ALTERATIONS IN FOLIC ACID, VITAMIN B12, THYROID HORMONES AND ANTIOXIDANT/OXIDANT LEVELS IN DIFFERENT AGE GROUPS OF SPRAGUE DAWLEY RATS

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ABSTRACT

***Introduction**: In the course of time, many structural and functional changes occur at different levels, and they are all known to contribute towards aging. The purpose of the study was to determine the changes in the levels of folic acid, vitamin B12, free T3 and T4, TOS (total oxidant status) and TAS (total antioxidant status) as the rat ages from 6 to 36 months old.*

***Materials and methods**: Sprague-Dawley rats were obtained, grown, and divided into five equal groups as follows: Group I (6 months old), Group II (12 months old), Group III (18 months old), Group IV (24 months old) and Group V (36 months old). The levels of folic acid, vitamin B12, free T3 and T4, total oxidant and antioxidant capacity in the blood were analyzed.*

***Results**: Folic acid level in Group I was detected to be significantly higher compared to the other groups (P<0.001), and vitamin B12 levels in Group V were determined to be significantly lower than those of the other groups (P<0.001). Free T3 level in Group II and free T4 level in Group I were measured significantly higher compared to the other groups (P<0.005, P<0.01), and TAS level in Group V was determined to be significantly lower compared to Group I and II (P<0.05).*

***Conclusions**: Changes in the levels of folic acid, vitamin B12, thyroid hormones, and total oxidant and antioxidant capacities, might contribute to the proper diagnosis and treatment procedures of age-related diseases.*

***Key words**: Aging, antioxidant system, folic acid; free T3 and T4, vitamin B12.*

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Introduction

Aging is defined as the progressive accumulation of diverse deleterious changes in cells and tissues that increase the risk of disease and death[1]. Deformation, which occurs in tissues over time, is known as one of most important causes of aging. However, while there are various assumptions about the mechanisms or developmental processes of this damage; currently, there is no clear understanding. The cause of tissue degeneration has been attempted to be explained through various mechanisms. Some researchers claim that external factors as heat energy fluctuations from cosmic rays, heavy metal accumulation, and even gravity might be the cause of this damage[2,3].

Other researchers, on the other hand, suggest that natural factors such as autointoxication, autoantibodies and somatic mutation cause tissue degeneration[1,2,3].

Changes in the functions of organisms, such as folic acid and vitamin B12 absorption, transportation and metabolism were recorded during aging[4,5,6,7]. Folic acid is necessary for many methylation reactions about phospholipid, DNA, protein and neurotransmitter synthesis in the organism[8]. The most important function of vitamin B12, which plays an important role in methylation reactions and many cellular processes, is to act as a reserve with folic acid to DNA synthesis, and to enable folic acid usage in the cell[9]. Previous studies have reported that, folic acid and vitamin B12 levels decrease in elderly[6,7,10,11] and the beneficial effects of the supplementation of these two vitamins might be preventing age related diseases, such as, Alzheimer, vascular dementia and mental disorders[12].

Aging also affects the endocrine system of living organisms[13]. Thyroid-stimulating hormone (TSH) has important roles in the endocrine system and shows differences in normal individuals in terms of production and metabolic effects according to age, sex, nutrition and race[14,15]. Tightly regulated physiological levels of thyroid hormones are necessary for the continuation of developmental process in a healthy way and needed for cognitive functions to be performed throughout life[16,17,18].

It is well known that thyroid dysfunction plays a role in pathogenesis of many diseases. Besides this fact, it is also known to increase mitochondrial oxidation rate and form a resource for the constitution of many reactive oxygen species, by making changes in the activity and numbers of mitochondrial respiratory chain components[19]. It was stated that a decrease in thyroid hormone release occurs with age, and this situation causes increase in thyroid diseases and increase the incidence based on age[20].

Increase in oxidant molecules is most frequent in older age groups. Although cells have antioxidant defense systems, which can decrease or remove the harmful effects of free radicals, diseases due to aging emerge as a result of antioxidant systems to be insufficient together with aging[21]. In case of aging, changes in hydrophilic (ascorbate, urate, glutathione), lipophilic radical scavengers (tocopherol, carotenoids), metal chelating agents, and activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase were reported[22,23,24]. In some studies, effect of plasma and tissue on total antioxidant capacity was examined, and antioxidant capacity was recorded to be not changed or decreased[25,26]).

In this study, we determined the serum folic acid, vitamin B12, free T3 and T4, total oxidant capacity (TOS) and total antioxidant capacity (TAS) levels in rats that have same nutrition and environment conditions and are at different ages (6, 12, 18, 24 and 36 months old).

Materials and methods

Animals

Sprague Dawley rats were obtained while they were around 5-6 months old age and grown until they reached 6, 12, 18, 24 and 36 months of age. The weight of the animals ranged between 172 and 318 grams at those age groups. Before the experimental procedure, consent for the study was taken from Kafkas University Animal Experiments Local Ethics Committee. Pre-experimental conditions were accepted as optimal zone for rats, such as temperature (23 ± 2 °C), humidity (50 ± 10 %) and light (12 h light/dark cycles). All animals were allowed free access to standard chow (Bayramoglu-Erzurum) and ad-lib freshwater. Same management conditions were applied for all experimental groups throughout the experimental period.

Methods

Fifty Sprague Dawley rats were divided into 5 groups, as there would be 10 animals in each group: Group I (6-months-old, 172-215 g), Group II (12-months-old, 196-239 g), Group III (18-months-old, 219-268 g), Group IV (24-months-old, 220-281 g) and Group V (36-months-old, 260-318 g).

Blood samples from animals were taken at the end of 6th, 12th, 18th, 24th and 36th months from their hearts under ether anesthesia. Serum was separated by centrifugation of the blood samples at 3000 rpm for 10 minutes. Samples were preserved at -20ºC until the analysis was performed. Serum folic acid, vitamin B12, free T3 and T4 levels were measured in analyzer with the device's own kit (Beckman Coulter), and TAS and TOS levels were colorimetrically detected with commercial kits (REL Assay Diagnostics, Gaziantep-Turkey) in spectrophotometer.

Statistical analysis

Statistical analyses were performed by using statistical packages for the social sciences (SPSS) Windows 16.0 packaged software by SPSS Inc. Between-group average values were determined by one-way analysis of variance (ANOVA) and differences between-groups were determined by Duncan test. Results were given as mean and standard deviation (Mean ± SD).

Results

Folic acid, vitamin B12, free T3 and T4, TOS and TAS levels of rats in same and different age groups were presented in Table 1. It was determined that folic acid level of Group I was significantly higher ($P<0.001$) compared to the other groups. No significant difference was noted among the other groups. B12 level of Group V was determined to be significantly lower ($P<0.001$) compared to other groups, and significantly higher ($P<0.001$) in Group I. However, a significant difference in vitamin B12 levels of Groups II, III and IV was not identified. Free T3 level in Group II was

identified to be significantly higher (P<0.005) compared to the other groups, and significantly lower (P<0.005) in Group III. However, a significant difference in Group IV and Group V free T3 levels were not determined. Group I free T4 level was detected to be significantly higher (P<0.01) compared to the other groups. While Group V total antioxidant capacity level was determined to be significantly lower (P<0.05) compared to Group I and Group II a significant difference in Group III and Group IV was not noted. While Group V total oxidant capacity level was higher (P=0.127) compared to Group I, a significant difference in Group II, III and IV was not identified.

Parameters	Months					P
	6th (Group I)	12th (Group II)	18th (Group III)	24th (Group IV)	36th (Group V)	
Folic acid (ng/mL)	53.07±6.56^{a}	30.72±2.66^{b}	35.27±4.64^{b}	31.95±2.60^{b}	31.30±3.47^{b}	<0.001
Vitamin B12 (pg/mL)	1002.37±76.84^{a}	810.81±38.33^{b}	740.14±61.03^{b}	741.0±36.41^{b}	570.0±27.79^{c}	<0.001
Free T3 (pg/mL)	5.14±0.35^{ab}	5.66±0.22^{a}	4.04±0.19^{c}	4.53±0.14^{bc}	4.60±0.37^{bc}	<0.005
Free T4 (ng/dL)	2.79±0.13^{a}	2.29±0.10^{b}	2.22±0.12^{b}	2.19±0.10^{b}	2.27±0.11^{b}	<0.01
TAS (µmol Trolox Eqv./L)	0.519±0.04^{a}	0.503±0.02^{a}	0.485±0.02^{ab}	0.485±0.03^{ab}	0.397±0.03^{b}	<0.05
TOS (µmol H2O2 Eqv./L)	9.01±1.32^{b}	11.08±1.01^{ab}	11.04±1.75^{ab}	12.57±0.76^{ab}	12.84 ±0.85^{a}	0.127

Table 1: Serum Folic Acid, Vitamin B12, Free T3 and T4, TOS and TAS Levels in Rats with Different Ages.

Discussion

Age is an important factor for folic acid, vitamin B12, thyroid hormones and oxidant/antioxidant system parameters. In this study, serum folic acid, vitamin B12, free T3 and T4, and TOS and TAS levels of rats at ages of 6, 12, 18, 24 and 36 months were determined. We found that these parameters might change as the organism ages, and serum folic acid, vitamin B12 and free T3 and T4 levels significantly changes especially in older animals compared to the youngers.

In the present study, we found that the serum folic acid levels started to decrease after 6th month of age; however after this point, there were no further decrease as the animal ages. In a study conducted by Papandreou and Mavromichalis with 524 children in age groups of 6-9, 10-12, 13-15, serum folate levels between age groups 6-9 and 6-15, and 10-12 and 13-15 (11.8, 7.5 ng/mL) are identified to be significant. Furthermore, in the same study, serum vitamin B12 levels showed significant differences for all three age groups: 1048, 805, 700 pg/mL, respectively. In their study, they have reported that homocysteine level increases based on age, and this might be related to low folic acid and vitamin B12 levels[6].

In a study performed by Akanji et al. on youngsters in 10-14 year and 14-19 year age groups, it has been reported that serum folate and vitamin B12 levels decreased significantly with age while total homocysteine level increased[7]. Similarly, in another study by Delvin et al which was conducted in children, they identified that while plasma homocysteine concentration has a negative correlation with vitamin B12 and folate, it had a positive correlation with age[11]. The decrease in vitamin B12 and folic acid levels during aging has been thought to be a result of their common participation in methionine synthase reaction. Low folic acid level in old age has been stated to be caused by various reasons, such as insufficient nutrition, a decrease in liver functions, and a deterioration of intestinal folate absorption during aging[5].

In our study, serum free T3 levels were determined to decrease with age. Additionally, serums free T4 level was found to decrease only in rats after 6th month, without a difference with other age groups. Studies conducted in healthy elders has reported a decrease in serum TSH and free T3 levels accompanying reverse T3 level (rT3) depended on age, and no changes occur in serum free T4 level, exist[27,28,29,30]. TSH oscillation was reported to be much lower in the young individuals compared to old ones, due to the decrease in thyroid releasing hormone (TRH) oscillation and thyroid hormone concentration[31]. Bremner et al suggested that the increase in TSH oscillation throughout aging might be a consequence of changes in TSH bioactivity that might depend on age[30]. Consequently, changes emerging in hormone levels, which are subject to hypophysis-thyroid axe, are thought to be related to the aging process.

Previous studies have indicated that aging is a result of long-term oxidative side effects of two important biological events. One of these has been associated with development and cellular differentiation, the other has been associated with metabolism to produce energy. When metabolic rate increases, free oxygen radicals also rise as a result of increasing oxygen consumption. Accordingly, free radicals might damage biomolecules, and accelerate aging[32,33]. In our study, serum TAS levels were determined to decrease in rats beginning from 18th month, and serum TOS levels were determined to increase with aging. Parallel to our study, in a study that was done by Sivonova et al on 6, 15 and 26 month old rats; it has been reported that plasma and antioxidant capacity level in rats decreases depending on age[33].

Moreover, DNA damage in lymphocytes and lipid peroxidation increase and an important decrease occurs in the amount of compounds that include total sulfhydryl groups. In a study, which was performed by Kim et al on rats, it has been determined, that serum peroxide levels in old rats significant increased while antioxidant capacity significantly decreased, and serum redox balance changed on behalf of oxidation throughout aging[25]. In a study conducted by Hernanz et al. on healthy 43 (72±7) elders and 27 (28±3) youngsters at different ages, it has been reported that plasma total glutathione (GSH) level increased, but erythrocyte GSH level decreased in elderlies[28]. A decrease in plasma vitamin E level and an increase in plasma thiobarbituric acid reactive substances (TBARS) were determined in elders compared to youngsters. In the same study, although there was an increase in homocysteine level, this increase was reported to share similarities with folic acid and vitamin B12 levels compared to youngsters[28].

In our study, change in oxidant and antioxidant metabolism on behalf of oxidant show similarities with the above-mentioned and some other studies[24,33]. Although in some prior studies oxidative stress was recorded to increase with age; there are alternative findings regarding what kind of changes happen in antioxidant defense system parameters.

In a study, in which Messarah et al created hypothyroidism and hyperthyroidism in rats, serum TAS levels were reported to be considerably high in rats with hyperthyroidism[34]. In the same study, they claimed that ingestion of high thyroxin in old rats might change activities of malondialdehyde (MDA) and some enzymes related to free radical metabolism. In the study, which Adali et al conducted on patients with hyperthyroidism, they determined that complicated application of propylthiouracil, propranolol and vitamin E decreases MDA levels, and increases activities of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px)[35]. Increase of thyroid hormone oscillation causes the formation of reactive oxygen types by stimulating metabolic rate. We hypothesize that the increase in oxidant capacity might be due to an increase in lipid peroxidation products in cells.

Oxidative stress is a condition, which takes place in many chronic inflammatory diseases. The ability of cobalamin to regulate inflammatory cytokines shows that it has an antioxidant characteristic. In an in vitro study, thiolatocobalamins, N-acetyl-L-cysteinylcobalamin and glutathionylcobalamin has been reported to be quite effective antioxidants[36].

In conclusion, aging negatively affects antioxidant status by increasing free radical damage and decreasing folic acid, vitamin B12 and thyroid hormone levels possibly leads to dysfunctions in many organs. Folic acid, vitamin B12, thyroid hormone and antioxidant reinforcement in normal individuals can be recommended in increased age to be important in decreasing or preventing diseases depends on age.

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