



Journal of Biological Sciences

ISSN 1727-3048

science
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Changing of Total Protein and Plasma Cholinesterase Levels Depending on Age in Human

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Abstract: In this study, differences of acetylcholinesterase and total protein levels which plays very important role in nervous system and some of the related diseases like Alzheimer, Down syndrome, Parkinson in the different age groups in human were investigated. Cholinesterase and total protein levels of 200 healthy people's blood plasma that are between 7-60 years old were investigated by using spectrophotometric techniques. There was no correlation between plasma cholinesterase activity and age groups. Total protein levels that are 7-12 years old group shows differences than the other groups. Disfunction of food uptake and situation of social cultural cause decreasing the total blood protein in 7-12 years old groups. Meanwhile, this difference in total protein for this group is not enough to predict for some of the disease which to become fact in future. There was no any study on differences of blood plasma acetylcholinesterase and total protein levels in the different age groups in human for Turkey. Because of this specificity this study has original quality and necessary for investigators which study in this area.

Key words: plasma cholinesterase (PChE), total protein, human blood

INTRODUCTION

Acetylcholinesterase (AChE) is an enzyme that is used by the body to break down unused acetylcholine. Acetylcholine (ACh) is one important neurotransmitter and acetylcholinesterase is an enzyme that inactivates unused acetylcholine, by breaking it down into its components (acetate and choline)^[1]. These components can then be reabsorbed into the neurons and recycled back into acetylcholine for future use. Acetylcholine (ACh) is one of the first neurotransmitter ever to be identified in human bodies.

Neurotransmitter is a chemical substance which neurons, muscular and secretion glands use to related with each other. Acetylcholinesterase (AChE) plays very important role which is a key enzyme in human nervous system. AChE is a serine hydrolase that belongs to the esterase family within higher eukaryotes. ACh is one of the best understood because it is also a peripheral transmitter and thus much more accessible to investigation. Cholinergic inhibitors are drugs that increase the efficiency of ACh by interfering with acetylcholinesterase. AChE is targeted by such things as nerve agents, insecticides and drugs for treating various neurological diseases. Recently it has been the focus of drug developers who are searching for a way to handle

Alzheimer's disease^[2]. The most important function of ACh in human depends on movement and mentality. Total protein in human blood has combination of albumin and globulin. Albumin is a water-soluble protein made by the liver that is one of the main constituents of blood. Serum proteins are also made in the liver. Albumin and serum protein are important in maintaining correct pressure between the fluids of the vascular system and surrounding cells. In addition to this function, they carry chemicals which include different drugs, in human blood. They also are important in transporting chemicals, including some medications, throughout the blood system. Insoluble materials, such as barbiturates, bilirubin, fatty acids and some hormones, "hitch" a ride on them^[3]. Normal level of total protein amount in human blood is 6.4-8.3 g/dL^[4]. The level of total protein can increase when the situation chronic infection is happened with dehydration. This increasing in total protein can show different kind of leucemia. Decreasing level of total protein in blood indicate that liver and kidney disfunctions, digestion disfunctions and insufficient food uptake. In the some of search on children, there are some illness like hepatitis, kidney disfunctions and also when this kind of illnesses, albumins and the other serum protein levels are getting decrease. The aim of this study was to find the differences

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of plasma cholinesterase and total protein levels in the different age groups in human. These are the important characteristic findings for understanding the human population's development in a different region of country.

MATERIALS AND METHODS

Chemicals: Copper sulfate ($\text{CuSO}_4 \times 5\text{H}_2\text{O}$), Na-K tartrate, NaOH, Na_2CO_3 , Folin-Ciocalteu reagent, BSA, Sorensen phosphate buffer, 5,5' dithiobis-2-nitrobenzoic (DTNB), Acetylthiocholin.

Blood samples which used in this research obtained from Çanakkale State Hospital Blood Bank and Blood Center. In this study, 200 healthy people's blood samples were used in four different age groups which are between 7/12, 12/20, 20/35 and 35/60 years old.

Preparing of blood plasma: Blood samples were taken to tubes with EDTA. Six hundred microliter of sample were centrifugated for 5 min in 2500 g at 4°C and supernatant (plasma) were collected. Four hundred microliter cold extraction buffer (etanol/chloroform, 62.5/37.5) and 250 µL plasma mixed in the glass tubes. After that mixture were mixed for 30 sec and centrifugated for 10 min in 3000 g at 4°C. Supernatant were collected and kept in -18, -20°C till analyze.

Determination of total protein: Proteins were assayed by the method of Lowry *et al.*^[5] using bovine serum albumin as standard. Under alkaline conditions the divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan and cysteine react with Folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue. In addition to standard liquid handling supplies a Shimadzu spectrophotometer with infrared lamp and filter were used. 0.01 mL blood plasma diluted 1000 times with distilled water, after dilution 0.5 mL of sample mixed with 2.5 mL alkaline copper reagent. Tubes were incubated for 10 min at room temperature and 0.25 mL folin reagent added to each tube and after 30 min absorbance measured at 660_{nm} in spectrophotometer cuvettes. After calculations and statistical analyses amount of protein given as g/dL.

Determination of plasma cholinesterase (PChE) enzyme: Plasma Cholinesterase (PChE) activity was determined using the method of Ellman *et al.*^[6] modified for microassays. Acetylthiocholine was used as substrate at a final assay concentration. All samples, including reagents and homogenates, were kept on crushed ice

before incubation. Enzyme activity was determined in duplicate for samples and blanks. For enzyme activity 2.8 mL of sorensen's phosphate buffer (67 mmol L⁻¹, 37°C and pH 7.4), 0.1 mL Ellman's reagent (DTNB) (0.27 mmol L⁻¹) and 0.1 mL acetylthiocholine (2.98 mmol L⁻¹) was pipetted into a tube. After all 20 µL plasma sample added and the tubes were incubated for 10 min in a shaking water bath at 37°C. Blanks were obtained by adding 2.9 mL of sorensen's phosphate buffer (67 mmol L⁻¹, 37°C and pH 7.4), 0.1 mL Ellman's reagent (0.27 mmol L⁻¹) and 20 µL plasma sample. The tubes were incubated for 10 min in a shaking water bath at 37°C. After standing for 15 min at room temperature, samples were read with 30 sec intervals in a Shimadzu spectrophotometer at 410_{nm}. The activity of plasma cholinesterase were reported as nmol thiocholine formed min⁻¹ mg protein⁻¹. The assay was set up to allow the reaction to be linear for both tissue concentration and incubation time.

RESULTS AND DISCUSSION

In this study total 200 healthy people's blood plasma samples were used for total protein and plasma cholinesterase analyses. Protein levels between age groups shows that there was no corellation between protein amount and age (Table 1). Normal total protein levels in human blood are between 6.4- 8.3 g dL⁻¹^[3].

Plasma cholinesterase activities between age groups shows that there was no corellation between protein amount and age (Table 2). Normal plasma ChE activities in human blood are between 1800-5000 nmol/dk/mL.

According to statistical analysis in 7-12 years old group this group shows differences from the other groups at the point of p<0.05 statistically and this is important point. Total protein levels that are 7-12 years old group shows differences than the other groups. Disfunction of food uptake and situation of social cultural cause decreasing the total blood protein in 7-12 years old groups. Meanwhile, this difference in total protein for this

Table 1: Total protein changing depending on age

Age groups	Total protein (g dL ⁻¹)
07-12	6.45±0.45
12-20	7.02±0.52
20-35	7.61±0.61
35-60	8.05±0.72

Table 2: Cholinesterase enzyme activity changing depending on age

Age groups	ChE activity (nmol min ⁻¹ mg protein ⁻¹)
07-12	1913±62.8
12-20	1843±47.7
20-35	1909±68.3
35-60	1951±76.3

group is not enough to predict for some of the disease which to become fact in future. Total protein amount in blood can increase depend on chronical inflammation because of dehydration. This increasing in total protein is an indicator for blood cancer, bone marrow cancer. Decreasing in total protein amount indicate that disfunction of food uptake and digestion in other hand liver and kidney diseases. Research on children during the hepatitis, kidney disease and some of the inflectional diseases albumin and other serum protein levels shows decreasing^[3] but this differences in total protein amount are not enough to predict for disease diagnosis and other analysis have to realize for certain result. Protein-energy insufficiency in children effects growth and development.

In other study plasma activity of butyrylcholinesterase and benzoilcholinesterase in 70 healthy people, plasma activity of aspirin esterase and acetylcholinesterase in 43 people between 18-85 years old were analyzed spectrophotometrically and there was no corelation between enzyme activity and age groups^[7]. In this study there was no corelation between plasma cholinesterase activity and age groups cause all of the ChE activity values very close to each other and all of the activity levels between the normal levels of ChE in all of the age groups. This results shows us that 200 people are healthy at least in ChE levels.

ACKNOWLEDGMENT

The methods, data and results which given in this manuscript to get from a Miss N.Unat's M.Sc Thesis which completed successfully year 2002.

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