Proteins expression clustering of Alzheimer disease in rat hippocampus proteome

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ABSTRACT

Because of the huge amounts of proteomic data and demand for new methods of laboratory analysis results, proteins collective analysis, in addition to taking less time, biostatistician assist at identification of new patterns in the data set. In this study, rat hippocampus proteome in normal and Alzheimer's disease (AD) were analyzed by using proteomic techniques and bioinformatics' analysis. Protein extracts from normal and Alzheimer's rats were separated by using two-dimensional electrophoresis (2DE). The silver staining method was used for detecting spots. Bioinformatics analysis of proteome were performed by progensis same spots software. Bioinformatics and statistical analysis of 2DE gel techniques obtained 760 protein spots were detected in both normal and AD rats. Comparisons between controls and Alzheimer gel containing 20 common proteins were expressed significantly differences. 16 new proteins were expressed in AD, while 36 proteins were suppressed. Proteins clustering by using correlation analysis evaluated 3 clusters in the proteome; Principal component analysis also confirmed the results of clustering. Finally, we can conclude that a significant expression of Alzheimer changes in the hippocampus proteome which are associated with specific biological processes summarized in 3 main clusters indicated 3 principal biological pathways of AD.

Keywords: Alzheimer; Proteomics; Clustering; Progenesis; Same Spot Software

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia, is characterized by intracellular accumulation of neurofibrillary tangles (NFT) and extracellular beta amyloid (Aβ) plaque [1, 2]. Aβ is a toxic pro-inflammatory agent that promotes inflammatory process in the brain [3]. AD patient Also have defect in cholinergic system[4,5]. In AD, hippocampus is the first brain region changes, in addition the greatest concentration of amyloid plaques [5] are also present, so it is detected early neurodegenerative symptoms include significant deficits in the performance of hippocampus dependent cognitive abilities such as spatial learning and memory [6]. AD is the result of both genetic and etiologic factor (environmental, trauma, and immunological changes such as viral invasion and inflammation) and changes in the brain may start several decades before the onset of clinical symptoms. The other side, there is no cure for AD so far, and the disease causes progressive decline in daily activities, eventually resulting in death [7,8]. Different Proteomic investigations by using classical and advanced methods on human and animal models of Alzheimer's demonstrated changes in protein expression in different parts of the brain [9-12]. Several classes of proteins involved in different stages of the AD such as proteins responsible for synaptic transmission (synaptosomes proteomics), a set of heat shock proteins (HSC71) and mitochondrial proteins were expressed in response to oxidative stress [11]. As well biomarkers that ways have been identified as targets for diagnostic and therapeutic proteins include presenilin 1 and 2, and beta amyloid precursor (APP) [13] in the first stage and the apolipoprotein E allele e4 [14] in the final stages as behavioral disorders Alzheimer's genes responsible. Mutations in the presenilin genes cause of 80% AD development [15]. Because of the huge amounts of proteomic data, new analysis methods for data mining is necessary so analysis of proteins as protein set can also spend less time

and help researchers to identify new patterns in the data set. Today, the use of software clustering imperative seems to be one of the main ways to get quick access to information and analysis of experimental data (6). In this study, hippocampus tissue proteome of rats in AD and normal groups are analyzed by using proteomic techniques and bioinformatics analyzes. Increased or decreased protein expression of two groups gels are cluster by using progensis same spots software. This software can cluster protein in different groups with co-expression [6] that indicates every cluster has biological correlation. Relationships based on co-expression capture the overall tendency for a pair or group of genes to have similar expression levels across conditions (as opposed to being high in one set of conditions and low in another)[7]. In every disorder, proteomic study employ in the area of disease particularly to reveal disease relevant biomarkers. Thus, in this study detect protein co-expression of rat hippocampus to identify biomarkers for molecular diagnosis as well as new targets for medical intervention of the disease process.

MATERIALS AND METHODS

Animals

A total number of 10 male Wistar rats, weighing 220-280 g, were employed in the present study. The animals were kept at constant temperature under a 12:12 h light/dark cycle with free access to food and water. The subjects were first divided into control and Alzheimer group.

Establishment of AD model

Animal model of AD was created by intracerebroventricular (i.c.v.) injection of 10 μg of Aβ1-42 peptide (Sigma Aldrich, St. Louis, MO, USA) dissolved in distilled water. The injection site (AP=Bregma, LR=1.5 mm, D=4 mm) was determined according to the Stereotaxic Atlas (15). The animals in control group were treated with the same procedure except that they received distilled water.

Sampling

The Congo red staining verified the formation of Aβ plaque in the hippocampal area of brain in Aβ-treated animals (16, 17). Hippocampus tissue samples were taken from both groups of rats for proteomics analysis.

Protein purification

Fresh tissue samples of hippocampus tissue were snap frozen and kept in liquid nitrogen until use. Each tissue sample was added to an appropriate amount of lysis buffer containing 7 M Urea, 2 M Thiourea, 4% CHAPS(3-(3-Cholamidopropyl) dimethylammonio)-1-propanesulfonic acid), 40 mM Tris, 50 mM DTT (Dithiothreitol), and protease inhibitor (one tablet in 2 ml lysis buffer) (Roche). Tissue samples were homogenized by pestle under ice conditions. Homogenates were left for one hour at room temperature and sonicated on ice for 10 s every 15 min. After that centrifuged at 20000 g for 60 minutes at 4 °C. Protein concentration of all samples was estimated using a Bradford based microassay. 200 **μg** from each sample was resuspended in rehydration buffer containing 8 M urea, 4% CHAPS, 0.5% Ampholyte, 50 mM DTT for 7 hr and then loaded onto 7 cm immobilized (pH=3-10) nonlinear gradient strips (Bio-Rad, Hercules, CA, USA).

Two dimensional SDS-PAGES

The first dimension of 2D electrophoresis was performed on the PROTEAN IEF Cell system (Bio-Rad). Next, gels were equilibrated for 15 min in equilibration buffer I (6M urea, 2% SDS, 0.375 M Tris Hcl pH8.8, 20% glycerol, 130mM DTT). A 12% SDS-Polyacrylamide slab gel was used for the second dimension gel electrophoresis. Equilibrated IPG strips were placed on the surface of the second dimension gels and then sealed with 0.5% agarose in SDS electrophoresis buffer (25mM Tris base, 192mM glycine, 0.1%SDS) and were run vertically. Resulting gels were stained with silver nitrate.

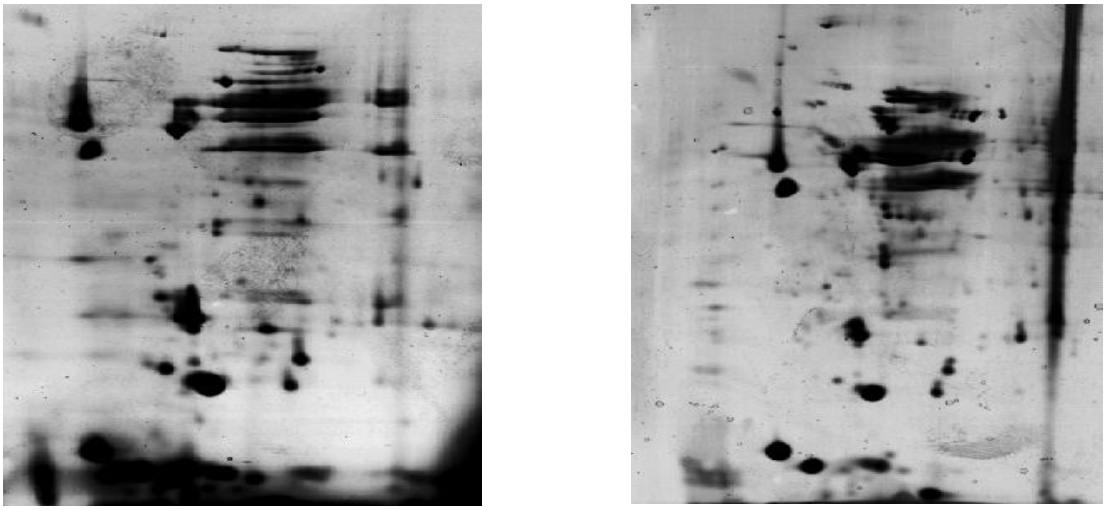
Bioinformatics analysis

2DE gels were scanned by Densitometer GS-800 (BioRad) and gels were analyzed by non linear progenesis same spot software to compare gels together and compare the spots in one statement in gels and get the density of same spot in each of gel. Statistical significance achieve by ANOVA (P value <0.05). Hierarchical clustering and principal component analysis were used for comparing two groups.

RESULT

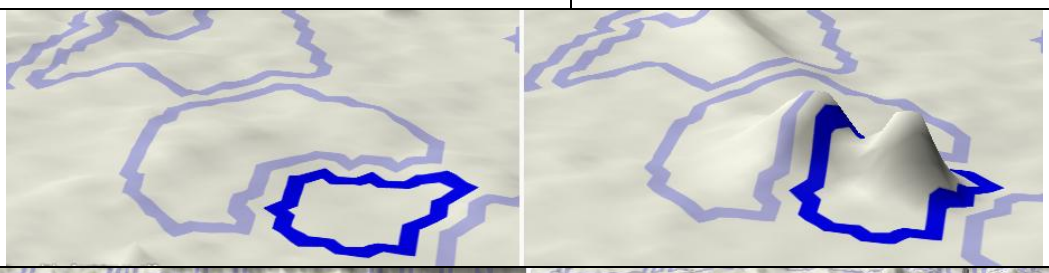
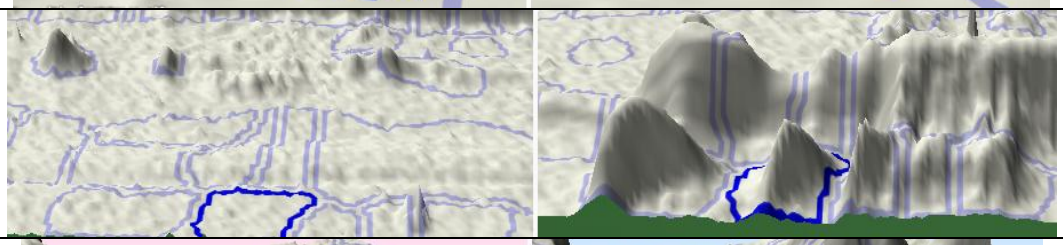
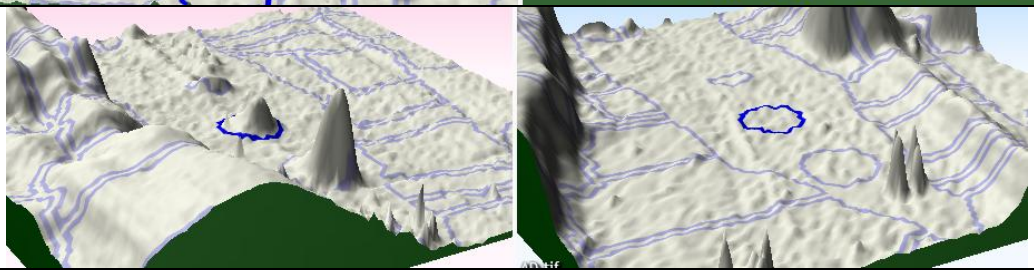
In this study two-dimensional gel electrophoresis of hippocampus proteome of normal and Alzheimeric rat were analysis by progenesis same spot software. Figure 1 shows 2DE gel images of hippocampus proteom in normal (A) and Alzheimeric (B) rats. Gel analysis with progenesis software identified 760 protein spots with different expression. Protein expression changes in hippocampus of

alzhiemeric rats compared with the control group determine that there are 20 common proteins significantly differences expression in both groups. 16 new proteins expressed in alzheimeric condition while suppressed expression of 36 proteins. 3D image of some protein spots with different expression in both normal and AD groups were represented in Figure 2. Software is also analyzed protein clustering by using correlation analysis to evaluate the relationship between expression profiles. Cluster of co-expression proteins represented as dendrogram with vertical distance between pairs of spots depicted in Figure 3 that dendrogram represent the similarity of expression profiles in both groups. To determine the outlier data and confirm doing appropriate clustering perform principal component analysis on control and AD groups data 2 dimensional PCA shown in Figure 4 a and b, respectively.



A B

Figure 1. 2DE gels of normal (A) and AD (B) rat hippocampus. Proteins were separated on a pH 3-10 NL IPG strips in the first dimension and on 12% SDS polyacrylamide gel in second dimension.

spot number	**Normal**	***AD***
157		
182		
227		

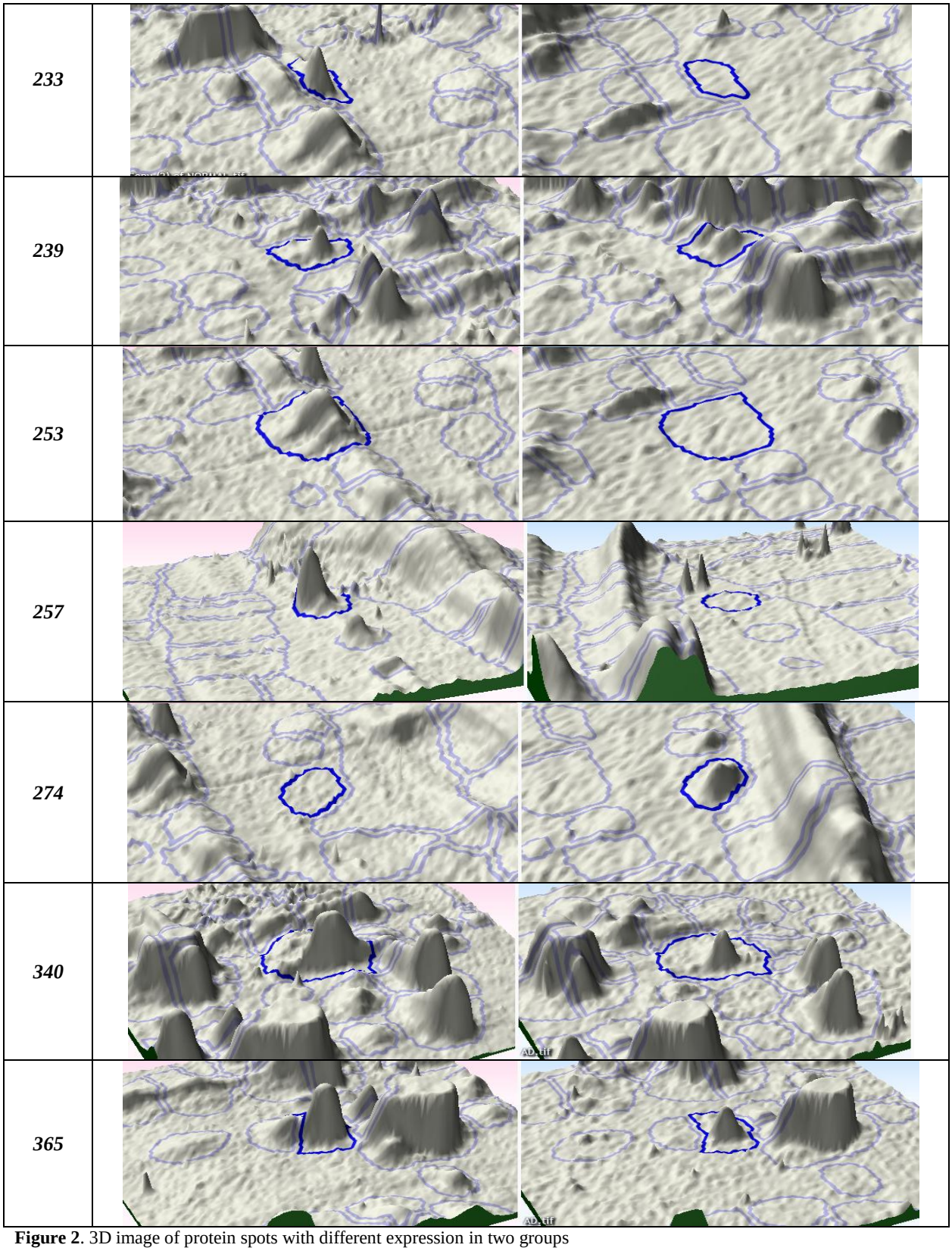


Figure 2. 3D image of protein spots with different expression in two groups

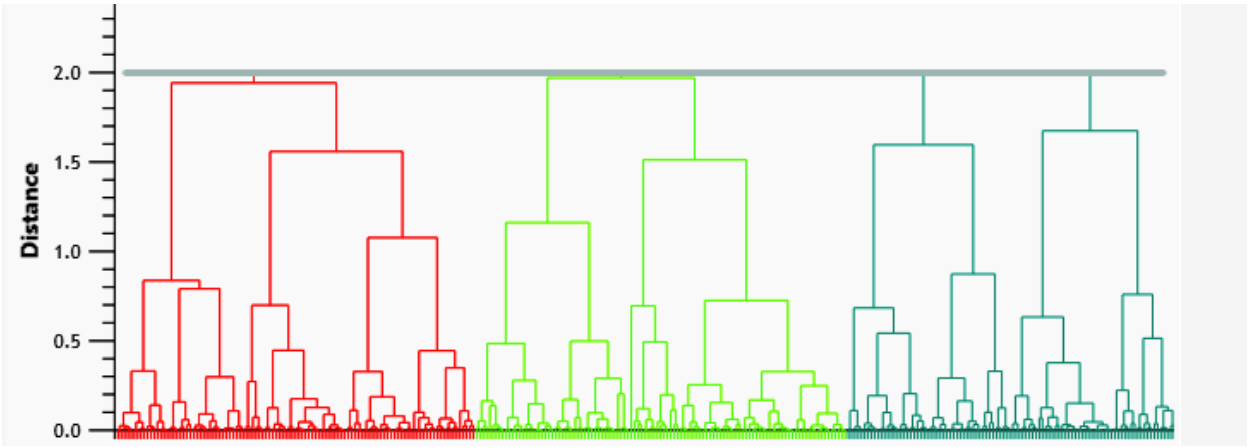
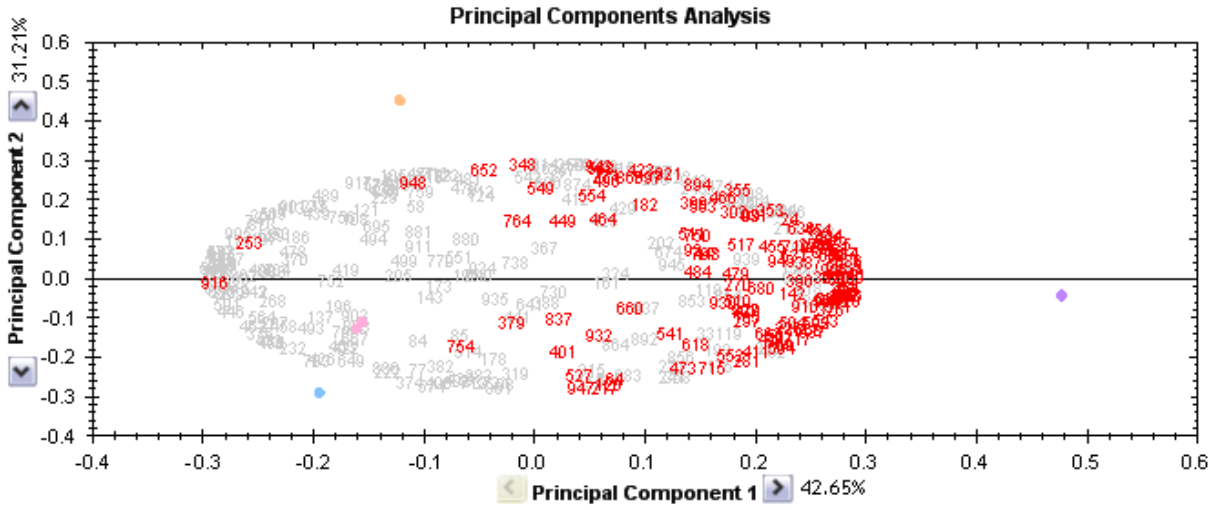
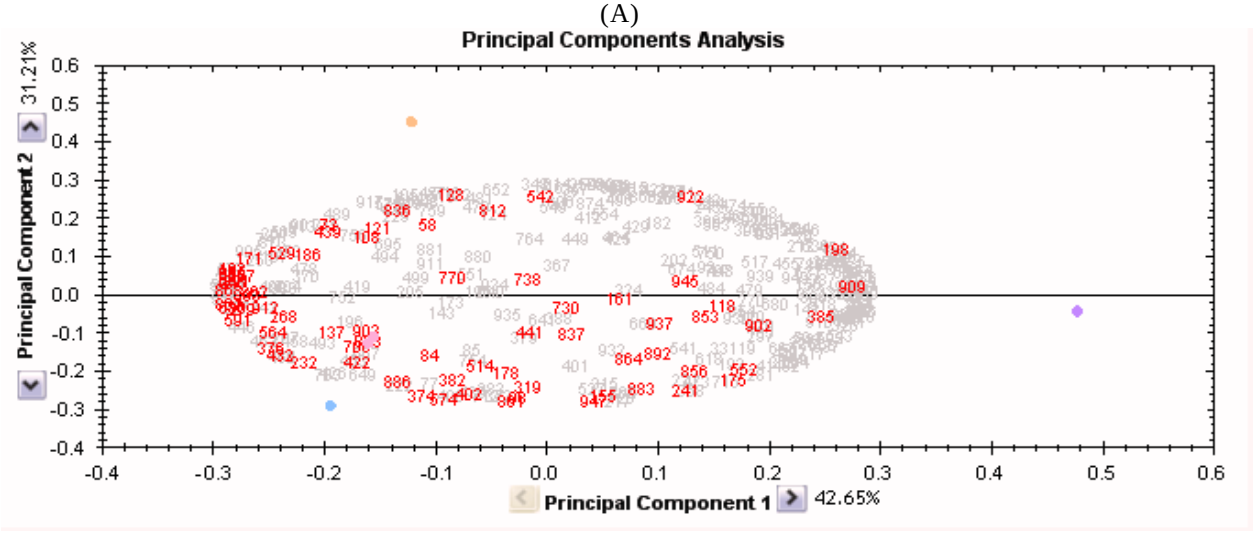


Figure 3. Protein expression clustering of normal and AD groups



(A)



(B)

Figure 4 (A) - PCA for control group (B) - PCA for AD group

DISCUSSION

World's population is getting old and older and the medical community face to new challenges and complexities of diagnosis and treatment of diseases associated with the aging. Alzheimer's disease is a common degenerative disease of the central nervous system [1]. According to the alzheimer's world association, more than 35 million people worldwide are affected by Alzheimer's. In Iranian population

about 600000 people are suffering from AD (18). Accumulation of plaques and tangles in the cortical areas of brain, leading to the emergence of other Alzheimer's symptoms such as lack of orientation, impaired speech and difficulty doing working skills. Neurofibrillary tangles have toxic effects on brain areas involved in learning particularly hippocampus and cause inflammation of brain tissue [19]. The release of acetylcholinesterase from amyloid plaques Also reduces the amount of acetylcholine, thus will impair spatial memory [16]. In present study, rats were investigated on Alzheimer's process by proteomics approach. Rat hippocampus proteome of Normal and AD are analyzed with progenesis same spot software. Figure 1 and 2 depicted images of 2DE gel of both groups and its analysis by software. 760 protein spots identified which some of them have increased expression and other have decreased expression. Comparison between controls and AD gel protein contain 20 proteins statistically significant differences in their expression in both groups. AD expressed 16 new proteins and suppressed 36 protein expressions. several proteins with change of expression are involved in different stages of AD such as the proteins responsible for the transmission of synapse, heat shock proteins and mitochondrial proteins in response to oxidative stress [11] or AD biomarkers like presenilin proteins 1 and 2 , beta amyloid precursor (APP) [13] and apolipoprotein E allele e4 [14]. Because of hyper-phosphorylation of Tau protein, the stabilizing effect of forming neural pathways become weak and creates slender coils Neurofilaments [19]. Recent evidence by Shi X et al. reported that four proteins which abundance was significantly altered in AD rats corresponded to synapsin Ib, protein disulfide-isomerase A3 precursor, tubulin β chain and ATP synthase β subunit [20]. Although, the role of plaques and tangles in AD is not fully understood, but there is a theory which states that amyloid plaques are toxic only indirectly compel glial brain cells to produce ROS and cytokines to remove or break up the plaques in the brains of patients and causes the nerve cells to disrupt homeostasis which these created inflammation causing destruction neurons and emerge symptoms of pathologic disease [21]. In this study as represented in Figure 3, alteration responsible proteins in AD cluster by progenesis same spot software. Cluster analysis considered as a shortcut way to discover the biomarkers, consists of a heterogeneous population divide into the number of homogeneous subsets that is called cluster. The objective of cluster analysis, finding a group that very different with each other, but its members are very similar [5, 6]. In this study, as depicted in figure 3, there are 3 main protein clusters base on the correlation analysis that aid in the identification of genes that share similar expression patterns across a variety of experimental conditions. Genes displaying such patterns are often controlled by the same transcriptional regulatory program, functionally related, or members of the same pathway or protein complex. Using unsupervised learning (hierarchical clustering) and principal component analysis on the mixture parameter estimates the samples were distributed into groups with similar co-expression patterns. Principal component analysis was used to assess the reliability of the classification of samples to classes. The comparison of the resulting classes or groups against the conditions and the distribution of samples within group provided information on the changes in co-expression patterns between conditions [22-24]. So to determine any outlier data and confirm appropriate clustering, principal component analysis for the control and AD groups is shown respectively in Figure 4 (a) and (b). Result illustrated that there are no outlier in protein set of both groups. PCA Analysis represents scattering of members of each cluster around a vector that the proteins close to each other belong to a class, so in this way in proteomics can be classified variation among data sets. On the other hand, if the data of the different species used can also specify any clusters and demonstrate that all instances of a species are placed around a vector and all the clusters are close together[23]. Several proteomic investigations used PCA to classify the protein, such as PCA in three-dimensional images of proteomic patterns used to detect instances of classes in a data set [24-26]. In conclusion, there are significant difference in the molecular level between control and Alzheimeric groups that changes conclude increasing or decreasing expression of proteins and the emerge or disappearance of proteins. Multivariate statistical analyzes such as clustering, principal component analysis and correlation analysis

were well revealed these changes and classified protein set. More ever to determine the molecular mechanisms of AD in rat hippocampus needed to clarify the types of proteins should be identified by mass spectrometry.

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