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ARAŞTIRMA MAKALESİ　　RESEARCH PAPER

Thiol/disulfide Homeostasis and Some Minerals in Aging

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Abstract: The aim of the study was to investigate how aging affects thiol/disulphide balance and serum mineral levels in rats of different ages. Twenty-four healthy male Sprague-Dawley rats in good health were used as study subjects. Group 1 consisted of 1.5-month-old baby rats, followed by 6-month-old, 12-month-old and 18-month-old groups. Sodium (Na), potassium (K), chloride (Cl), phosphorus (P), calcium (Ca), iron (Fe) and magnesium (Mg) concentrations in serum were measured by spectrophotometric method. Natural Thiol (NTL) and Total Thiol (TTL) concentrations were determined using Rel Assay Diagnostics Equipment. The difference between Na, Fe and Mg levels was not statistically significant ($P>0.05$). Compared with group 1, Ca levels of groups 2 and 3 were shown to be significantly lower. Growth resumed in group 4 ($P<0.05$). K levels were lower in groups 2 and 3 compared to group 1. In group 4, it started to increase ($P>0.05$). Cl level started to increase with increasing age and this increase was significant between groups 1 and 4 ($P<0.05$). While the difference between Group 3 and Group 4 was significant ($P<0.05$), the difference between Group 2, which had the highest TTL and NTL levels, and Group 1, which started to decrease with age, was not significant ($P>0.05$). It has been found that disulfide levels begin to increase as we age ($P>0.05$). Mineral amounts and thiol/disulphide balance were found to change with age.

Keywords: Aging, mineral, thiol/disulphide homeostasis.

Yaşlanmada tiyol/disülfür Homeostazisi ve Bazı Mineraller

Öz: Araştırmada farklı yaşlardaki sıçanlarda yaşlanmanın tiyol/disülfit dengesi ve serum mineral düzeylerine etkisinin araştırılması amaçlandı. Çalışma denekleri olarak sağlık durumu iyi 24 sağlıklı erkek Sprague-Dawley sıçanı kullanıldı. Grup 1'de 1,5 aylık yavru sıçanlar yer alırken bunu 6 aylık, 12 aylık ve 18 aylık gruplar takip etti. Serumdaki sodyum (Na), potasyum (K), klorür (Cl), fosfor (P), kalsiyum (Ca), demir (Fe) ve magnezyum (Mg) konsantrasyonları spektrofotometrik yöntem kullanılarak ölçüldü. Doğal Tiol (NTL) ve Toplam Tiyol (TTL) konsantrasyonları Rel Assay Diagnostics Equipment kullanılarak belirlendi. Farklı yaş gruplarındaki sıçanların Na, Fe ve Mg seviyeleri arasındaki fark istatistiksel olarak önemli bulunmadı ($P>0.05$). Grup 1 ile karşılaştırıldığında Grup 2 ve 3'ün Ca düzeylerinin anlamlı derecede düşük olduğu görüldü. Grup 4'te artmaya başladı ($P<0.05$). Grup 2 ve 3'te K düzeylerinin Grup 1'e göre daha düşük olduğu görüldü. Grup 4'te ise artmaya başladı ($P>0.05$).Yaş arttıkça Cl düzeyi artmaya başladı ve Grup 1 ile 4 arasında bu artış anlamlıydı ($P<0.05$). Grup 3 ve Grup 4 arasındaki fark anlamlı iken ($P<0.05$), Grup 2'de TTL ve NTL düzeyleri en yüksek olan ve yaşlanmayla birlikte düşmeye başlayan Grup 1 arasındaki farkın anlamlı olmadığı ($P>0.05$) tespit edildi. Yaş aldıkça disülfit seviyelerinin artmaya başladığı bulundu ($P>0.05$). Mineral miktarlarının ve tiyol/disülfür dengesinin yaşla birlikte değiştiği tespit edildi.

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Anahtar kelimeler: Mineral, tiyol/disülfür homeostazisi, yaşlanma.

INTRODUCTION

Aging changes occur in all cells, tissues and organs of the body, and these changes affect the functioning of all body systems (Flatt, 2012). A widespread loss in function affecting various organ systems is a sign of aging. It takes many different factors to age. Aging also sees an increase in oxidative stress (Bourgonje et al., 2021). The majority of theories about the basic mechanisms of aging assume that homeostatic metabolic, inflammatory, and/or redox processes in cells and tissues deteriorate over time (Barzilai et al., 2012). Oxidative stress occurs when the natural production of ROS cannot be balanced by the anti-oxidative capacity of tissues (Barja et al., 2013). Increases in oxidative damage to DNA, proteins, and lipids have all been found with normal aging (Fischer et al., 2012).

The maintenance of thiol/disulfide homeostasis (TDH) is essential for several metabolic pathways. Despite the fact that TDH imbalance has been linked to numerous diseases, including preeclampsia, ischemic stroke, and diabetes (Korkmaz et al., 2016; Ates et al., 2016). Thiol is an important antioxidant and plays a key role in scavenging free oxygen radicals by enzymatic and non-enzymatic means (Turell et al., 2013). The shaped disulphide bonds can be reversed into thiol groups and so preserve the homeostatic equilibrium between thiols and disulphide bonds (Jones and Liang, 2009; Turell et al., 2013). The protection of antioxidants, programmed cell death, cellular transmission processes, cellular enzymatic efficiency, detoxification, and transcription are all significantly impacted by the dynamic thiol/disulphide balance (Jones and Liang, 2009). A thiol/disulphide imbalance has been linked to disorders like cancer, diabetes mellitus, and cardiovascular conditions (Go and Jones, 2011; Matteucci and Giampietro, 2010).

Minerals are crucial for the body's growth and development, muscle contraction, electrolyte balance, nerve impulse transmission, enzyme activity, and bone production. As a mineral imbalance can result in metabolic problems and diseases, such as an excess or lack of vital minerals or an excess of poisonous minerals (Cambell, 2001). Along with the aging process, disruption of iron homeostasis also causes decreased mitochondrial function and probably plays an important role in various degenerative diseases, including age-related tissue dysfunction (Killilea et al., 2004).

Whether the electrolyte moves towards the anode or cathode depends on whether it has a positive or negative charge. The main electrolytes it contains are: sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chloride (Cl). The ions with the highest intracellular concentration are potassium and magnesium (Ganong, 1997). Mg affects the excitability of neurons, the contraction of muscles, and the regularity of heart beat through participating in the active transport of other ions across cell membranes, such as Ca and K (Barbagallo and Dominguez 2007). Mg's biological significance, clinical relevance, effects on aging-related disorders and health, as well as its molecular and physiological effects on aging, have all been studied over the past few decades. The most common metal in the human body is calcium (Lansdown, 2002; Higdon and Drake, 2012). It's interesting to note that younger persons have higher calcium requirements than elderly adults do. Along with promoting various important processes, most notably hemostasis, is calcium.

The primary intracellular and external electrolytes and osmolytes are potassium and sodium (Higdon and Drake, 2012), which are known for maintaining the cellular membrane potential. Osmotic pressure and proper fluid distribution depend on the electrolyte balance of bodily fluids. Two of the main positive ions in bodily fluid are sodium (Na) and potassium (K), while one of the main negative ions is chlorine (Cl).

The body needs iron to produce its oxygen transport proteins, such as hemoglobin and myoglobin, as well as other iron-containing enzymes involved in electron transfer and oxidation-reduction reactions (Hurrell, 1997). Iron is necessary for a number of vital biological functions in the brain, such as oxygen transport, DNA synthesis, mitochondrial respiration, myelin synthesis, and the production and metabolism of neurotransmitters (Crichton, 2009). Age-related increases in total iron concentration may be due to a variety of factors, including inflammation, redistribution of iron within the brain, increased blood-brain barrier permeability, and modifications in iron homoeostasis. The main risk factor for neurodegeneration is aging. Iron buildup brought on by aging could be a significant contributor to neurodegenerative processes (Conde and Streit, 2006).

The purpose of this study was to look at how aging affects the levels of serum minerals and the homeostasis of thiol/disulphide in rats of various ages.

MATERIAL AND METHOD

Research Site and Animal Material: The study material consisted of 24 Sprague-Dawley male rats. Rats were obtained from Ondokuz Mayıs University Experimental Animals Application and Research Center. The study was started with the permission of Ondokuz Mayıs University Animal Experiments Local Ethics Committee numbered 2021/14. During the study, rats fed ad libitum, which were clinically healthy and provided with a room temperature of 22±2 ^{0}C, a humidity of 60 %, and a 12/12 hour light/dark environment, were divided into groups according to age range.

Animal study design: Group 1 (n= 6) 1.5 months old (12.5 puberty), Group 2 (n= 6) 6 months, (18 years), Group 3 (n= 6) 12 months, (30 years) and Group 4 (n= 6) 18 months (45 years) (Karaman et al., 2013).

Collecting Blood Samples and Obtaining Serum: After 12 hours of fasting in rats of different age groups, the abdomen and chest areas of the rats were opened and blood was drawn directly from their hearts with xylazine (10 mg/kg body weight intraperitoneal) and ketamine (60 mg ketamine hydrachloride/kg body weight, intraperitoneal) general anesthesia. Sacrification/euthanasia was performed by exsanguination method during surgery.

Following a 20-minute incubation period and coagulation, the blood samples were centrifuged at +4 °C for 10 minutes at 1550 x g. The serum was then extracted and separated into aliquots. The serums were kept cold until they were analyzed.

Determination of major mineral levels in serum: The concentrations of Mg, Ca, P, and Na, K, and Cl in serum were determined using a spectrophotometric method in an autoanalyser (Beckman Coulter Inc., Brea, CA, USA) using Beckman kits (Michaylova and Illkova 1971,).

Thiol/Disulphide Homeostasis: The components of total thiol (-S-S- + -SH) are native and reduced thiol. Erel and Neselioglu devised a novel automatic and spectrophotometric method for measuring thiol/disulfide assays. The thiol/disulfide measuring method's basic idea is to use sodium borohydride (10 µL) as a reductant solution to convert dynamic disulfide bonds (-S-S-) into functional thiol groups (-SH). Subsequently, the unused $NaBH_4$ was entirely eliminated using formaldehyde. Measurements were made of the total thiol groups following their reaction with 5,50-Dithiobis-(2-nitrobenzoic acid) (DTNB), both reduced and native. The number of dynamic disulphide bonds is determined by dividing the difference between the total and native thiol by half, as the reduction of a disulphide bond results in the formation of two thiol groups. Additionally, the ratios of native thiol/total thiol, disulphide/native thiol, and disulphide/total thiol were computed (Erel and Neselioglu, 2014).

Statistical Analyses: The results of the study were analyzed using Statistical Package for Social Sciences, version 23 software. The test results were shown as mean ± standard deviation values in the Independent Samples Test. A value of P<0.05 was considered statistically significant. Correlations between the variables were evaluated using a Pearson correlation analysis.

RESULTS

In our study, it was determined that as the age progressed, Na, Mg and Fe levels decreased slightly compared to Group 2 compared to Group 1, while Group 3 and Group 4 increased slightly compared to Group 2, but this was not statistically significant (P>0.05). K level decreased in Group 2 and Group 3 compared to Group 1, while it increased in Group 4 and approached Group 1. The Cl level, on the other hand, started to increase with increasing age, and this increase was determined to be the highest in Group 4 (P<0.05). P level decreased in Group 2, 3 and Group 4 compared to Group 1, and this decrease was the highest in Group 3. It was determined that Ca level decreased in Group 1 compared to Group 2 and Group 3 (P<0.05), on the other hand, it started to increase again in Group 4 and approached Group 1 (P<0.05) (Table 1, Fig. 1, 2 and 3).

When the correlation relationship of mineral level between the groups was examined, it was determined that there was a significant positive correlation between Na level and Cl and Fe levels (r=0.844**, 0.523**).

It was determined that there was a significant positive correlation between K level and P, Ca and Mg levels (r=0.635**, 0.568**, 0.614**). The connection between P level and Ca and Fe levels was found to be positive (r=0.821**, 0.417**).

Ca level and Fe level were discovered to be positively correlated (r=0.426**) (Table 2). TTL and NTL levels were highest in Group 2 and began to decline with age. It was found that the difference between Groups 1 and 2 was not significant (P>0.05), however the difference between Groups 3 and 4 was significantly compared to Group 2 (P<0.05). The disulfide level was shown to start rising with aging (P>0.05) (Table 3). TTL and NTL levels were found to be significantly positively correlated (r=0.918**) (Table 4, Fig. 4).

Table 1. Sodium (Na), potassium (K), chlorine (Cl), phosphorus (P), calcium (Ca), Iron (Fe) and magnesium (Mg) levels in serum.

	Group 1	Group 2	Group 3	Group 4
Na (mmol/L)	137,83±2,93	135,83±1,66	140,33±0,80	141,33±1,40
Cl (mmol/L)	99,00±1,54^{a}	99,83±0,79^{ab}	102,33±0,71^{ab}	102,83±1,16^{b}
K (mmol/L)	6,40±0,26^{a}	5,79±0,13^{ab}	5,17±0,19^{b}	6,16±0,42^{a}
P (mg/dL)	9,90±0,32^{a}	6,76±0,24^{b}	5,36±0,38^{c}	6,85±0,24^{b}
Ca (mg/dL)	11,71±0,26^{a}	9,73±0,23^{b}	9,75±0,13^{b}	10,46±0,22^{c}
Fe (ug/dl)	201,33±43,77	152,16±10,87	152,66±10,31	160,00±10,04
Mg (U/L)	2,61±0,16	2,48±0,04	2,55±0,05	2,70±0,12

$^{a, b, c}$: The difference between the groups indicated with different letters in the same column is significant (P<0.05).
Group 1 (1.5 months old), Group 2 (6 months), Group 3 (12 months), Group 4 (18 months).

Table 2. Correlation relationship between groups.

	Na	K	Cl	P	Ca	Fe	Mg
Na	1	-0.29	0.844**	-0.08	0.326	0.523**	0.047
K		1	-0.02	0.635**	0.568**	0.134	0.614**
Cl			1	-0.382	0.057	0.275	0.067
P				1	0.821**	0.417*	0.222
Ca					1	0.426*	0.320
Fe						1	-0.123
Mg							1

Table 3. Serum Total Thiol (TTL), Native Thiol (NTL) and Disulphide levels.

	TTL(µmol/L)	NTL(µmol/L)	Disulphide (µmol/L)
Group 1	456,37±47,12[ab]	324,66±41,96 [ab]	65,85±5,75
Group 2	495,84±30,17 [b]	366,08±37,31[b]	64,87±4,5
Group 3	361,24±34,4 [a]	230,01±37,45 [a]	65,61±5,84
Group 4	352,47±21,47 [a]	217,08±32,19 [a]	67,69±16,03

[a, b, c] :The difference between the groups indicated with different letters on the same line is significant (P<0.05)

Table 4. Correlation relationship between groups.

	TTL	NTL	Disulphide
TTL	1	0,918**	0,064
NTL		1	-0,338
Disulphide			1

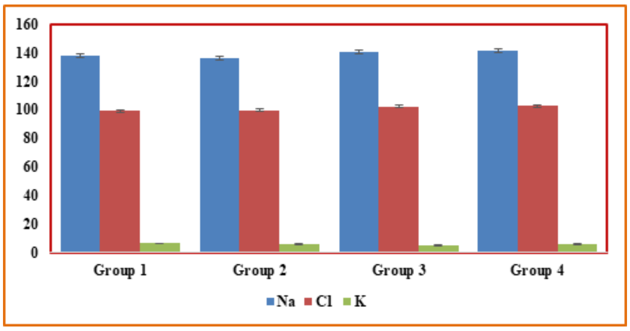

Figure 1 Sodium (Na), potassium (K) and chlorine (Cl) levels in serum.

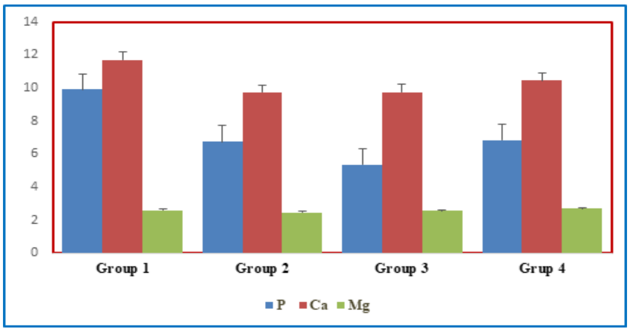

Figure 2. Phosphorus (P), calcium (Ca) and magnesium (Mg) levels in serum.

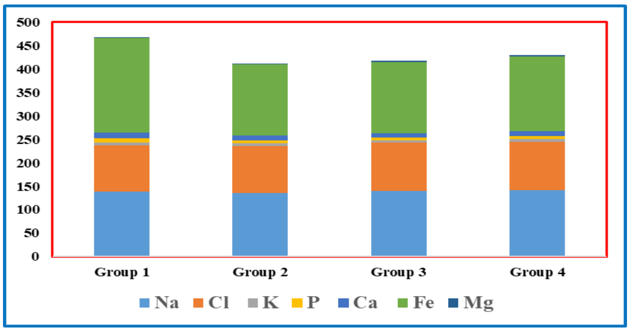

Figure 3. Sodium (Na), potassium (K), chlorine (Cl), phosphorus (P), calcium (Ca), Iron (Fe) and magnesium (Mg) levels in serum.

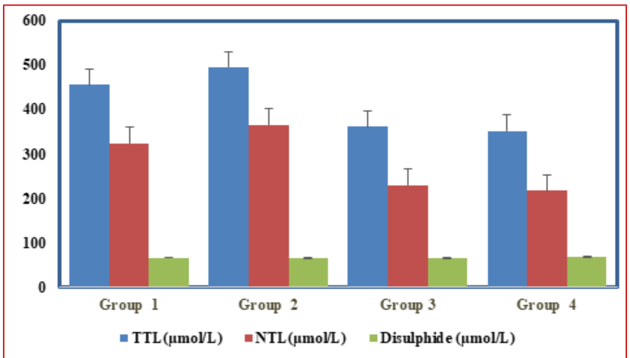

Figure 4. Serum Total Thiol (TTL), Native Thiol (NTL) and Disulphide levels.

DISCUSSION AND CONCLUSION

The process of aging is defined by the gradual loss of organ and tissue functions. The oxidative stress theory of aging, which was formerly known as the free radical theory of aging, is predicated on the structural damage theory, which holds that the buildup of oxidative damage in macromolecules (lipids, DNA, and proteins) is what causes age-related functional deficits. This imbalance is brought on by an increase in reactive oxygen species (ROS) and/or a decrease in antioxidant capacity, as well as oxidative stress (Halliwell, 2020). When the level of oxidants is larger than the level of antioxidants, or when there is an imbalance between the oxidant and antioxidant systems, it is said to be under oxidative stress (Thanan et al., 2014). Recently, thiol-disulfide balance has been recognized as an indicator of oxidative stress, which is the main cause of most diseases. Thiol groups remove reactive oxygen species (ROS) as part of the antioxidant cascade, and total thiol levels can be utilized to assess oxidative status (Bal et al., 2016). Applying the oxidation method demonstrated a linear decrease in native thiol capacity (Erel and Neselioglu, 2014). Thiols are critical to the antioxidant cascade because they include a sulfhydryl group (-SH) that functions as an enzymatic or nonenzymatic defense against ROS (Elmas et al., 2017). Protein thiol groups are oxidized to create disulfide bonds during oxidative stress. The equilibrium between thiols and disulfide groups may be preserved if the disulfide bonds that were created were reduced back to thiol groups. Thiol/disulfide homeostasis (TDH) has a vital role in many metabolic pathways. Despite the fact that TDH imbalance has been linked to numerous diseases, including preeclampsia, ischemic stroke, and diabetes (Korkmaz et al., 2016; Ates et al., 2016).

In our study, it was determined that the TTL and NTL levels were highest in Group 2 and the difference between Group 1, which started to decrease with age, was not significant (P>0.05), while the difference between Group 3 and 4 was significant compared to Group 2 (P<0.05). It was determined that the disulfide level started to increase with aging (P>0.05). It was determined that there was a significant positive correlation between TTL and NTL levels. Children with diabetic axonal polyneuropathy, Alzheimer's disease, and febrile convulsions had statistically lower NT and TT levels than controls. On the contrary, the disulfide/TT ratio was statistically higher than the controls (Gümüşyayla et al., 2016; Elmas et al., 2017). Research indicates that patients with multiple sclerosis have significantly greater disulfide levels and ratios of disulfide to NT and disulfide to TT (Vural et al., 2016). Comparing patients with acute ischemic stroke, panic disorder, and Parkinson's disease to healthy controls, it was also shown that their NT and TT levels were significantly lower. Furthermore, a bigger infarct volume and a higher National Institutes of Health Stroke Scale (NIHSS) score were linked to decreased NT levels (Vural et al., 217; Kulaksızoğlu and Kulaksızoğlu, 2017; Bektaş et al., 2016).

It was determined that there was a significant positive correlation between K level and P, Ca and Mg levels. The most common anion in internal fluid and one of the most significant extracellular anions is chloride (Cl). It supports a variety of bodily processes, including as the preservation of osmotic and acid-base balance, the functioning of the muscles and nervous system, and the transport of water and solutes between fluid compartments. In our study, it was determined that the difference between Group 1 and Group 4 was statistically significant ($P<0.05$).

Diabetes mellitus and its consequences were frequently linked to magnesium depletion and hypomagnesemia (Touitou et al., 1987; Mikhail and Ehsanipoor, 1999). As a result, a magnesium deficiency makes one more vulnerable to immune system dysfunction and oxidative stress, which can impair membrane integrity and function and cause a number of age-related changes to the mitochondria, including decreased number, altered morphology, increased DNA mutations, decreased biogenesis, decreased autophagy, and increased apoptosis (Barbagallo and Dominguez 2010). In our study, it was determined that Group 2 was the lowest and slightly increased with age, but this was not statistically significant ($P>0.05$).

Iron is a necessary element for all living organisms since it is utilized in mitochondria to produce ATP, cytochromes, hemoglobin, and many other compounds. For an organism to grow and maintain itself, iron is required. Iron is closely related to aging, and controlling iron stores in the body can be an important way to extend human lifespan. The most reactive oxygen species, hydroxyl radicals, induce DNA chain breaks and lipid peroxidation, which encourage oxidative damage in tissues and result in ferroptosis, or the aging and death of cells. Overconsumption of iron has been linked to speeding the development of age-related disorders like diabetes, carcinogenesis, and cancer (Torti and Torti, 2013; Boult et al., 2008). It was determined that the iron level was highest in Group 1 and decreased with age, while in Group 4 it increased slightly and the difference between the groups was not statistically significant ($P>0.05$).

In conclusion, the results of this study showed that thiol-disulfide homeostasis and some mineral levels were determined to change during the aging process. Antioxidant therapy to slow down the aging process can positively affect clinical damage caused by oxidative stress.

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