miRNAs are small non-coding RNAs that regulate gene expression by interaction with target miRNAs. It was shown that miRNA binding sites are located not only in 3′UTR but also in 5′UTR and Coding Sequence (CDS) [1, 2]. There are plenty of programs for miRNA binding sites prediction in 3′UTR of mRNA [3, 4]. While miRNA binding sites in CDS and 5′UTR are not studied well. However, researching miRNAs and alteration of their concentration is an essential for understanding of miRNA involvement in the regulation of gene expression and pathological processes. It was shown that miRNAs are involved in Alzheimer’s disease (AD) [5]. Among neurodegenerative diseases AD is the most common form of dementia in the world. World Alzheimer Report in 2015 estimates about 46.8 million people worldwide have dementia. Epidemiological data predict that by 2050 more than 131 million of people will be affected by AD. It was shown that some miRNAs were significantly differentially expressed between patients with AD and control groups [6]. Moreover, miRNAs can be used as potential biomarkers of AD [7]. Therefore, studying of miRNA binding sites in mRNA of genes involved in AD is essential for understanding molecular pathology of this disease.

Materials and Methods. Using databases and publications 74 most involved genes in Alzheimer’s disease were chosen. Nucleotide sequences of these genes were taken from NCBI (http://www.ncbi.nlm.nih.gov). MiTarget program developed previously in our laboratory was used for miRNA binding sites prediction [8]. This program searches the miRNA binding site within entire sequence of mRNA in 3′UTR, CDS, 5′UTR taking into account the free energy of miRNA:mRNA hybridization (ΔG, kJ/mole). The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals to the free energy of miRNA binding with its fully complementary nucleotide sequence). Nucleotide sequences of 2568 miRNAs were taken from mirBase (http://mirbase.org) and 3701 miRNAs from article of Londin et al. [9]. miRNA binding sites with high ΔG/ΔGm ratio were chosen.

Results. It is predicted that among 74 genes involved in Alzheimer’s disease development 64 are targets for miRNAs. Therefore, the expression of most genes associated with AD could be regulated by miRNAs. It was also revealed that binding sites of some of the miRNAs are arranged sequentially or with overlapping each other forming a cluster. Clusters were characterized according to their: 1) localization within mRNA; 2) average value of free energy of interaction; 3) compactness – a degree, which evaluate the ratio of length of all miRNA binding sites on the length of cluster in mRNA. There are eleven clusters with three miRNA binding sites in miRNAs of genes ACHE (in 5′UTR, CDS and 3′UTR), APP (in 5′UTR), BACE (in 5′UTR), BIN1 (in 5′UTR), CHAT (in 5′UTR), CYP46A1 (in CDS), MAPT (in 5′UTR), MEF2C (in 5′UTR), MTHFR (in 5′UTR). For clusters with four miRNA binding sites were found in miRNAs of genes CBRN52 (in 3′UTR), HDG1 (in CDS and 3′UTR), and SORL1 (in 5′UTR). Three clusters with five miRNA binding sites were found in genes BACE1 (in 5′UTR), CHRN7 (in 5′UTR), and PARG (in 5′UTR). There are two clusters with six miRNA binding sites in miRNAs of genes TOMM40 (in CDS) and P1N1 (in 5′UTR). Five miRNAs have binding sites in CDS of mRNA of ACE gene in position from 60 to 106 nucleotide (nt) with an average value of interaction energy equal to -126 kJ/mole. This cluster is 47 nt in length, 3.5 times less than the total length of all binding sites equal to 162 nt. There are four clusters of miRNA binding sites in mRNA of ACHE gene. Two of them are in 5′UTR of ACHE from 3 to 43nt and from 117 to 164 nt with an average ΔG equal to -118 kJ/mole and -122 kJ/mole respectively. Other clusters consist of binding sites for three miRNAs in CDS (from 1841 to 1869 nt with ΔG = -124 kJ/mole) and in 3′UTR (from 2133 to 2162 nt with ΔG = -119 kJ/mole). The mRNA of GSK3B gene contains three clusters of mRNA binding sites. First cluster of 41 binding sites for 23 miRNAs in 5′UTR from 2 to 40 nt with an average ΔG equal to -126 kJ/mole. Second cluster in 5′UTR from 352 to 378 nt with ΔG = -123 kJ/mole. There is a cluster of polysites for 4 miRNAs in 3′UTR of GSK3B from 4705 to 4748 nt with ΔG = -104 kJ/mole. The ratio of the total length of miRNA binding sites to the length of clusters in the mRNA of this gene is 22, 6 and 14 respectively. There are clusters in 5′UTR of following genes: BIN1 has binding sites for miR-4-11761-3p, miR-8-21445-5p and miR-7-20193-5p; PPARGC1A has 23 binding sites for eight miRNAs with an average ΔG equal to -111 kJ/mole; five binding sites for three miRNAs in PPARG with ΔG = -124 kJ/mole; 13 binding sites for nine miRNAs in RELN with ΔG = -124 kJ/mole. Clusters of mRNA binding sites were found in 3′UTR of following genes: polysites for six miRNAs in CD2AP with average ΔG equal to -105 kJ/mole; binding sites for miR-101-27078-5p and miR-3-5147-5p in
AN IMPACT OF DIABETES DURATION ON SERUM ASYMMETRIC DIMETHYLARGININE CONCENTRATION

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Recent studies point to significant role of endothelial dysfunction in the pathogenesis of diabetes mellitus type 2 (DMT2) and its complications. However, an impact of diabetes duration on markers of endothelial dysfunction such as asymmetric dimethylarginine (ADMA) has not been sufficiently investigated. The aim of the present study was to assess an impact of diabetes duration on serum ADMA concentration.

Materials and Methods. Participants for this cross-sectional study were randomly selected from Out-Patient Family Medicine Clinic “Višnjik”, Sarajevo, Bosnia and Herzegovina. DMT2 was defined by American Diabetes Association criteria. Based on diabetes duration patients with DMT2 were divided into: up to 10 years diabetes duration group

Keywords: Alzheimer’s disease, miRNA, cluster of binding sites, target genes

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