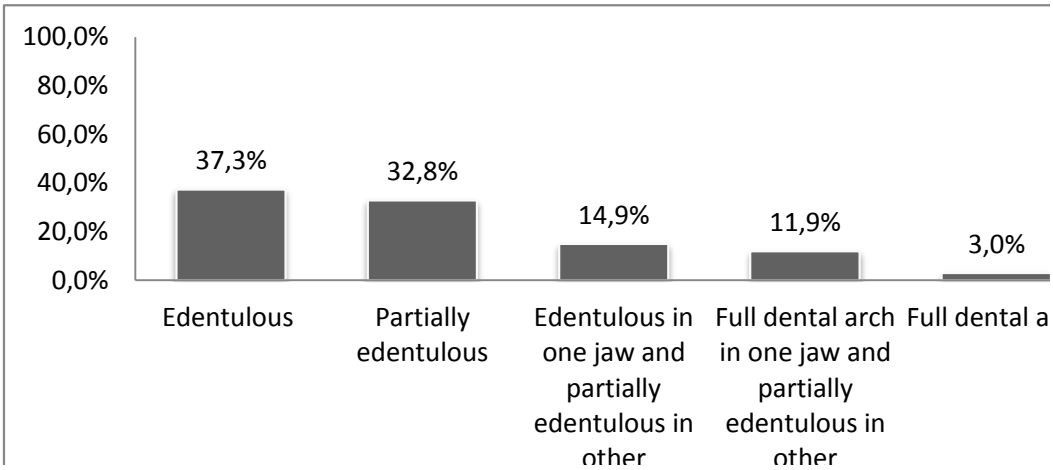

Figure 2. Distribution of dental statuses among demented patients.

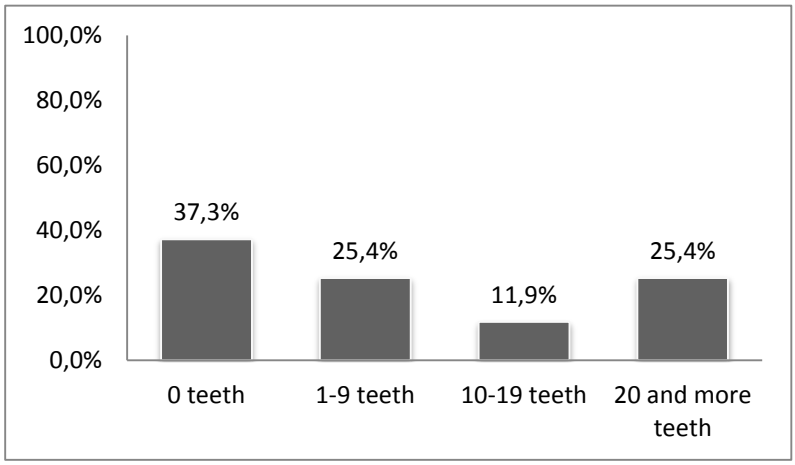

Figure 3. Distribution of teeth number among demented patients.

DISCUSSION

It has been well documented that E4 contributes to Alzheimer's disease (LAHIRI *et al.*, 2004; SUNDSTROM *et al*., 2007; TILVIS *et al.*, 1998; ROSICH-ESTRAGO *et al*., 2004; FRIKKE-SCHMIDT *et al*., 2001; BENNETT *et al.*, 2003; LITTLE *et al.*, 2009) with accelerated development of cognitive impairment (SUNDSTROM *et al.*, 2007; TILVIS *et al.*, 1998; LITTLE *et al.*, 2009). Moreover, association between apoE4 and mortality was shown, and dementia was strongly associated with shorter survival (MCCARRON *et al*., 1999; SPARKS STEIN *et al*., 2007). It is considered that apoE4 carriers are more prone to beta-amyloid deposits and senile plaque formation in brain tissue than carriers of other isoforms of apolipoprotein. Our results confirm the association between dementia and the presence of apoE4 allele. The main finding of this study is the existence of positive association between the presence of apoE4 allele, very few teeth and dementia.

In our study group there was no case with genotype E4/E4, and allele E4 was almost in all cases incorporated in the genotype E3/E4 (55.2%). This is in contrast with the USA population where E3/E3 genotype was the most frequent among dementia patients with 51.3%, followed by E3/E4 present in 33.0% (LITTLE *et al*., 2009). ApoE allele and genotype frequencies found in the present study in AD patients were considerably different compared to the reported control values from the study of Kecmanović et al. on Serbian population (KECMANOVIĆ *et al.*, 2010). Interestingly, Kecmanović and associates in their study did not find association between APOE polymorphism and schizophrenia.

In our study, most demented patients were edentulous or partially edentulous with very few teeth, which is in accordance with similar studies where authors concluded that having less teeth increased the risk for dementia. In the population of Sweden, the edentulous group had a higher frequency of APOE epsilon 4 than the dentate group (BERGDAHL *et al*., 2008), which is in agreement with our results.

Jae Min et al. concluded that having fewer teeth is significantly associated with dementia and Alzheimer disease (JAE-MIN *et al*., 2007). Shimazaki and associates in their prospective study

showed that the probability of the development of mental impairment was higher in subjects who were older, had worse dentition and stayed in an institution (SHIMAZAKI *et al.*, 2001). These associations could be potentially explained by common underlying risk factors both for tooth loss and dementia, which may be chronic infection or inadequate nutrition (JOSHIPURA *et al.*, 2003; TAGUCHI *et al.*, 2004). Tooth loss can have effects on diet and nutrition contributing to neuropathology and cognitive decline (SPARKS STEIN *et al*., 2007; KAMER *et al*., 2008; STEWART *et al*., 2008). If the reason of tooth loss is periodontal disease, the chronic recurrent infection and inflammation originating from periodontal tissue may be responsible for this association. In particular in the case of Alzheimer's disease inflammatory involvement has been described both in neuropathological and epidemiological studies (KAMER *et al*., 2008; HOLMES *et al*., 2009). In addition, early experiments on animal have shown that spatial memory and cholinergic neuronal system are impaired by the loss of molar teeth, suggesting that tooth loss is one of the risk factors for dementia (KATO *et al*., 1997), but these assumptions were not until recently proved in humans. It must be emphasized that some studies have not been able to show any association between oral condition and occurrence of dementia (ARRIVE *et al.*, 2012). On the opposite, they concluded that having eleven or more missing teeth was associated with a minor risk of dementia possibly due to the suppression of source of chronic inflammation originating from periodontal disease.

Some association between dental status and dementia exists, since most of partially edentulous patients had apoE4 allele. A larger sample is needed to confirm these findings. In the only study that investigated both dental status and apoE in demented patients, a similar interaction was found (STEIN *et al.*, 2007).

It cannot be concluded whether dental status has influenced dementia onset or having dementia has worsened dental status. However, dementia itself is unlikely to have caused loss of teeth over the relatively brief period of diagnosis (mostly less than 3 years), and patients were with unchanged dental status for a minimum of 5 years, but usually much longer. Since the differences in chewing performance and physiology of orthognatic system, with all their negative effects, are huge between patients with and without dentures, prosthetic rehabilitation in AD patients should be strongly recommended.

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RAZLIKA U DISTRIBUCIJI APOLIPOPROTEINA E KOD STARIH LJUDI SA ALCHAJMEROVOM BOLEŠĆU RAZLIČITOG DENTALNOG STATUSA

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Izvod

Veza između demencije i gubitka zuba je pokazana u literaturi iako priroda ove povezanosti nije jasna. Takođe je pokazano da je rizik od demencije povećan kod nosilaca apolipoprotein E4 alela. Cilj ove studije je bio da se utvrdi učestalost APOE alela i njihova veza sa dentalnim statusom kod starijih ljudi sa demencijom.Dentalni status je zabeležen kod 67 ispitanika sa demencijom. DNK je izolovana iz bukalnog brisa, a genotipizacija je urađena metodom PCR-RFLP.. Genotip E3/E4 je bio statistički značajno češći od ostalih ($p<0.001$). Nije pronađena značajna veza između dentalnog statusa i genotipa i između broja zuba i genotipa. Međutim, krezubost sa veoma malim brojem zuba (1-9) je bila značajno učestalija među ispitanicima sa E3/E4 genotipom ($p=0.021$).Pacijenti sa Alcajmerovom bolešću najčešće imaju E3/E4 genotip i mali broj zuba ili su bezubi.

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