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CONTENTS/ İÇİNDEKİLER

TURKISH JOURNAL OF SCIENCE
VOLUME IV, ISSUE I, 1-14
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Osteoporoz Duyarlılık Risk Analizi

Osteoporosis Susceptibility Risk Analysis

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Öz: Bu çalışmada osteoporoz için risk teşkil eden bazı gen lokuslarında polimorfik yapıları araştırmayı amaçladık. Seçtiğimiz genler kemik sağlığı için gerekli olan proteinleri kodlayan genlerdir. Bu proteinler Kollajen Tip 1, Estrojen Reseptörü, D Vitamini Reseptörü, Laktaz, Osteoprotegerin ve İnterlökin 6'dır. Polimorfik yapı analizi bu altı molekülü kodlayan genlerin sekiz farklı noktasında yapıldı. Analizi yapılacak tam kan örnekleri Atatürk Üniversitesi Fizik Tedavi ve Rehabilitasyon kliniğine başvuran hastalardan alındı. Kemik Mineral Yoğunluğu hasta seçiminde kullandığımız temel kriterdi. Polimorfik Yapı Analizleri Atatürk Üniversitesi Biyokimya Anabilim Dalı Moleküler Laboratuvarında yapıldı. Genomik DNA tam kan örneklerinden manuel metot ile izole edildi. DNA miktar analizi UV spektrofotometre ile yapıldı. Primerler spesifik gen sekanslarının amplikasyonu ve etiketlenmesi Multiplex PCR'da yapıldı. PCR aplikasyonu sonucu elde edilen numuneler Agaroz Jel Elektroforeze uygulandı. DNA sekans amplikasyonları spesifik allel hibridizasyon ile Array Tüpte belirlendi. Hasta genotipi sonuçları Array Tüp okuyucu bir sisteme sahip Solas 1 Reader'den alındı. 150 gönüllüden alınan tam kan numunelerden elde edilen 1200 sonucun istatistiği yapıldı. Artan polimorfik yapı sayısı ile azalan kemik yoğunlukları arasında korelasyon olduğu gözlendi. Polimorfik yapıların osteoporoz risk oranları belirlendi. Analizi yapılan genlerdeki polimorfik yapılar osteoporoz için bir risk taşımaktaydı.Osteoporozun güçlü genetik bileşene sahip olduğu ancak diğer genetik olmayan faktörlerle birlikte değerlendirilmesi gerektiği sonucuna varıldı.

Anahtar Kelimeler — Osteoporoz, osteocheck, polimorfizim, risk analizi.

Abstract: In this study, we aimed to investigate polymorphic structures in some gene loci which are risk for osteoporosis. The genes we chose are genes that encode the proteins necessary for bone health. These proteins are collagen type 1, estrogen receptor, vitamin D receptor, lactase, osteoprotegerin and interleukin 6. Polymorphic structure analysis was performed at eight different points of the genes encoding these six molecules.

The whole blood samples to be analyzed were taken from the patients who applied to the Physical Therapy and Rehabilitation Clinic of Atatürk University. Bone Mineral Density is the basic criterion we use in patient selection. Polymorphic Structure Analysis was done at Atatürk University, Department of Biochemistry, and Department of Molecular Laboratory. Genomic DNA was isolated from whole blood samples by the manual method. DNA quantity analysis was performed with UV spectrophotometer. Primers specific gene sequences were amplified and labeled in Multiplex PCR. The samples obtained after the PCR applications were subjected to Agarose Gel Electrophoresis. DNA sequence amplifications were identified in the Array Tuple by specific allele hybridization. Patient genotype results were obtained from Solas 1 Reader with an Array Tube reader system. A total of 1200 results were obtained from whole blood samples taken from 150 volunteers.

It was observed that there was a correlation between increasing number of polymorphic structures and decreasing bone densities. The risk ratios of osteoporosis of polymorphic structures were determined. The polymorphic structures in the analyzed genes pose a risk for osteoporosis. It was concluded that osteoporosis has a strong genetic component but should be evaluated together with other non-genetic factors.

Keywords — Osteoporosis, osteocheck, polymorphism, risk analysis.

INTRODUCTION

Osteoporosis is a polygenetic skeletal system disease characterized [Bonnie 2006] by prevalence [Long 2004], progressive [Murray 2004, Anderson 1993], low bone density [Uitterlinden 1997], bone microarchitecture disruption [Fordham 2006], and increased brittleness [Raltson 2005]. The major outcomes of osteoporosis are increased rates of brittleness [Raltson 2005] and rising economic costs, high morbidity and mortality. [Raisz 2005]

Risk factors for osteoporosis include age [Ueland 2007], gender [Fordham 2006], hormonal balance [Murray 2004], nutrition [Guyton 2001], physical activity [WHO 2007], other diseases [Raltson 2005], drugs [Delmas 2000; Hannon 2000] and genes [Choi 2005; Wang 2006]. Age-related osteoporosis is an important cause of hip fracture that leads to injury and death. [Akçay 2000; Ganong 2002] Osteoporosis is more common in women than in men, and is more common in light-skinned individuals than in dark-skinned. [Sen 2005] Factors supporting osteoporosis include endocrine, metabolic and mechanical factors, parathyroid hormone and calcitonin secretion abnormalities, insufficient vitamin D and calcium intake, postmenopausal hormonal status, pregnancy, nutritional diseases, inactivity and drugs such as cortisol. [Ginaldi 2005] The risk of osteoporosis in postmenopausal women is higher than in other woman. [Sen 2005] Osteoporosis is often postmenopausal or develops slowly during menopause. A few cases are associated with mutations in Collagen 1 alpha 1 (COL1A1), Collagen 1 alpha 2 (COL1A2) and Vitamin D Receptor (VDR) [Murray 2004]. There is an increased risk of osteoporosis in inadequate and unbalanced nutritional status. The lack of adequate protein matrix due to malnutrition is important for osteoporosis. Parameters such as nutrition, exercise, smoking and alcohol consumption are risk factors for osteoporosis [WHO 2007].

Increased Parathyroid Hormone (PTH) values have been reported to be associated with increased mortality in subtle-poor ages. [Raisz 2005] Although genetic factors are effective on body shape, development in the mother's womb and childhood nutrition play a role in the pathogenesis of osteoporosis. [Fordham 2006] It has been reported that overweight may protect against osteoporosis either by increasing burden or by leptin hormone [WHO 2007]

With decreased physical activity and a sedentary lifestyle there is an increased risk of osteoporosis. Throughout their lives, the bodies of physically active people increase bone turnover in response to physical stress and have a lower risk of osteoporosis. The most effective physical activity type is weight lifting exercises. The puberty has been reported to be the most effective period to strengthen bone density. In adults, physical activity may help maintain bone mass, but the increase in bone mass is about 1-2%. Excessive exercise can lead to progressive damage to the bone. In women, heavy exercise causes menstrual cycle suppression associated with decreased estrogen levels. Bedridden people are at significantly higher risk of osteoporosis [WHO 2007].

There are many inflammatory, gastrointestinal, endocrine and genetic diseases (these include diseases such as Rheumatoid Arthritis, Chronic Liver Disease, Hypogonadism, Osteogenesis Imperfecta, Myeloma) that pose a are at risk of osteoporosis. In addition, corticosteroids, GNRH antagonists, thyroxine, aromatase inhibitors, anticonvulsants, anticoagulants, sedatives are associated with osteoporosis and increase the risk of osteoporosis [Murray 2004].

Three mechanisms have been proposed for osteoporosis, a multifactorial disease 3 resulting from the complex interaction between bone turnover, bone mass, skeletal geometry, and genetic and environmental factors affecting fall risk; inadequate bone formation, inability to reach peak bone mass, and excessive bone loss. All factors affecting the bone tissue are involved in the pathogenesis of osteoporosis through one, two, or all three of these

mechanisms. Genetic factors are confirmed to be interactions with these three mechanisms [WHO 2007].

In the literature, more than one gene name participates in the pathogenesis of osteoporosis. Some of them have a low effect on the formation of osteoporosis, but some have a high effect. For example, it has been reported that some base changes of genes encoding the Collagen 1 alpha 1 (COL1A1), Estrogen receptor (ESR), Vitamin D receptor (VDR), Osteoprotegerin (OPG), Interleukin 6 (IL-6) and Lactase (LCT) have a risk of osteoporosis. COL1A1 is the most important component of bone and connective tissue whereas ESR is an important molecule for estrogen hormone activity. VDR is an important regulator for vitamin D and calcium metabolism. While OPG has an important link between the bone and the vascular system, IL-6 inflammation is important for continuing bone health as a symptom. LCT is one of the digestive system enzymes involved in lactose breakdown in the milk [Osteocheck 2006].

In our study, we performed polymorphic analysis at eight different points on six different genes (COL1A1, ESR, VDR, OPG, LCT, IL-6). We aimed to investigate the risk ratios of these polymorphic structures to osteoporosis.

MATERIALS AND METHODS

Those with systemic disease and continuous drug use were not included in the study. 150 volunteers were included in this study and majority of the participants were woman. They were divided into groups 3 groups (osteoporosis, osteopenia, healthy) using dual energy x-ray absorptiometry (DEXA) measurements, the gold standard for the diagnosis of voluntary osteoporosis. All volunteers were examined at the Atatürk University Physical Therapy and Rehabilitation Clinic and sent to Radiology Department for DEXA measurements.

Whole Blood Specimens taken from the veins of all volunteers were sent to the Biochemistry Molecular Analysis Laboratory of the same hospital. DNA was isolated and amplified by PCR multiplex method using specific primers. The amplicons were subjected to microchip hybridization with oligonucleotide probes, and polymorphism analysis was performed in eight different gene regions.

Osteocheck microarray systems are molecular biochemical methods used to determine genetic polymorphism. Osteocheck microarray systems include the following polymorphisms: COL1A1 Sp1 G2046T polymorphism, ESR Xbal A351G polymorphism, ESR Pvull T397C polymorphism, VDR b / B INT 8C → T BsmI polymorphism, OPG G209A polymorphism, OPG T245G polymorphism, LCT T13910C polymorphism, IL-6 G174C polymorphism.

The following protocols were followed: The sampling and storage of the sample was done according to the method of Osteocheck and Invisorb [Osteocheck 2006; Invisorb 2004]. Materials required for manual DNA isolation were monitored using the Invisorb protocol. A standard registered commercial kit (Invictek's registered trademark, Invisorb) was used to determine the genomic DNA [Invisorb 2004]. Quantitative analysis of isolated DNA samples was carried out on a UV spectrophotometer at a wawelength of 260/280 [Osteocheck 2006; Invisorb 2004]. The Osteocheck protocol was followed for multiplex PCR [Osteocheck 2006]. Materials required for PCR were identified based on invisorb kit content [Invisorb 2004]. The materials required for electrophoresis were prepared according to the manual protocol. The genomic DNA was electrophoresed by the method of analysis by Agarose Gel Electrophoresis. [Sarıkaya 2004]. Array Tube Hybridization Protocol was applied for hybridization. DNA sequencing was performed by an automated method [Osteocheck 2006]. Routine Ogham Solas 1 system was used for analysis of both normal and mutant genes. 'Ogham Solas 1' was loaded on the laboratory instrument 'PrimoLas' and the results were obtained from my computer system.

A control DNA sample was used to confirm the results. The results were reported by loading it into a computer and hospital automation system in the laboratory. The SPSS Statistic program (version 15.0) was used to analize the data. Significance values were determined at p <0.05 using Pearson Chi-Square Test. The ethical approval of the study was given by the Ethics Committee of Atatürk University Medical Faculty (26/04/2007, Jan.1, 2007). The kits used for the study were received by the hospital. To all volunteers were given Osteoporosis Susceptibility Risks Analysis report. There is no the interest relationship between the parties in the study.

RESULTS

150 volunteers were included to work. Volunteers were divided into three groups, according to DEXA measurements. Polymorphic structure analysis was performed at the gene locus of COL1A1 Sp1 G2046T, ESR Xbal A351G, ESR Pvull T397C, VDR b / B INT 8C? T BsmI, OPG G209A, OPG T245G, LCT T13910C, IL-6 G174C. Polymorphic structure was detected in all gene loci analyzed. A total of 1200 polymorphic results were obtained for each volunteer. The results were reported as Wildtype, Heterozygote and Homozygote. In Table 1, the percentages of DEXA and polymorphic structures are given. There was no significant correlation between bone mineral densities and polymorphic structures (Pearson Chi-Square Test (p <0,486) (Table 1).

Table 1: Bone Mineral Density and polymorphic structure percentages

	Normal	Osteopenia	Osteoporosis
Wildtype	53,44%	51,25%	49,58%
Heterozigot	26,25%	26,50%	27,92%

Homozigot	20,31%	22,25%	22,50%
	100,00%	100,00%	100,00%
Pearson Chi-Square Test (p< 0,486)			

[Percentage values of normal, osteopenia and osteoporosis, which are voluntary base groups according to DEXA measurements, and wildtype, heterozygote and homozygote percentage values according to polymorphic analysis results.]

The most common genotype (wildtype) and 4 polymorphic structures were detected in each volunteer (35,33%). The polymorphic structure was observed at 5 points (19,33%), 3 points (17,33%), 2 points (14,67%) and 6 points (9,33%) respectively (Table 2).

Table 2: Polymorphic structure frequency

	GENE								
	1 gene	**2 gene**	**3 gene**	**4 gene**	**5 gene**	**6 gene**	**7 gene**	**8 gene**	
%	***2,67***	**14,67**	**17,33**	**35,33**	**19,33**	**9,33**	**1,34**	**0**	**100**
Total	4	**22**	**26**	**53**	**29**	**14**	**2**	**0**	**150**

[The table gives the number of normal genotypes at eight points studied. Or, if the gene is not a normal genotype, it means polymorphic structure. 4 (2,67%) with 1 polymorphic structure, 22 (14,67%) with 2 polymorphic structures, 26 (17,33%) with 3 polymorphic polymorphic structures, 53 (35,33%), 29 (19,33%) with 5 polymorphic structures, 14 (9,33%) with 6 polymorphic structures, and 2 (1,34%) with 7 polymorphic structures were detected].

R^2 values were obtained by plotting the graphs showing the relationship between wildtype numbers and bone mineral density of individuals with the wildtype genotype. The relationship between the number of wildtype genotypes and normal bone mineral densities in an individual is shown in Figure 1, the relationship between those with osteoporosis in Figure 2 and the relationship between those with osteopenia in Figure 3.

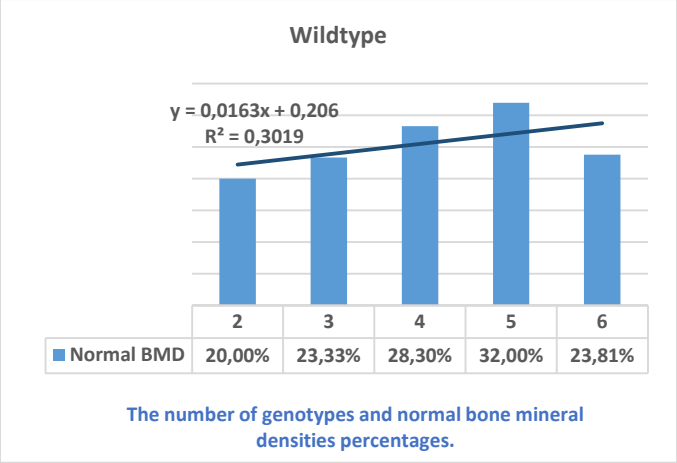

Figure 1: Wildtype; The number of genotypes and normal bone mineral densities percentages. Numbers 2, 3, 4, 5 and 6 give the number of wildtype in eight points analyzed in one person. Accordingly, the number of wildtype residues increases in proportion to having normal bone density. The percentage of individuals with normal bone mineral density having 2 wildtype genotypes was 20.00%

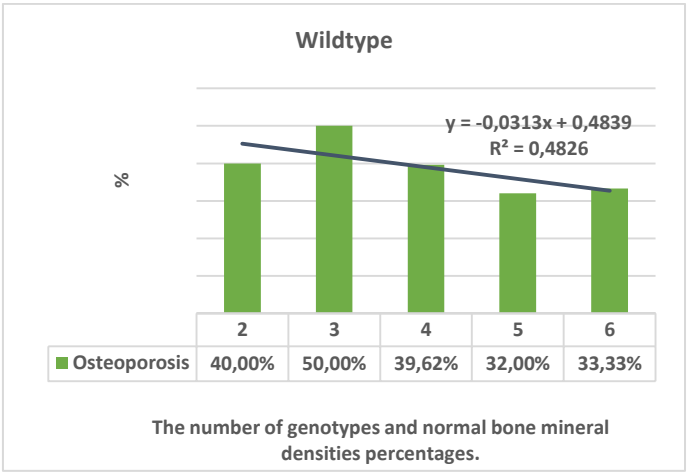

Figure 2: Wildtype; The number of genotypes and osteoporosis percentages. Numbers 2, 3, 4, 5 and 6 give the number of wildtype in eight points analyzed in one person. According to the figure, the percentage of osteoporosis is decreasing while the number of wildtypes is increasing.

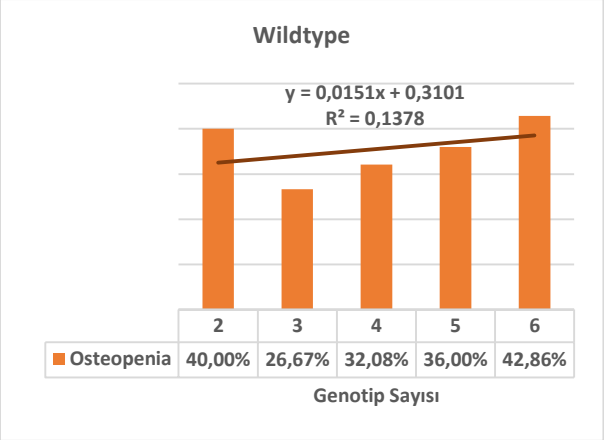

Figure 3: Wildtype; The number of genotypes and osteoporosis percentages. Numbers 2, 3, 4, 5 and 6 give the number of wildtype in eight points analyzed in one person. According to the figure, the percentage of osteopenia is increasing while the number of wildtypes is decreasing.

According to the study done between normal bone mineral density, the wildtype genotype percentage of normal bone density was higher than heterozygous and homozygous cases (Figure 4), and the wildtype percentage was lower than that of heterozygous and homozygous ones in osteoporosis cases (Figure 5). Those with osteopenia had the same genotype results as those with normal bone density (Figure 6).

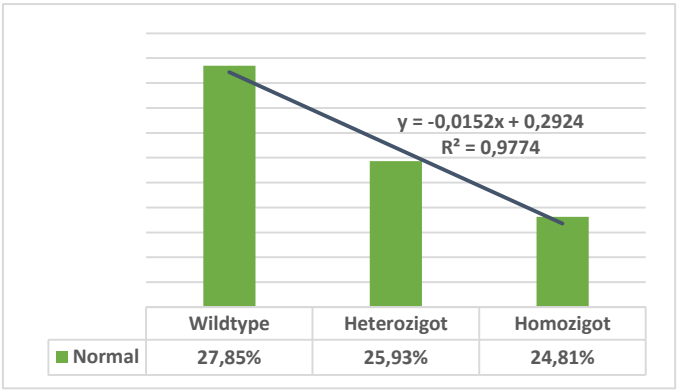

Figure 4: In those with normal bone density, the percentage of wildtype is highest (27.85%) and the percentage of homozygotes is lowest (24.81%).

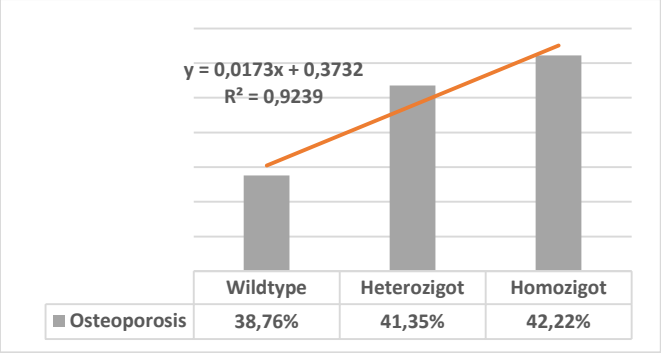

Figure 5: Genotype percentages in those with osteoporosis. In the cases of osteoporosis, the percentage of wildtype is lowest (38.76%) and the percentage of homozygote is highest (42.22%).

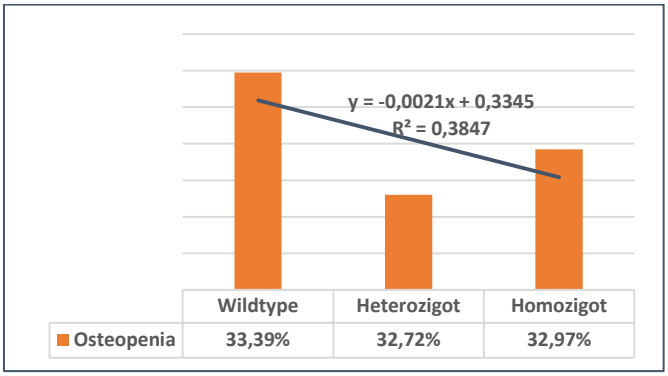

Figure 6: Genotype percentages in those with osteopenia. In osteopenic subjects, the percentage of wildtype was highest (33.39%), the percentage of heterozygotes was lowest (32.72%)

DISCUSSION

Osteoporosis is one of the diseases with great social and economic burden. The number of people affected by gives statistics the disease is also increasing in comparison with the increasing elderly population in the world. For this reason, genetic risk analysis tests performed for many different situations are performed for osteoporosis, which increases the susceptibility to disease. The desire for a healthier aging is acceptable to reduce the incidence of osteoporosis.

Polymorphisms in several genes are associated with different mass and bone fragility. It is now even more probable that osteoporosis can be predicted by these polymorphisms, the calculation of fracture risk and the approach of treatment [Raisz 2005].

A polymorphic structure every 500 nucleotides are normally expected. These polymorphic structures contribute to people being different. These differences can sometimes be neutral, sometimes positive, and sometimes negative [Hannon 2000]. Risk analysis tests conducted to detect nucleotide changes in the genes of healthy bone development and persistent direct and indirectly related molecules are drawn to bone health by providing us with information on this topic.

The role of transcriptional factors for polymorphisms in osteoporosis has not yet been elucidated [Raisz 2005]. There was no significant association between polymorphic structures and age, as seen in figure 1 in our study. With increasing age, the increased risk of osteoporosis was associated with no significant association with bone density of polymorphic structures.

In general, the interaction between polymorphisms and osteoporosis is associated with moderate effects [Uitterlinden 2004]. According to the results shown in Table 1, there was no significant difference in the incidence of polymorphic structure among the groups.

In recent years, intensive research on genetic markers has reported that several genetic polymorphisms are associated with osteoporosis. These polymorphisms are associated with decreased bone turnover and a high risk of osteoporosis [Osteocheck 2006]. The polymorphic structure of the entire gene locus of COL1A1 Sp1 G2046T, ESR Xbal A351G, ESR Pvull T397C, VDR b/B INT 8C/T BsmI, OPG G209A, OPG T245G, LCT T13910C and IL-6 G174C in the assay was determined. But these polymorphic structures were at different points in different individuals. In all groups 35.33% of polymorphic structures were detected in at

least four points (Table 2). Polymorphic structure was observed in 5 points, 3 points, 2 points and 6 points respectively. Bone mass is under the control of many genes [Murray 2004; Brandi 2001]. In different societies different polymorphic structures and their different effects have been observed [Brandi 2001]. According to the results obtained in our study, there was no one who did not have any polymorphic structure. Only at one point is the polymorphic structure number / percentage very low (2,67%). However, polymorphic structures were found to be the most common at 4 points. Polymorphic structure was observed in 5 points, 3 points, 2 points and 6 points respectively (Table 2).

Our data provide valuable rewards for the literature regardless of the age and gender about the regional polymorphic structure frequency. According to DEXA results, there was no significant relationship between osteoporosis, osteopenia and the groups that we normally formed and the incidence of polymorphic structure. If the relationship between DEXA and the polymorphic structure were made in the same age, sex, diet, and physical activity, the data would be more tangible.

It is known that genetic factors play a role in the micro-architectural properties of the bone. It is even reported that genetic factors account for 70-80% of changes in bone phenotype. In addition, osteoporosis in the family history indicates that the person has genetic background [Hannan 2000]. It has been stated that daughters of mothers with osteoporotic fractures have low bone density [Albrand 2003].

Despite the fact that the number of polymorphic structures seen and the percentage of osteoporosis figures are open to debate in many respects, it is obvious that the probability of osteoporosis increases as the polymorphic structure frequency increases in a person.

The greater the number of wildtype genotypes in a person, the greater the percentage of having normal bone density (figure 1), whereas the lower the number of wildtypes, the greater

the percentage of osteopenia (figure 2) and osteoporosis (figure 3). In those with normal bone density, the percentage of wildtype is highest (27.85%) and the percentage of homozygotes is lowest (24.81%) (Figure 4). In osteoporosis cases, the percentage of wildtype was lowest (38.76%) and the percentage of homozygotes was highest (42.22%) (Figure 5). In osteopenic subjects, the percentage of wildtype was highest (33.39%) and the percentage of heterozygotes was lowest (32.72%) (Figure 6).

Bone mineral density is known to be a corporate result of environmental and genetic factors. Likewise, our data show that it has a corporate effect on genetic factors (Figures 1, 2, 3, 4, 5, and 6). It was determined that polymorphic structures at eight different points analyzed have osteoporosis contribution, but this contribution is not statistically significant. It is understood that this contribution is quite large when considering hundreds of proteins participating in the mechanisms of bone formation and destruction.

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Sığır Sütündeki Laktoperoksidaz Enzimi Üzerine Bazı Flavonoid Türevlerinin İnhibitör Etkisinin Belirlenmesi

Determination of Some Flavonoid Derivatives Inhibitory Effect on Bovine Milk Lactoperoxidase Enzyme

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Öz: Peroksidazlar (PODs), bakteri, mantar, bitki ve hayvanlarda yaygın olarak bulunan, gıda, ilaç endüstrisi ve klinik teşhislerde önemli kullanım alanına sahip olan enzim gruplarıdır. Peroksidaz enzim sınıfına dahil olan Laktoperoksidaz (LPO) enzimi ise memelilerde süt, tükürük ve gözyaşında bulunur. Tiyosiyanat ve hidrojen peroksitle beraber enfeksiyonlara karşı vücudun savunma sistemlerinden birini oluşturur. Flavonoid türevleri bitkilerde bol miktarda bulunur ve diyetin önemli bir parçasını oluşturur. Sentetik olarak üretilen veya doğal olarak bulunan bu türevler, birçok farmakolojik aktiviteye sahiptir. Bu çalışmada laktoperoksidaz enzimi üzerine bazı flavonoid türevlerinin (5,7-dihidroksi-2-(3-hidroksi-4-metoksifenil)-4H-kromen-4-on (a), 3,5,7-Trihidroksi-2-(3,4,5-trihidroksifenil)-4H-kromen-4-on (b), 7-hidroksi-4'-nitroizoflavon (c), 6-Floroflavon (d), 7-Hidroksi-3-(4-metoksifenil)-4H-kromen-4-on (e), 7-metoksi-2-fenil-4H-kromen-4-on (f)) etkisi incelenmiştir. İlk olarak Sefaroz-4B-L-tirozin-sülfanilamid afinite kromatografisi ile sığır sütünden LPO enzimi 65 kat ve %23 verim ile saflaştırılmış ve bu enzim kullanılarak flavonoid türevleri ile kinetik çalışmalar yapılmıştır. 6 molekülün Ki değerlerinin 7.85 µM ile 0.023 µM arasında değiştiği bulunmuştur. 6-Floroflavon, 0.023 µM Ki değeri ile en etkili inhibitördür.

Anahtar Kelimeler — Laktoperoksidaz, flavonoid türevleri, inhibisyon.

Abstract: Peroxidases (PODs) are a group of enzymes that are commonly found in bacteria, fungi, plants and animals and have important uses in the food, pharmaceutical industry and clinical diagnostics. Lactoperoxidase (LPO) enzyme, which is included in peroxidase enzyme class, is found milk, saliva and tears in mammals. With thiocyanate and hydrogen peroxide, it forms one of the body's defense systems against infections. Flavonoid derivatives are abundant in plants and constitute an important part of the diet. These derivatives, which are produced synthetically or naturally, have many pharmacological activities. In this study, the effect of some flavonoid derivatives (5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one (a), 3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one (b), 7-Hydroxy-4'-nitroisoflavone (c), 6-Fluoroflavone (d), 7-Hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (e), 7-Methoxy-2-phenyl-4H-chromen-4-one (f)) on the lactoperoxidase enzyme was investigated. Firstly, by using Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography, the LPO enzyme was purified 65 fold (with a yield of 23%) from bovine milk and kinetic studies were carried out with flavonoid derivatives using this enzyme. The Ki values of six molecules were found in ranging from 7.85 µM to 0.023 µM. 6-Fluoroflavone was the most effective inhibitor with Ki value of 0.023 µM.

Keywords — Lactoperoxidase, flavonoid derivatives, inhibition.

INTRODUCTION

Peroxidases are heme containing enzyme groups that found in bacteria, fungi, plants and animals [1]. LPO is a glycoprotein that is included in the peroxidase family and is found in saliva, milk and tears. LPO catalyzes the oxidation of thiocyanate to cyanide in the presence of hydrogen peroxide and shows antimicrobial effect [2]. The reactive products produced in the LPO/SCN^-/H_20_2 system oxidize sulfhydryl groups (- SH) of proteins in the bacterial cell membrane and inhibit the transport of nutrients, DNA and RNA synthesis and respiratory chain [3].

Flavonoids are a class of plant-derived polyphenolic compounds used in human health, pharmaceutical and industrial research [4, 5]. These compounds are predominantly found in vegetables, fruits and teas. These derivatives are formed by binding different groups to the two phenyl rings and a heterocyclic ring. There are more than 4000 types of flavonoid and are divided into six main groups according to their chemical structure: Flavones, flavonols, flavanols, flavanones, anthocyanidins and isoflavonoids [6, 7]. Flavonoids are an important part of our diet, especially in Japan, daily intake is up to 68.2 mg. Quercetin, one of the most known flavonoids, is abundant in apple and onion and contributes significantly to the daily intake of flavonoids [8, 9].

Studies have shown that flavonoids have many pharmacological effects, including antioxidant, anticancer and anti-inflammatory properties [10, 11]. These molecules have been shown to inhibit many metabolic enzymes such as monooxygenase, glutathione S-transferase, mitochondrial succinate-oxidase, nicotinamide adenine dinucleotide hydrate-oxidase, tyrosine and serine-threonine protein kinase [12].

In this study, it was aimed to investigate the inhibition effect of flavonoid derivatives with antioxidant activity on LPO enzyme. This study will define the interaction of these substances with the lactoperoxidase enzyme. For this purpose, the bovine milk LPO enzyme was purified and the inhibition effect of six flavonoid derivatives on the enzyme was investigated in detail.

MATERIAL AND METHOD

Chemicals and materials

The bovine milk used in the study was obtained from the local market. Amberlit CG-50-NH_4^+, CNBr-activated Sepharose 4B, L-tyrosine and sulphanilamide were purchased from Sigma-Aldrich and 5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one, 3,5,7-

Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one, 7-Hydroxy-4'-nitroisoflavone, 6-Fluoroflavone, 7-Hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one, 7-Methoxy-2-phenyl-4H-chromen-4-one were purchased from Fluorochem UK. All other chemicals used in this study were analytical grade.

Determination of LPO Enzyme Activity

Lactoperoxidase enzyme activity was calculated by absorbance increase at 412 nm of the coloured compound formed by the oxidation of the 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) chromogenic substrate in the presence of hydrogen peroxide (H_2O_2) [13].

Purification of LPO Enzyme

For the purification of LPO enzyme, bovine milk was first centrifuged at 3000g for 10 min and the milk fat was removed, then Amberlit CG-50-NH_4^+ was treated with fat-removed milk and washed with the appropriate solution to obtain a homogenate which, amounts of proteins were determined and enzyme activities were measured. The homogenate was applied to the Sepharose 4B-L-tyrosine-sulphanamide affinity column which equilibrated with 10mM phosphate buffer (pH 6.8). The affinity gel was washed with 25mM phosphate buffer (pH 6.8). The bovine LPO enzyme was eluted with same buffer (1 M NaCl/25, pH 6.8) [14]. Protein concentrations in the homogenates and purification steps were determined by the modified Lowry method [15].

Inhibition studies

The inhibition effects of flavonoid derivatives on LPO enzyme purified from Sepharose 4b-L-Tyrosine sulphanilamide affinity gel were investigated. Activity values for each derivative were measured at constant substrate (ABTS) concentration and at five different inhibitor concentrations. Then, activity %-inhibitor graphs were drawn and IC_{50} values were calculated from these graphs. Three different inhibitors and five different substrate concentrations were used for determination of K_i values. K_i and the inhibition type were calculated from Lineweaver–Burk graphics (1/V-1/[S])[16].

RESULTS AND DISCUSSION

Flavonoids are mostly phenolic compounds, abundant in fruits, vegetables and tea and are an important part of the human diet. Synthetic or naturally occurring flavonoid derivatives have many pharmacological activities. These are antitumor, anticonvulsant, vasorelaxant,

analgesic, antioxidant and anti-inflammatory activities [17]. It is known that natural and synthetic flavonoid derivatives are potent antioxidants as well as their regulatory effects on metabolism.

The effects of flavonoid derivatives on enzymes have been studied by many researchers and these studies are increasing day by day. In the literature, there are studies on the inhibitory effects of these derivatives on ACE (Angiotensin Converting Enzyme), Monoamine oxidase, lipase, acetylcholine esterase enzyme. Exemplarily, quercetin, quercetin-3-glucoside, quercetin-3-galactoside, cyanidin-3-galactoside were studied on the ACE and IC_{50} values were found to be 151 μM, 71 μM, 180 μM, 206 μM, respectively [18]. The effect of flavonoid derivatives on MAO-A (Monoamine oxidase-A) and MAO-B enzymes was investigated and it was observed that these derivatives showed an inhibition effect at μM level [19]. Luteolin and chrysoeriol have a noncompetitive and mixed inhibition on lipase enzyme with values of IC_{50}: 63 and 158 μM respectively [20]. In addition, synthetic flavonoid derivatives have been identified as potential inhibitors of AChE (Acetylcholinesterase) [21].

In this study, the effect of commercially available flavonoid derivatives on bovine milk LPO enzyme was investigated. First, the bovine milk LPO enzyme was purified using Sepharose 4B-L-tyrosine-sulphanilamide affinity gel. The results of purification are shown in Table 1.

Table 1. The Purification results of LPO enzyme from bovine milk.

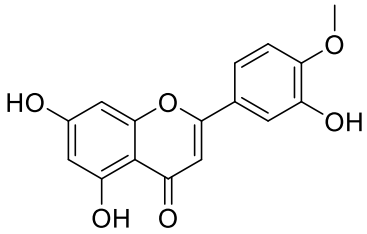
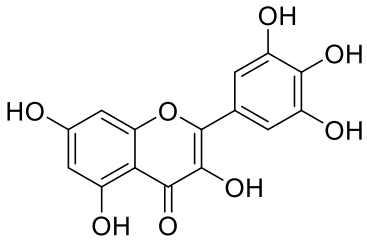
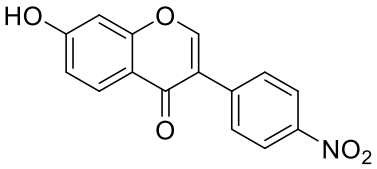
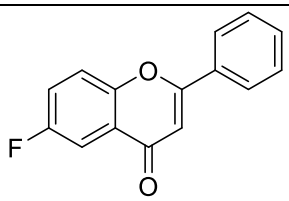
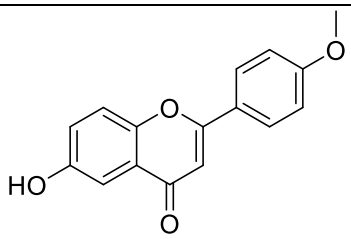
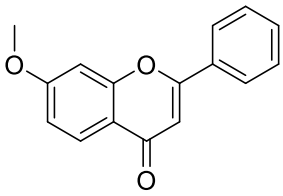
STEP	Total Volume (mL)	Activity (EU/mL)	Protein (mg/mL)	Total Activity (EU)	Total Protein (mg)	Specific activity (EU/mg)	Yield %	Fold
[a]***Step1***	6	0,9	8,2	5,4	49,2	0,109	100	1
[b]***Step2***	2	0,62	0,087	1,24	0,174	7,12	23	65

[a]*Step1: Amberlite CG-50-NH_4^+*

[b]*Step2: Sepharose 4B-L-tyrosine- sulphanilamide*

To determine the effect of flavonoid derivatives on purified LPO enzyme, detailed kinetic studies were performed and IC_{50}, K_i values and inhibition types were determined. The kinetics results are shown in Table 2.

Table 2. IC_{50} value, K_i constant and inhibition type of flavonoid derivatives for LPO enzyme

Inhibitors	**Name**	**IC_{50} (µM)**	**K_i (µM)**	**Inhibition type**
	5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one **(a)**	0,921	0,512 ± 0,0231	Competitive
	3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one **(b)**	0,434	0,452 ± 0,0918	Competitive
	7-Hydroxy-4'-nitroisoflavone **(c)**	0,832	0,623 ± 0,123	Competitive
	6-Fluoroflavone **(d)**	0,035	0,0233 ± 0,0081	Non-competitive
	7-Hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one **(e)**	1,81	2,86 ± 0, 261	Non-competitive
	7-Methoxy-2-phenyl-4H-chromen-4-one **(f)**	5,92	7,85 ± 0,712	Non-competitive

When the molecules and inhibition values are examined, the most effective inhibitor is 6-Fluoroflavone (d). The electronegative fluorine atom of the molecule **d**, increased the interaction of the inhibitor with the LPO enzyme and caused a stronger inhibition (See molecule **d,** K_i: 0.0233 μM). Table 2 shows that the inhibition effects of the molecules increase with the effect of OH groups. When the number of OH groups increases, the inhibition effect of the molecules is strengthened. Among the molecules containing the OH group, the highest effect was observed in molecule **b**. (K_i: 0.434 μM). This molecule contains six OH groups. The weakest inhibition value is K_i: 7.85 and molecule **f**. This is because molecule f does not contain the OH group and the electronegative group. It was observed that various groups that bind to flavon ring changed the inhibition effect to 350 fold.

In the literature, the inhibitory effect of nine different phenolic compounds on LPO enzyme was investigated and the K_i value of quarcetin was calculated as 5.99 μM. In the same study, K_i values of caffeic acid, ellagic acid, ferulic acid and syringic acid ranged from 2.83 to 17.76 μM [22]. In our study with flavonoid derivatives, the inhibition values ranged between 0.023 and 7.85 μM. This indicates that these derivatives, other than the molecule **f**, are more effective inhibitors.

CONCLUSIONS

Reducing the effect of LPO by the flavonoid derivatives, which is the most important part of the LPO/SCN^-/H_20_2 system, has negative consequences. The defense mechanism against bacteria is attenuated by the decrease in the activity of this enzyme. This makes especially babies less resistant to bacterial infections. In this study, six flavonoid derivatives were identified as the new reversible inhibitors of LPO enzyme and kinetic studies were performed. Ki, IC_{50} and inhibition types were determined for the first time.

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Çekal Ligasyon ve Delme ile oluşturulan Polimikrobiyal Sepsis Modelinde Akut Organ Hasarına Karşı Fraxin'in Antioksidan Etkileri

The Antioxidant Effect of Fraxin against Acute Organ Damage in Polymicrobial Sepsis Model induced by Cecal Ligation and Puncture

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Öz: Amaç: Bu makalede, ratlarda çekal ligasyon ve delme (CLP) modeli ile indüklenen akut organ hasarına karşı fraksin' in antioksidan etkisinin değerlendirilmesi amaçlandı. **Gereç ve Yöntemler**: Bu amaçla çalışmamızda, tüm deney hayvanları randomize şekilde gruplandırıldı. Bu gruplar sham kontrol, CLP, CLP+Fraxin 50 mg/kg, CLP+Fraxin 100 mg/kg gruplar olarak belirtildi. Tüm sıçanların böbrek ve akciğer dokularında TAS, TOS, OSI, MDA düzeyleri, MPO ve SOD aktiviteleri değerlendirildi. **Bulgular**: Böbrek ve akciğer dokularının TOS, OSI, MDA düzeyleri ve MPO aktivitesi, CLP grubunda sham kontrol grubundan daha yüksekti, ancak TAS düzeyi ve SOD aktivitesi daha düşüktü. Bununla birlikte, bu sonuçlar fraxin tedavi gruplarında önemli ölçüde değişmiştir. **Sonuç**: Bu sonuçların ışığında, fraksin' in, CLP' nin neden olduğu sepsis modelinde böbrek ve akciğer dokusu üzerindeki TOS, MPO ve MDA seviyelerini düşürerek SOD ve TAS seviyesini yükselterek olumlu bir etki gösterdiği ifade edilebilir.

Anahtar Kelimeler — Çekal ligasyon ve delme, fraksin, sepsis, rat.

Abstract: Aim: In this paper, it was purposed to evaluate the antioxidant effect of fraxin against acute organ damage induced by cecal ligation and puncture model (CLP) in rats**. Material and Methods**: For this purpose in our study, all experimental animals were randomly grouped. These groups were stated as sham-control, CLP, CLP+Fraxin 50 mg/kg, CLP+Fraxin 100 mg/kg groups. TAS, TOS, OSI, MDA levels, MPO and SOD activities were evaluated in kidney and lung tissues of all rats. **Results**: TOS, OSI, MDA levels and MPO activity of kidney and lung tissues were higher in the CLP group than in the sham-control group, but TAS level and SOD activity were lower. However, these results were significantly changed in the fraxin treatment groups. **Conclusion**: In light of these results, it can be stated that fraxin shows a positive effect by raising the level of SOD and TAS by decreasing TOS, MPO and MDA levels on kidney and lung tissue in the model of sepsis caused by CLP.

Keywords — Cecal ligation and puncture, fraxin, sepsis, rat.

1.Introduction

Sepsis is a life-threatening clinical condition leading to organ dysfunction in which a host response to infection occurs [1, 2]. The cecal ligation and puncture (CLP) that we have applied in our study is very similar to sepsis seen in the clinic, and CLP in experimental animals is commonly used to simulate sepsis animal modelling and imitation clinical appendicitis or diverticulitis perforation [3, 4]. For this reason, to date, the efficacy of a large number of agents to minimize or completely eliminate organ damage caused by sepsis with high morbidity and mortality rates has been investigated [5, 6]. Fraxin has anti-inflammatory,

hepatoprotective, antioxidant, immunomodulatory activity, analgesic effects and is known to be the main active compound obtained from Cortex Fraxini, which is preferred in clinic therapies such as hyperuricemia, diarrhea and liver therapies [7-9]. However, when a literature review was performed, it was not come across the studies that evaluated the therapeutic or protective efficacy of fraxin in a polymicrobial sepsis model induced by CLP So, the aim of this study was to evaluate an antioxidant effect of the fraxin on the kidney and lung tissues in the sepsis model induced by CLP in rats.

2. Material and Methods

The experimental animals used in this study were obtained from the Atatürk University Experimental Animal Research and Application Center (ATADEM) and the experimental stages of our study were performed in the same place. This study has been approved by Atatürk University Local Animal Ethics Committee (Date/Number of ethical approval:28.06.2018/138). All experimental animals were kept in the same standard laboratory conditions and were fed with standard rat food and water.

2.1. Design of Experimental Study

Firstly, 32 Wistar albino rats used in this study were divided into 4 groups. These groups were designed as follows;

1. Sham-Control group (n=8): In this group, only laparatomy and bowel manipulation were applied. But cecal ligation puncture was not done.

2. CLP (cecal ligation puncture) group (n=8): The animals in this group were anesthetized with a mixture of ketamine / xylazine (60/10 mg / kg; i.p.). Polymicrobial sepsis was formed by the CLP method which was used in previous studies [10, 11]. After the abdominal area was cleared, an incision was made on the midline. The cecum was taken out and two holes were drilled using an 18-ga needle. Then the punctured cecum was placed in the peritoneum and the abdominal wall was closed again.

3. CLP+Fraxin 50 mg/kg group (n=8): After CLP was performed, the subjects were treated with fraxin as 50 mg/kg intraperitoneally.

4. CLP+Fraxin 100 mg/kg group (n=8): After CLP was performed, the subjects were treated with fraxin as 100 mg/kg intraperitoneally.

Approximately 18 hours after the formation of sepsis induced by CLP, all rats were sacrificed. Later the lungs and kidney tissues were rapidly removed.

2.2. Biochemical Analysis

MDA/ Malondialdehyde (μmol/g protein) level, SOD/ Superoxide dismutase (U/mg protein) and MPO/ Myeloperoxidase (U/mg protein) activities were measured taking reference from previous studies [12-14]. Also, TAS/ Total antioxidant status (mmol/L) and TOS/ Total oxidant status (μmol/L) levels were analysed using commercial kits. OSI/ oxidative stress index value was determined as formula: OSI = [(TOS, μmol H_2O_2 equivalent/L)/(TAS, mmol Trolox equivalent/L) × 10].

2.3. Statistical Analysis

All of our biochemical data were analyzed statistically with One-Way ANOVA and Tukey HSD tests. Statistical significance was considered significant at the 0.05 level. All our results are given as Mean±Standard Error Mean (SEM).

3. Results

MDA/ Malondialdehyde (µmol/g protein) levels, SOD/ Superoxide dismutase (U/mg protein), MPO/ Myeloperoxidase (U/mg protein) activities, TAS (mmol/L) and TOS (µmol/L) levels and OSI value of lung and kidney tissues.

MDA level as an indicator of lipid peroxidation and MPO activities were significantly higher in CLP groups compared to sham-control groups in lung and kidney tissues. On the contrary, in the analysis of both lung and kidney tissues, MDA levels and MPO activities were found to be significantly decreased in the groups given fraxin at 50 and 100 mg/kg doses (See Fig 2a and b; $p<0.05$).
When SOD activity of lung and kidney tissues were evaluated, it was determined that SOD activities decreased especially in the CLP group when compared with the sham-control group and also increased with fraxin treatments (See Fig 2c; $p<0.05$).

Table 1 and 2 show that TAS levels of lung and kidney tissues were significantly decreased in CLP group and increased due to Fraxin treatments. However, in the CLP group, TOS and OSI values decreased significantly compared to the rise in TAS. Besides, fraxin treatment was able to decrease the TOS level and OSI value.

Table 1a: TAS, TOS levels and OSI values belonging to kidney tissue of all groups are presented as Mean±SEM. There is a statistically significant relationship between the groups with the same letters.

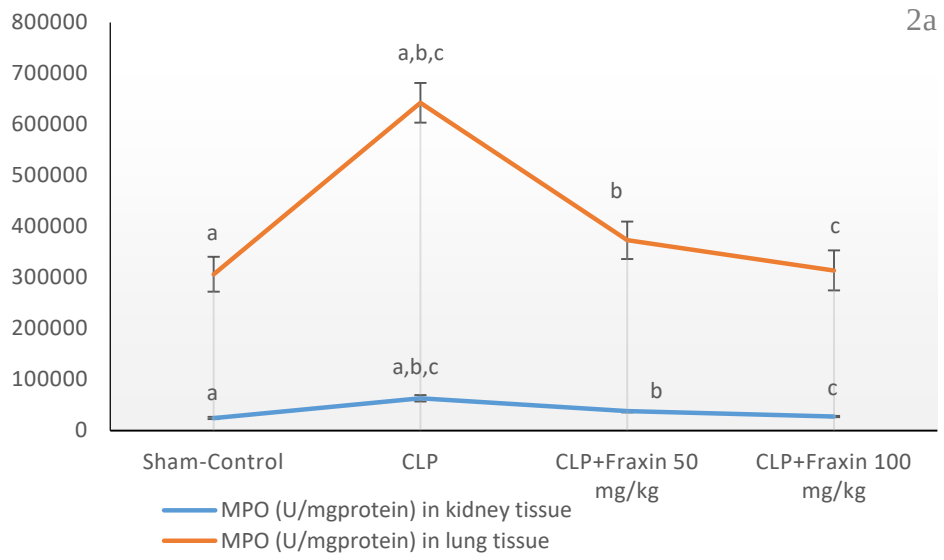
Kidney Tissue			
	TAS (mmol/L)	**TOS (µmol/L)**	**OSI**
Sham-Control	2,94±0,06[a]	5,33±0,19[a]	0,18±0,008[a]
CLP	0,86±0,02[a,b,c]	9,01±0,23[a,b,c]	1,04±0,04[a,b,c]
CLP+Fraxin 50 mg/kg	2,78±0,12[b]	6,66±0,27[b]	0,24±0,01[b]
CLP+Fraxin 100 mg/kg	3,00±0,11[c]	5,58±0,26[c]	0,18±0,01[c]

[a]: Comparison with Sham-Control group ($p<0.05$), [b]: Comparison with CLP group ($p<0.05$), [c]: Comparison with CLP group ($p<0.05$).

Table 1b: TAS, TOS levels and OSI values belonging to lung tissue of all groups are presented as Mean±SEM. There is a statistically significant relationship between the groups with the same letters.

Lung Tissue			
	TAS (mmol/L)	**TOS (µmol/L)**	**OSI**
Sham-Control	0,36±0,01[a]	11,48±0,45[a]	3,22±0,27[a]
CLP	0,17±0,01[a,b,c]	19,61±0,60[a,b,c]	11,37±0,69[a,b,c]
CLP+Fraxin 50 mg/kg	0,30±0,02[b]	13,71±0,38[b]	4,58±0,33[b]
CLP+Fraxin 100 mg/kg	0,36±0,03[c]	11,80±0,56[c]	3,51±0,37[c]

[a]: Comparison with Sham-Control group ($p<0.05$), [b]: Comparison with CLP group ($p<0.05$), [c]: Comparison with CLP group ($p<0.05$).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b
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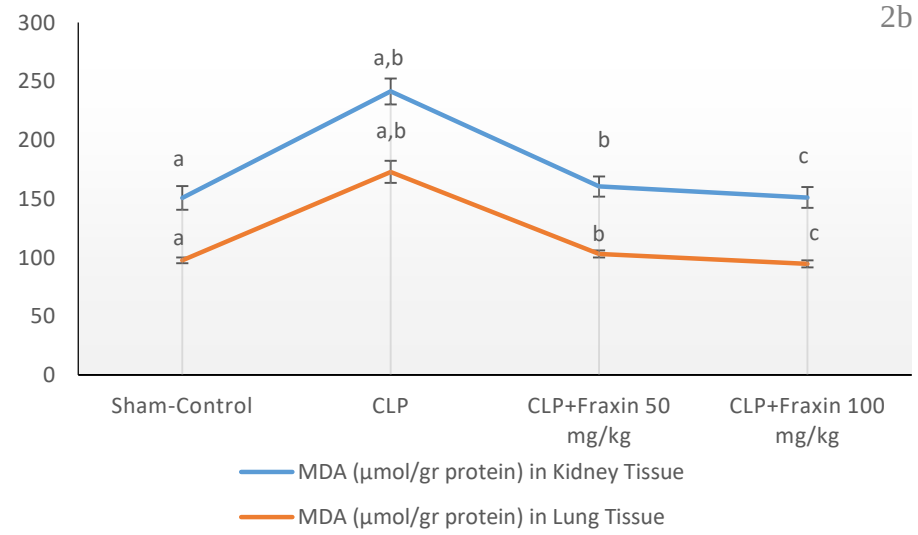
Sham-Control
CLP
CLP+Fraxin 50 mg/kg
CLP+Fraxin 100 mg/kg
MPO (U/mgprotein) in kidney tissue
MPO (U/mgprotein) in lung tissue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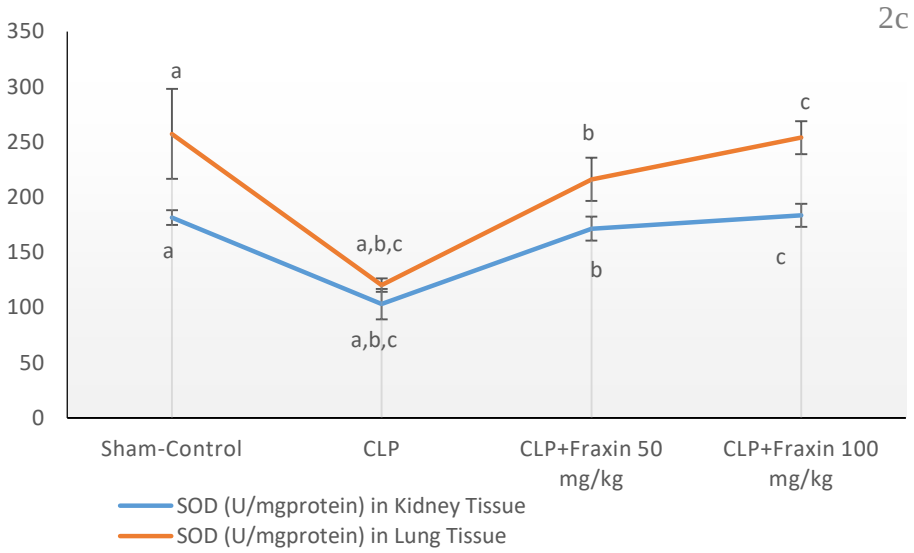
Sham-Control
CLP
CLP+Fraxin 50 mg/kg
CLP+Fraxin 100 mg/kg
MDA (µmol/gr protein) in Kidney Tissue
MDA (µmol/gr protein) in Lung Tissue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c
Sham-Control
CLP
CLP+Fraxin 50 mg/kg
CLP+Fraxin 100 mg/kg
SOD (U/mgprotein) in Kidney Tissue
SOD (U/mgprotein) in Lung Tissue

[a]: Comparison with Sham-Control group ($p<0.05$), [b]: comparison with CLP group ($p<0.05$), [c]: comparison with CLP group ($p<0.05$).

Figure 2a: MPO activities of all groups were given in lung and kidney tissues. **2b:** MDA level of all groups were given in lung and kidney tissues. **2c:** SOD activities of all groups were given in lung and kidney tissues. There is a statistically significant relationship between the groups with the same letters.

4. Discussion and Conclusion

Sepsis has been one of the problems that have been difficult to treat and mortality for many years. Sepsis and septic shock, which need to be treated urgently, are still considered to be a pathology with high mortality [15]. This is a clinical and pathophysiological process in which the majority of patients have a continuous increase in the severity of the clinical conditions leading to sepsis, severe sepsis, septic shock and multiorgan failure involving acute lung injury, acute respiratory distress syndrome, acute/chronic kidney disease and end-stage renal disease and even death [15-17]. Therefore, this situation, which has a high mortality rate, is defined as acute in the early period and the chance of survival of the patients can be increased with the application of an emergency and effective treatment [15]. Today, studies on sepsis have focused on experimental models. Sepsis was formed with different models on experimental animals. Although it is currently aimed at revealing the pathophysiology of sepsis with a wide variety of experimental studies and developing new treatment strategies, the current treatment protocol is often limited to routine life support treatment [6, 18]. However, cecal ligation and puncture (CLP) method was frequently used on some studies [19, 20]. Acute lung and kidney damage is the most common type of endotoxemia induced by CLP. Therefore, research focuses on the prevention or improvement of lung and kidney damage caused by sepsis [5, 21]. It is reported that acute lung and kidney damage were occured by the overproduction of reactive oxygen species (ROS), neutrophil accumulation and increased production of proinflammatory cytokines in kidney and lung [5, 22, 23]. ROS is thought to be an important defence mechanism for the protection of the organism against bacterial infections and the generation of free oxygen radicals is increased against sepsis. In some studies, malondialdehyde (MDA) levels, which are a marker of lipid peroxidation due to oxidative stress, were found to be high in the presence of sepsis [24, 25]. Previous studies have reported that lipid peroxidation is increased, especially in patients with sepsis, whereas antioxidant enzyme activities have decreased [26]. Oxidative stress, which results from the unbalance of ROS production and antioxidant enzymes. Increased oxidant levels in sepsis directly result in cellular damage by attacking biological molecules such as cellular proteins, lipids and nucleic acids. In this respect, treatment procedures with antioxidant agents are studied experimentally and clinically in sepsis. In several studies, it was demonstrated that MDA levels increased in CLP-induced sepsis and glutathione (GSH) and superoxide dismutase (SOD) activities decreased [5, 6, 27]. Myeloperoxidase (MPO) enzyme is a hemoprotein found in defensive cells such as neutrophils and monocytes. It shows indispensable properties in the formation of an inflammatory response by catalyzing the formation of ROS which is effective in microbial killing [28]. MPO activity and oxidant products are considered responsible for shaping many pathologies [29]. As accordance with the results of the previous scientific studies in our study, it was found that MDA level and MPO activities increased and SOD activity decreased in both lung and kidney tissues in CLP-induced sepsis model. The total antioxidant status (TAS) is known as a marker of total antioxidant capacity and is a total measure of the cumulative effect of all antioxidants, either by incorporation of all antioxidants, including in unexplored form and total oxidant status (TOS) reflects the total effect of all oxidants [30-32]. OSI value is obtained based on the ratio

of TOS level to the TAS level. In this study, it was determined that TAS level decreased due to sepsis and a significant increase in the TOS and OSI levels were observed in lung and kidney tissues. It was observed that these oxidant levels, which we evaluated in sepsis, decreased significantly with the fraxin treatment and antioxidant activities increased.
In conclusion, we think that fraxin as an antioxidant support therapy can be overcome in order to alleviate the damage of kidney and lung tissue in polymicrobial sepsis induced by CLP and may have a positive effect on sepsis treatment. We believe that this experimental study data will shed light on the wider experimental series and clinical prospective randomized human studies.

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Conflict of interest

There is no conflict of interest between authors.

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İki-Boyutlu Harmonik Konveks Fonksiyonlar ve İlgili Genelleştirilmiş Eşitsizlikler

Two-Dimensional Operator Harmonically Convex Functions and Related Generalized Inequalities

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Öz: Bu çalışmada, fonksiyonların harmonik konveksliği üzerine çalıştık. İlk olarak, reel sayı doğrusu üzerinde bu fonksiyonlar için bazı yeni genelleştirilmiş Hadamard tipi ve Ostrowski tipi eşitsizlikleri elde ettik. Ayrıca, harmonik konveks fonksiyonlar için iki-boyutlu operatörü kullanarak yukarıda söz edilen eşitsizlikleri genelleştirdik.

Anahtar Kelimeler — harmonik konveks fonksiyon, Hermite-Hadamard tipli eşitsizlikler, Ostrowski tipli eşitsizlikler.

Abstract: In this study, we studied on the harmonically convexity of functions. Firstly, we obtained some new generalized Hadamard's type and Ostrowski's type inequalities for these functions on the real number line. Besides, we generalized the above mentioned inequalities using two-dimensional operator for harmonically convex functions.

Keywords — harmonically convex function, Hermite-Hadamard type inequalities, Ostrowski's type inequalities.

1. Introduction and Preliminaries

It becomes famous the Hermite-Hadamard inequality for convex functions [1] in the recent years. Alomari [2] generalized this classical Hermite-Hadamard type inequality for every convex function and obtained the Ostrowski's type inequalitiy for every positive convex function on $[a,b]$. Recently, Dragomir [3] proved the classical Hermite-Hadamard type inequality for convex functions on the coordinates. Like as Alomari [2], Mwaeze [4] proved some generalizations inequalities of the above mentioned inequalities on the coordinates.

The Hermite-Hadamard type integral inequality for convex functions has received renewed attention in recent years and the remarkable varieties of refinements and generalizations have been found in [1]-[13]. In this context, the Hermite-Hadamard type inequalities for harmonically convex functions were obtained by many researchers in literature (see, [8]-[13]). Also, İşcan [8] showed definition of harmonically convex as follows:

Definition 1.1 ([8]). *Let $I \subset \mathbb{R} \setminus \{0\}$ be a real interval. A function $f: I \subset \mathbb{R} \setminus \{0\} \to \mathbb{R}$ is said to be harmonically convex, if*

$$f\left(\frac{xy}{\lambda x+(1-\lambda)y}\right)\leq \lambda f(y)+(1-\lambda)f(x)$$

for all $x,y\in I,\quad \lambda\in[0,1]$. *If the above inequality is reversed, then* f *is said to be harmonically concave.*

Proposition 1.1 ([8]). *Let* $f:I\subset\mathbb{R}\setminus\{0\}\to\mathbb{R}$ *be a function then*

i) if $I\subset(0,\infty)$ *and* f *is convex and nondecreasing function then* f *is harmonically convex,*

ii) if $I\subset(0,\infty)$ *and* f *is harmonically convex and nondecreasing function then* f *is convex,*

iii) if $I\subset(0,\infty)$ *and* f *is harmonically convex and nondecreasing function then* f *is convex,*

iv) if $I\subset(0,\infty)$ *and* f *is convex and nondecreasing function then* f *is harmonically convex.*

Theorem 1.1 ([8]). *Let* $f:I\subset\mathbb{R}\setminus\{0\}\to\mathbb{R}$ *be harmonically convex function and* $a,b\in I$ *with* $a<b$. *If* $f\in L[a,b]$ *then the following inequality holds*

$$f\left(\frac{2ab}{a+b}\right)\leq\frac{ab}{b-a}\int_a^b f(x)dx\leq\frac{f(a)+f(b)}{2}.$$

Now, Let us see definition of two-dimensional harmonically convex function by Noor et al. [9]. Consider $\Delta=[a,b]\times[c,d]\subset\mathbb{R}^2\setminus\{0\}$ with $a<b$ and $c<d$.

Definition 1.2 ([9]). *A function* $f:\Delta\to\mathbb{R}$ *is said to be a harmonically convex on* Δ, *if the following inequality holds*

$$f\left(\frac{ac}{\lambda a+(1-\lambda)c},\frac{bd}{\lambda b+(1-\lambda)d}\right)\leq(1-\lambda)f(a,b)+\lambda f(c,d)$$

for all $(a,b),(c,d)\in\Delta$ *and* $\lambda\in[0,1]$. *If the above inequality is reversed, then* f *is said to be a harmonically concave on* Δ.

Definition 1.3 ([9]). *A function* $f:\Delta\subset\mathbb{R}^2\setminus\{0\}\to\mathbb{R}$ *is said to be a harmonically convex on the co-ordinates if the partial mappings* $f_s:[a,b]\subset\mathbb{R}\setminus\{0\}\to\mathbb{R}, f_s(u):=f(u,s)$ *and* $f_t:[c,d]\subset\mathbb{R}\setminus\{0\}\to\mathbb{R}, f_t(v):=f(t,v)$ *defined for all* $t\in[a,b]$ *and* $s\in[c,d]$ *are harmonically convex.*

Theorem 1.2 ([9]). *Suppose that* $f:\Delta\subset\mathbb{R}^2\setminus\{0\}\to\mathbb{R}$ *is harmonically convex on the co-ordinates. Then the following inequality holds*

$$f\left(\frac{2ab}{a+b},\frac{2cd}{c+d}\right)\leq\frac{1}{2}\left[\frac{ab}{b-a}\int_a^b\frac{1}{t^2}f\left(t,\frac{2cd}{c+d}\right)dt+\frac{cd}{d-c}\int_c^d\frac{1}{s^2}f\left(\frac{2ab}{a+b},s\right)ds\right]$$

$$\leq\frac{abcd}{(b-a)(d-c)}\int_a^b\int_c^d\frac{1}{(ts)^2}f(t,s)dtds$$

$$\leq\frac{1}{4}\left[\frac{ab}{b-a}\int_a^b\frac{1}{t^2}\big(f(t,c)+f(t,d)\big)dt+\frac{cd}{d-c}\int_c^d\frac{1}{s^2}\big(f(a,s)+f(b,s)\big)ds\right]$$

$$\leq\frac{f(a,c)+f(a,d)+f(b,c)+f(b,d)}{4}.$$

Concordantly, in present study, we obtained some important generalized inequalities for harmonically convex functions on real number line and on the coordinates, respectively.

2. Main Results

This section contains two sub-sections. In the first sub-section we obtained the generalized Hadamard's type and Ostrowski's type inequalities for harmonically convex functions on the real number line. In the second sub-section we verified the above mentioned inequalities for harmonically convex functions on the coordinates.

2.1. Generalized Inequalities for Harmonically Convex Functions on the Real Line

Let us obtain the generalized Hadamard's type inequality for related functions.

Theorem 2.1. *Let* $f: I \subset \mathbb{R} \setminus \{0\} \to \mathbb{R}$ *be a harmonically convex function and* $f \in L[a,b]$. *Then the double inequality holds*

$$\frac{b-a}{nab}\sum_{k=1}^{n} f\left(\frac{2t_{k-1}t_k}{t_{k-1}+t_k}\right) \le \int_a^b \frac{f(t)}{t^2}dt \le \frac{b-a}{2nab}\left[f(a)+2\sum_{k=1}^{n-1} f(t_k)+f(b)\right] \tag{2.1}$$

where $t_k = a + k\frac{b-a}{n}$, $k = 1,2,\dots,n;\ n \in N$.

Proof. Using harmonically convexity of f on each sub-interval $[t_{k-1}, t_k] \subseteq [a,b]$, $k = 1,2,\dots,n$, then for all $\lambda \in [0,1]$

$$f\left(\frac{t_{k-1}t_k}{\lambda t_{k-1}+(1-\lambda)t_k}\right) \le \lambda f(t_k) + (1-\lambda)f(t_{k-1}). \tag{2.2}$$

Integrating (2.2) with respect to λ on $[0,1]$

$$\int_0^1 f\left(\frac{t_{k-1}t_k}{\lambda t_{k-1}+(1-\lambda)t_k}\right)d\lambda \le \frac{f(t_{k-1})+X(t_k)}{2}. \tag{2.3}$$

Changing of variable $t = \frac{t_{k-1}t_k}{\lambda t_{k-1}+(1-\lambda)t_k}$ in (2.3)

$$\int_{t_{k-1}}^{t_k} \frac{f(t)}{t^2}dt \le \frac{t_k - t_{k-1}}{2t_{k-1}t_k}\big(f(t_{k-1})+f(t_k)\big).$$

Taking the sum over k from 1 to n, we get

$$\sum_{k=1}^{n}\int_{t_{k-1}}^{t_k}\frac{f(t)}{t^2}dt = \int_a^b \frac{f(t)}{t^2}dt \le \sum_{k=1}^{n}\frac{t_k - t_{k-1}}{2t_{k-1}t_k}\big(f(t_{k-1})+f(t_k)\big).$$

$$\le \frac{1}{2}\max_k\left\{\frac{t_k - t_{k-1}}{t_{k-1}t_k}\right\}\sum_{k=1}^{n}\big(f(t_{k-1})+f(t_k)\big)$$

$$= \frac{b-a}{2nab}\left[f(t_0)+f(t_1)+\sum_{k=2}^{n-1}\big(f(t_{k-1})+f(t_k)\big)+f(t_{n-1})+f(t_n)\right]$$

$$= \frac{b-a}{2nab}\left[f(a) + 2\sum_{k=1}^{n-1} f(t_k) + f(b)\right]. \tag{2.4}$$

Because of harmonically convexity of f on $[t_{k-1}, t_k]$, then for $\lambda \in [0,1]$

$$f\left(\frac{2t_{k-1}t_k}{t_{k-1}+t_k}\right) \leq \frac{1}{2}\left[f\left(\frac{t_{k-1}t_k}{\lambda t_{k-1}+(1-\lambda)t_k}\right) + f\left(\frac{t_{k-1}t_k}{\lambda t_{k-1}+(1-\lambda)t_k}\right)\right]$$

$$\leq \frac{1}{2}[f(\lambda t_k + (1-\lambda)t_{k-1}) + f((1-\lambda)t_k + \lambda t_{k-1})]. \tag{2.5}$$

Applying on (2.5) by using similar way in (2.2)-(2.4)

$$\frac{b-a}{nab}\sum_{k=1}^{n} f\left(\frac{2t_{k-1}t_k}{t_{k-1}+t_k}\right) \leq \int_a^b \frac{f(t)}{t^2}dt. \tag{2.6}$$

From (2.4) and (2.6), we have (2.1).

Let us give firstly the generalized Ostrowki's type inequality for related functions

Theorem 2.2. *Let $f: I \subset \mathbb{R}\setminus\{0\} \to \mathbb{R}_+$ be a positive harmonically convex function and $f \in L[a,b]$. Then the inequality holds*

$$\int_a^b \frac{f(t)}{t^2}dt - \left(\frac{b-a}{2ab}\right)f(s) \leq \frac{b-a}{2nab}\left[f(a) + 2\sum_{k=1}^{n-1} f(t_k) + f(b)\right] \tag{2.7}$$

for all $s \in [a,b]$, where $t_k = a + k\frac{b-a}{n}$, $k = 1,2,\dots,n$; $n \in N$.

Proof. Fix $s \in [t_{k-1}, t_k]$, $k = 1,2,\dots,n$. Since f is harmonically convex on $[a,b]$, then f so is harmonically convex on each subinterval $[t_{k-1}, t_k]$, in particular on $[t_{k-1}, s]$, then

$$f\left(\frac{t_{k-1}s}{\lambda t_{k-1}+(1-\lambda)s}\right) \leq \lambda f(s) + (1-\lambda)f(t_{k-1}), k = 1,2,\dots,n \tag{2.8}$$

Integrating (2.8) with respect to λ on $[0,1]$ we get

$$\int_0^1 f\left(\frac{t_{k-1}s}{\lambda t_{k-1}+(1-\lambda)s}\right)d\lambda \leq \frac{f(t_{k-1})+f(s)}{2}. \tag{2.9}$$

Changing of variable $t = \frac{t_{k-1}s}{\lambda t_{k-1}+(1-\lambda)s}$ in (2.9), then

$$\int_{t_{k-1}}^{s} \frac{f(t)}{t^2}dt \leq \frac{s-t_{k-1}}{2t_{k-1}s}\big(f(t_{k-1}) + f(s)\big). \tag{2.10}$$

Similarly for $[s, t_k]$

$$\int_s^{t_k} \frac{f(t)}{t^2}dt \leq \frac{t_k - s}{2t_{k-1}s}\big(f(s) + f(t_k)\big). \tag{2.11}$$

Adding the inequalities (2.10) and (2.11)

$$\int_{t_{k-1}}^{s}\frac{f(t)}{t^2}dt+\int_{s}^{t_k}\frac{f(t)}{t^2}dt=\int_{t_{k-1}}^{t_k}\frac{f(t)}{t^2}dt$$

$$\leq\frac{s-t_{k-1}}{2t_{k-1}s}\big(f(t_{k-1})+f(s)\big)+\frac{t_k-s}{2t_ks}\big(f(s)+f(t_k)\big)$$

$$\leq\frac{t_k-t_{k-1}}{2t_{k-1}t_k}\{f(t_{k-1})+f(t_k)\}+\frac{b-a}{2ab}f(s). \tag{2.12}$$

Taking the sum over k from 1 to n, we get

$$\sum_{k=1}^{n}\int_{t_{k-1}}^{t_k}\frac{f(t)}{t^2}dt=\int_{a}^{b}\frac{f(t)}{t^2}dt\leq\sum_{k=1}^{n}\frac{t_k-t_{k-1}}{2t_{k-1}t_k}\big(f(t_{k-1})+f(t_k)\big)+\sum_{k=1}^{n}\frac{b-a}{2ab}f(s).$$

$$\leq\frac{1}{2}\max_k\left\{\frac{t_k-t_{k-1}}{t_{k-1}t_k}\right\}\sum_{k=1}^{n}\big(f(t_{k-1})+f(t_k)\big)+\frac{b-a}{2ab}f(s)$$

$$=\frac{b-a}{2nab}\left[f(t_0)+f(t_1)+\sum_{k=2}^{n-1}\big(f(t_{k-1})+f(t_k)\big)+f(t_{n-1})+f(t_n)\right]+\frac{b-a}{2ab}f(s)$$

$$=\frac{b-a}{2nab}\left[f(a)+2\sum_{k=1}^{n-1}f(t_k)+f(b)\right]+\frac{b-a}{2ab}f(s).$$

Remark 2.2. In Theorem 2.1 for $n=1$, then the clasicall Hermite-Hadamard inequality for harmonically convex functions is obtained.

2.2. Generalized Inequalities for Two-dimensional Harmonically Convex Functions

Throughout this section, consider $\Delta:=[a,b]\times[c,d]\subset\mathbb{R}^2\setminus\{0\}$ with $a<b$ and $c<d$. Let us obtain the generalized Hadamard's inequality for related functions:

Theorem 2.3. *Let $f:\Delta\subset\mathbb{R}^2\setminus\{0\}\to\mathbb{R}$ be harmonically convex on the coordinates on Δ. The following inequality satisfies*

$$\frac{d-c}{2ncd}\sum_{k=1}^{n}\int_{a}^{b}\frac{\left(t,\frac{2s_ks_{k-1}}{s_{k-1}+s_k}\right)}{t^2}dt+\frac{b-a}{2nab}\sum_{k=1}^{n}\int_{c}^{d}\frac{\left(\frac{2t_{k-1}t_k}{t_{k-1}+t_k},s\right)}{s^2}ds$$

$$\leq\frac{abcd}{(b-a)(d-c)}\int_{a}^{b}\int_{c}^{d}\frac{f(t,s)}{(ts)^2}dtds$$

$$\leq\frac{d-c}{4ncd}\int_{a}^{b}\frac{[f(t,c)+(t,d)]}{t^2}dt+\frac{b-a}{4nab}\int_{c}^{d}\frac{[(a,s)+f(b,s)]}{s^2}ds$$

$$+\frac{d-c}{2ncd}\sum_{k=1}^{n-1}\int_a^b \frac{f(t,s_k)}{t^2}dt+\frac{b-a}{2nab}\sum_{k=1}^{n-1}\int_c^d \frac{f(t_k,s)}{s^2}ds\,, \tag{2.13}$$

where $t_k = a + k\frac{b-a}{n}$, $s_k = c + k\frac{d-c}{n}$, $k = 1,2,\dots,n;\ n \in N$.

Proof. Using Theorem 2.1 for $f_t: [c,d] \subset \mathbb{R}\setminus\{0\} \to \mathbb{R}$, $f_t(s) = f\big((t,s)\big)$

$$\frac{d-c}{ncd}\sum_{k=1}^{n} f_t\left(\frac{2s_k s_{k-1}}{s_{k-1}+s_k}\right) \le \int_c^d \frac{f_t(s)}{s^2}ds \le \frac{d-c}{2ncd}\left[f_t(c)+2\sum_{k=1}^{n-1} f_t\,(s_k)+f_t(d)\right].$$

Thus

$$\frac{d-c}{ncd}\sum_{k=1}^{n} f\left(t,\frac{2s_k s_{k-1}}{s_{k-1}+s_k}\right) \le \int_c^d \frac{f(t,s)}{s^2}ds$$

$$\le \frac{d-c}{2ncd}\left[f(t,c)+f(t,d)+2\sum_{k=1}^{n-1} f\,(t,s_k)\right]. \tag{2.14}$$

Integrating all sides of (2.14) on $[a,b]$ and multiplying $\frac{1}{t^2}$, we have

$$\frac{d-c}{ncd}\sum_{k=1}^{n}\int_a^b \frac{f\left(t,\frac{2s_k s_{k-1}}{s_{k-1}+s_k}\right)}{t^2}dt \le \int_a^b\int_c^d \frac{f(t,s)}{(ts)^2}dtds$$

$$\le \frac{d-c}{2ncd}\left[\int_a^b \frac{f(t,c)}{t^2}dt+\int_a^b \frac{f(t,d)}{t^2}dt+2\sum_{k=1}^{n-1}\int_a^b \frac{f(t,s_k)}{t^2}dt\right]. \tag{2.15}$$

Similarly

$$\frac{b-a}{nab}\sum_{k=1}^{n}\int_c^d \frac{f\left(\frac{2t_{k-1}t_k}{t_{k-1}+t_k},s\right)}{s^2}ds \le \int_a^b\int_c^d \frac{f(t,s)}{(ts)^2}dtds$$

$$\le \frac{b-a}{2nab}\left[\int_c^d \frac{f(u_1,s)}{s^2}ds+\int_c^d \frac{f(u_2,s)}{s^2}ds+2\sum_{k=1}^{n-1}\int_c^d \frac{f(t_k,s)}{s^2}ds\right]. \tag{2.16}$$

Adding (2.15) and (2.16), one gets (2.13). That completes the proof.

Remark 2.3. *Under the assumptions of Theorem 2.3, the classical Hermite-Hadamard inequality for harmonically convex functions is obtained on the coordinates and we have the following inequality*

$$\sum_{k=1}^{n} f\left(\frac{2ab}{a+b},\frac{2s_k s_{k-1}}{s_{k-1}+s_k}\right)+\sum_{k=1}^{n} f\left(\frac{2t_{k-1}t_k}{t_{k-1}+t_k},\frac{2cd}{c+d}\right)$$

$$\leq \frac{ncd}{d-c}\int_c^d \frac{f\left(\frac{2ab}{a+b},s\right)}{s^2}ds + \frac{nab}{b-a}\int_a^b \frac{f\left(t,\frac{2cd}{c+d}\right)}{t^2}dt;$$

$$\frac{ncd}{d-c}\int_c^d \frac{[f(a,s)+f(b,s)]}{s^2}ds + \frac{nab}{b-a}\int_a^b \frac{[f(t,c)+f(t,d)]}{t^2}dt$$

$$\leq f(a,c)+f(a,d)+f(b,c)+f(b,d)+\sum_{k=1}^{n-1}[f(a,s_k)+f(b,s_k)+f(t_k,c)+f(t_k,d)].$$

Let us prove the generalized Ostrowki's type inequality for related functions.

Theorem 2.4. *Let* $f:\Delta \subset \mathbb{R}^2 \setminus \{0\} \to \mathbb{R}_+$ *be harmonically* convex *on the coordinates. Then the following inequality holds*

$$\int_a^b\int_c^d \frac{f(t,s)}{(ts)^2}dtds$$

$$\leq \frac{b-a}{4nab}\left[(n+1)\int_c^d \frac{f(a,s)}{s^2}ds + +2\sum_{k=1}^{n-1}\int_c^d \frac{f(t_k,s)}{s^2}ds\,(n+1)\int_c^d \frac{f(b,s)}{s^2}ds\right]$$

$$+\frac{d-c}{4ncd}\left[(n+1)\int_a^b \frac{f(t,c)}{t^2}dt + +2\sum_{k=1}^{n-1}\int_a^b \frac{f(t,s_k)}{t^2}dt\,(n+1)\int_a^b \frac{f(t,d)}{t^2}dt\right],$$

where t_k *and* s_k *are defined as in Theorem 2.3.*

Proof. Using Theorem 2.2 for $f_s:[a,b]\subset\mathbb{R}\setminus\{0\}\to\mathbb{R}$, $f_s(t)=f((t,s))$ at $t=b$,

$$\int_a^b \frac{f_s(t)}{t^2}dt - \left(\frac{b-a}{2ab}\right)f_s(b) \leq \frac{b-a}{2nab}\left[f_s(a)+2\sum_{k=1}^{n-1}f_s(t_k)+f_s(b)\right].$$

Thus

$$\int_a^b \frac{f(t,s)}{t^2}dt - \left(\frac{b-a}{2ab}\right)f(b,s) \leq \frac{b-a}{2nab}\left[f(a,s)+2\sum_{k=1}^{n-1}f(t_k,s)+f(b,s)\right]. \quad (2.17)$$

Integrating all sides of (2.17) on $[c,d]$ and multiplying $\frac{1}{s^2}$, we get

$$\int_a^b\int_c^d \frac{f(t,s)}{(ts)^2}dtds$$

$$\leq \frac{b-a}{2nab}\left[\int_c^d \frac{f(a,s)}{s^2}ds + (1+2n)\int_c^d \frac{f(b,s)}{s^2}ds + 2\sum_{k=1}^{n-1}\int_c^d \frac{f(t_k,s)}{s^2}ds\right]. \quad (2.18)$$

Using Theorem 2.2 for the mapping f_s at $t=a$ and integrating over $[c,d]$

$$\int_a^b \int_c^d \frac{f(t,s)}{(ts)^2} dtds$$

$$\leq \frac{b-a}{2nab}\left[(1+2n)\int_c^d \frac{f(a,s)}{s^2} ds + \int_c^d \frac{f(b,s)}{s^2} ds + 2\sum_{k=1}^{n-1}\int_c^d \frac{f(t_k,s)}{s^2} ds\right] \quad (2.19)$$

Using (2.18) and (2.19)

$$\int_a^b \int_c^d \frac{f(t,s)}{(ts)^2} dtds \leq \frac{b-a}{2nab}\left[\begin{array}{c}(n+1)\int_c^d \frac{f(a,s)}{s^2} ds + (n+1)\int_c^d \frac{f(b,s)}{s^2} ds \\ +2\sum_{k=1}^{n-1}\int_c^d \frac{f(t_k,s)}{s^2} ds\end{array}\right]. \quad (2.20)$$

Similarly

$$\int_a^b \int_c^d \frac{f(t,s)}{(ts)^2} dtds \leq \frac{d-c}{2ncd}\left[\begin{array}{c}(n+1)\int_a^b \frac{f(t,c)}{t^2} dt + (n+1)\int_a^b \frac{f(t,d)}{t^2} dt \\ +2\sum_{k=1}^{n-1}\int_{u_1}^{u_2} \frac{f(t,s_k)}{t^2} dt\end{array}\right]. \quad (2.21)$$

The desired inequality is obtained by adding (2.20) and (2.21).

Remark 2.4. *Under the assumptions of Theorem 2.4, then*

$$\int_a^b \int_c^d \frac{f(t,s)}{(ts)^2} dtds \leq \frac{d-c}{2cd}\int_a^b \frac{[f(t,c)+f(t,d)]}{t^2} dt + \frac{b-a}{2ab}\int_c^d \frac{[f(a,s)+f(b,s)]}{s^2} ds;$$

$$\int_a^b \int_c^d \frac{f(t,s)}{(ts)^2} dtds \leq \frac{d-c}{8cd}\int_a^b \frac{\left[3f(t,c)+2f\left(t,\frac{2cd}{c+d}\right)+3f(t,d)\right]}{t^2} dt$$

$$+\frac{b-a}{8ab}\int_c^d \frac{\left[3f(a,s)+2f\left(\frac{2ab}{a+b},s\right)+3f(b,s)\right]}{s^2} ds.$$

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4. Conclusion

In this paper, we obtained important generalized inequalities for harmonically functions on the real number line and on the coordinates. One can verify some generalized inequalities for various classes of convex functions.

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İkinci Mertebeden Kompleks Aileler için Ortak Diyagonal Çözümler

Common Diagonal Solutions for Second Order Complex Family

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Öz: Bu çalışmada ikinci mertebeden kompleks aralık matris aileleri için Lyapunov eşitsizliğinin ortak diyagonal çözümlerinin varlığı ve değerlendirilmesi problemi ele alınmıştır. Ele alınan probleme dair gerek ve yeter koşullar verilmiştir. Ayrıca bilinen yeterli koşulların ortak çözümleri vermediği durumlar için örnekler verilmiştir.
Anahtar Kelimeler — Kompleks aralık matris; Lyapunov eşitsizliği; Diyagonal kararlılık; Ortak Diyagonal çözüm.

Abstract: In this paper for second order complex interval matrix family we consider the problem of existence and evaluation of common diagonal solutions for the Lyapunov matrix inequality. Necessary and sufficient conditions are given. Numbers of examples are given, where known sufficient conditions do not give common solutions.
Keywords — Complex Interval Matrices; Lyapunov Inequality; Diagonal Stability; Common Diagonal Solution

1. Introduction

Let a 2×2 complex interval matrix family

$$\mathsf{A}=\left\{\begin{bmatrix} a_1+j\widetilde{a}_1 & a_2+j\widetilde{a}_2 \\ a_3+j\widetilde{a}_3 & a_4+j\widetilde{a}_4 \end{bmatrix}: a_i^- \le a_i \le a_i^+, \widetilde{a}_i^- \le \widetilde{a}_i \le \widetilde{a}_i^+ \ i=1,...,4\right\} \tag{1}$$

be given. Define the following 8-dimensional box

$$Q=[a_1^-,a_1^+]\times\cdots\times[a_4^-,a_4^+]\times[\widetilde{a}_1^-,\widetilde{a}_1^+]\times\cdots\times[\widetilde{a}_4^-,\widetilde{a}_4^+] \tag{2}$$

For $\lambda_1>0$, $\lambda_2>0$ define positive definite diagonal matrix $D=\mathrm{diag}(\lambda_1,\lambda_2)$.

Definition 1. If there exists positive diagonal D such that for all $A\in\mathsf{A}$

$$A^*D+DA<0, \tag{3}$$

where A^* is the conjugate transpose of A, that is $A^*=(\overline{A})^T$, the matrix D is called common diagonal solution to the Lyapunov matrix inequality (3). In the above the symbol "$<$" stands for the negative definiteness.

Diagonal stability problems of dynamical systems have many applications in economics, large scale systems, neural networks, see [1] and the references therein. The existence problems of the diagonal type solutions for different systems are considered in [1-16]. Among them it should be noted the works [1, 2, 7, 9, 13-16], more related to our present work. The monograph [1] presents a collection of results, observations on the results on diagonal stability and diagonal type Lyapunov functions. [2] gives sufficient conditions for the common diagonal stability of real interval matrices. [7] contains simple criterions of diagonal stability for second and third order matrices. In [9] an algorithm is presented for the checking of diagonal stability of a single real matrix. The algorithm is iterative and requires solving a linear programming problem at each step. In [13] a necessary and sufficient condition for the existence of a common diagonal solution of a pair of positive matrices is given.

In [14] the same problem has been solved for second and third order real interval matrix families. [14] contains a sufficient condition in the general case as well. For general complex interval families the same problem is studied in [15]. For third order real matrix polytopes the common diagonal solution problem is considered in [16]. For the general case the problem is solved by the cutting-plane algorithm. In the above mentioned works criterions for the existence and evaluation of common diagonal solutions for second order complex interval families did not considered. Our present work aims to fill this gap.

In [15], two sufficient conditions for common diagonal stability of complex interval systems are given. The first sufficient condition is stated as follows. Given $n \times n$ complex interval family $\mathcal{B} = [a_{ij}] + j[\tilde{a}_{ij}]$, where $a_{ij}^- \le a_{ij} \le a_{ij}^+$, $\tilde{a}_{ij}^- \le \tilde{a}_{ij} \le \tilde{a}_{ij}^+$ $(i,j = 1,2,\dots,n)$ define the real matrix $U = [u_{ij}]$, where

$$u_{ij} = \begin{cases} a_{ii}^+, & \text{if } i = j \\ \max\{|a_{ij}^- + j\tilde{a}_{ij}^-|, |a_{ij}^- + j\tilde{a}_{ij}^+|, |a_{ij}^+ + j\tilde{a}_{ij}^-|, |a_{ij}^+ + j\tilde{a}_{ij}^+|\}, & \text{if } i \neq j. \end{cases} \tag{4}$$

Theorem 1 ([15]). *The family $\mathcal{B}$ has a common diagonal solution if $\rho^H(U) < 0$, where $\rho^H(U) = \max\{\mathrm{Re}(\lambda_i(U)) : i = 1,2,\dots,n\}$.*

The formulation of the second theorem is cumbersome, therefore we do not give here its formulation. As noted in [15] the second theorem cannot be applied if

$$\rho^H\left(\begin{bmatrix} A_0 & \tilde{A}_0 \\ -\tilde{A}_0 & A_0 \end{bmatrix}\right) + \rho^H\left(\begin{bmatrix} R_A & R_{\tilde{A}} \\ R_{\tilde{A}} & R_A \end{bmatrix}\right) \ge 0, \tag{5}$$

where $A_0 = \left(\frac{a_{ij}^- + a_{ij}^+}{2}\right)$, $\tilde{A}_0 = \left(\frac{\tilde{a}_{ij}^- + \tilde{a}_{ij}^+}{2}\right)$, $R_A = \left(\frac{a_{ij}^+ - a_{ij}^-}{2}\right)$, $R_{\tilde{A}} = \left(\frac{\tilde{a}_{ij}^+ - \tilde{a}_{ij}^-}{2}\right)$.

In this paper for the family (1), we consider the problem of existence and evaluation of common diagonal solutions of Lyapunov inequality (3). We give necessary and sufficient conditions for the existence of common diagonal solutions. Number of examples are given, where common diagonal solutions are evaluated, whereas known results do not give such solutions.

The paper is organized as follows. In Section 2 we give a necessary and sufficient condition for robust diagonal stability for the second order family (1). In this stability each member is diagonally stable and each member has own diagonal solution for the Lyapunov matrix inequality (3). In Section 3 we give necessary and sufficient conditions for the existence of common diagonal solutions to the inequality (3). The cases $0 \notin [a_2^-, a_2^+] \cap [\tilde{a}_2^-, \tilde{a}_2^+]$ and $0 \in [a_2^-, a_2^+] \cap [\tilde{a}_2^-, \tilde{a}_2^+]$ are considered seperately. Number of examples are given. In Example 1 the familiy is robust diagonally stable, but there is no a common diagonal solution. In Examples 2 and 3 there are common diagonal solutions, which are obtained from Theorems 3 and 4, whereas known sufficient conditions from [15] do not give common solutions.

2. Robust diagonal stability

A necessary condition for the existence of a common diagonal solution is the robust diagonal stability. In this section we give a necessary and sufficient condition for the robust diagonal stability of the family $\mathcal{A}$.

Recall that, the family $\mathcal{A}$ is said to be robust diagonally stable if for every $A \in \mathcal{A}$ (equivalently for all $a = (a_1,\dots,a_4,\tilde{a}_1,\dots,\tilde{a}_4) \in Q$) there exists positive diagonal D such that

$$A^*D + DA < 0.$$

Proposition 2. *The family $\mathcal{A}$ (1) is robust diagonally stable if and only if*

$$a_1^+ < 0, \qquad a_4^+ < 0, \qquad \min_{a \in Q}(F^2 - 4EG) > 0, \qquad \min_{a \in Q} F > 0, \tag{6}$$

where

$$F = 4a_1a_4 - 2a_2a_3 + 2\tilde{a}_2\tilde{a}_3, \;\; E = a_2^2 + \tilde{a}_2^2, \;\; G = a_3^2 + \tilde{a}_3^2. \tag{7}$$

Proof. Without loss of generality all 2×2 positive diagonal matrices D may be normalized to have the form $D=\operatorname{diag}(t,1)$, where $t>0$. For $A\in\mathcal{A}$,

$$A=\begin{bmatrix} a_1+j\tilde{a}_1 & a_2+j\tilde{a}_2 \\ a_3+j\tilde{a}_3 & a_4+j\tilde{a}_4 \end{bmatrix},\quad D=\begin{bmatrix} t & 0 \\ 0 & 1 \end{bmatrix}$$

the matrix inequality $A^*D+DA<0$ becomes

$$A^*D+DA=\begin{bmatrix} 2a_1t & (a_2t+a_3)+j(\tilde{a}_2t-\tilde{a}_3) \\ (a_2t+a_3)-j(\tilde{a}_2t-\tilde{a}_3) & 2a_4 \end{bmatrix}<0$$

or

$$2a_1t<0,\qquad 2a_4<0\qquad\text{and}\qquad \det(A^*D+DA)=-Et^2+Ft-G>0, \tag{8}$$

where F, E and G are defined by (7).

We are looking for conditions under which for each $a\in Q$ there exists $t>0$ such that all three inequalities in (8) are satisfied. The first and the second are satisfied if and only if

$$a_1^+<0,\qquad a_4^+<0.$$

It is possible two cases.

Case 1. $0\notin[a_2^-,a_2^+]$ or $0\notin[\tilde{a}_2^-,\tilde{a}_2^+]$.

In this case $E>0$ for all $a\in Q$. The existence of $t>0$ such that $Et^2-Ft+G<0$ is equivalent to the following inequalities

$$\Delta=F^2-4EG>0,\qquad \frac{F+\sqrt{\Delta}}{2E}>0 \tag{9}$$

for all $a\in Q$. The second inequality is equivalent to

$$\min_{a\in Q}F>0. \tag{10}$$

Indeed, if (10) is satisfied the second inequality in (9) is true. Conversely, if $F+\sqrt{\Delta}>0$ then from $F^2\geq F^2-4EG$ we have $|F|\geq\sqrt{\Delta}\Leftrightarrow F\leq-\sqrt{\Delta}$ or $F\geq\sqrt{\Delta}$. The inequality $F\leq-\sqrt{\Delta}$ is impossible due to $F+\sqrt{\Delta}>0$. The second $F\geq\sqrt{\Delta}$ gives $F>0$ for all $a\in Q$, that is (10) is satisfied. Consequently (6) is true.

Case 2. $0\in[a_2^-,a_2^+]$ and $0\in[\tilde{a}_2^-,\tilde{a}_2^+]$.

In this case the inequality $E>0$ is not true. Define

$$Q_1=\{a\in Q:E(a)>0\},\qquad Q_2=\{a\in Q:E(a)=0\}. \tag{11}$$

Obviously, $Q_2=\{a\in Q:a_2=\tilde{a}_2=0\}$.

If $a\in Q_1$, the statement that for all $a\in Q_1$ there exists $t>0$ such that $Et^2-Ft+G<0$ is equivalent to (see Case 1)

$$\min_{a\in Q_1}(F^2-4EG)>0,\qquad \min_{a\in Q_1}F>0.$$

Additionally, $\min\limits_{a\in Q_2}(F^2-4EG)=\min\limits_{a\in Q_2}F^2=(4a_1^+a_4^+)^2>0$, $\min\limits_{a\in Q_2}F=4a_1^+a_4^+>0$.

If $a\in Q_2$, the statement that for all $a\in Q_2$ there exists $t>0$ such that $Et^2-Ft+G=-Ft+G<0$ is satisfied automatically since $F=4a_1a_4>0$, $G\geq 0$.

In summary the conditions (6) are necessary and sufficient for robust diagonal stability.

□

3. Common diagonal solutions

In this section we give necessary and sufficient conditions for the existence of common diagonal solutions.

Theorem 3. *Let the interval family $\mathcal{A}$ (1) be given and the inequalities (6) are satisfied. Assume that $0 \notin [a_2^-, a_2^+]$ or $0 \notin [\tilde{a}_2^-, \tilde{a}_2^+]$. There exists a common diagonal solution to the Lyapunov inequalities if and only if the following inequality is satisfied*

$$\alpha := \max_{a\in Q} x_1(a) < \beta := \min_{a\in Q} x_2(a) \tag{12}$$

where

$$x_1(a) = \frac{F - \sqrt{F^2 - 4EG}}{2E}, \qquad x_2(a) = \frac{F + \sqrt{F^2 - 4EG}}{2E}. \tag{13}$$

If (12) is satisfied, for every $t \in (\alpha, \beta)$ the matrix $D = \operatorname{diag}(t, 1)$ is a common diagonal solution.

Proof. Note that under the hypothesis of the theorem $E > 0$ for all $a \in Q$.

$\Rightarrow$: Assume that there exists a common $D = \operatorname{diag}(t_*, 1)$ $(t_* > 0)$, it means that there exists $t_* > 0$ such that for any $a \in Q$

$$A^*D + DA = \begin{bmatrix} 2a_1t_* & (a_2t_* + a_3) + j(\tilde{a}_2t_* - \tilde{a}_3) \\ (a_2t_* + a_3) - j(\tilde{a}_2t_* - \tilde{a}_3) & 2a_4 \end{bmatrix} < 0.$$

Then

$$a_1^+ < 0, \qquad a_4^+ < 0, \qquad Et_*^2 - Ft_* + G < 0$$

and for all $a \in Q$

$$x_1(a) < t_* < x_2(a).$$

where $x_1(a)$ and $x_2(a)$ defined by (13). Consequently, $\max\limits_{a\in Q} x_1(a) < t_* < \min\limits_{a\in Q} x_2(a)$ and (12) is satisfied.

$\Leftarrow$: Assume that (12) is satisfied and $t_* \in (\alpha, \beta)$. Then for all $a \in Q$ we have $x_1(a) < t_* < x_2(a)$ or $Et_*^2 - Ft_* + G < 0$. Consequently $D = \operatorname{diag}(t_*, 1)$ is a common solution.

□

Example 1. *Consider the following family*

$$\mathcal{A} = \begin{bmatrix} [-2,-1] + j\,[1,2] & [3,4] + j\,[1,2] \\ [-5,-1] + j\,[0.5,1] & [-2,-1.5] + j\,[1,2] \end{bmatrix}. \tag{14}$$

This family is robust diagonally stable. Indeed,

$$\begin{aligned} &a_1^+ = -1 < 0,\ a_4^+ = -1.5 < 0, \\ &\min_a(F^2 - 4EG) = 119 > 0, \qquad \min_a F = 13 > 0. \end{aligned}$$

By Proposition 2, the family (14) is robust diagonally stable. On the other hand there is no a common diagonal solution, since

$$\alpha := \max_a x_1(a) = 1.0213 > \beta := \min_a x_2(a) = 0.7122$$

and by Theorem 3 there is no a common solution.

Example 2. *Consider the family*

$$\begin{bmatrix} [-10.875, 0.865] + j\,[0.8, 0.9] & [0.1, 0.2] + 0.1\,j \\ [0.5, 0.501] + j\,[0.3, 0.4] & [-0.265, -0.165] + j\,[0.1, 0.3] \end{bmatrix}$$

This family is robust diagonally stable by Proposition 2, since $a_1^+ = -0.865 < 0$, $a_4^+ = -0.165 < 0$, $\min_a(F^2 - 4EG) = 0.31636 > 0$, $\min_a F = 0.5909 > 0$. The numbers α and β from (12) are $\alpha = 0.71249$,

$\beta = 13.196629$. *By Theorem 3, for every* $t \in (0.71249, 13.196629)$ *the matrix* $D = \mathrm{diag}(t, 1)$ *is a common solution. Note that for this example the sufficient conditions from [15] are not satisfied.*

Indeed, (see (4))

$$U = \begin{bmatrix} -0.865 & 0.223606 \\ 0.641093 & -0.165 \end{bmatrix}, \quad \rho^H(U) = \max_i \mathrm{Re}\lambda_i(U) = 0.000609 > 0$$

and Theorem 1 fails. The second sufficient condition does not work as well, since (see (5))

$$\rho^H\left(\begin{bmatrix} A_0 & \tilde{A}_0 \\ -\tilde{A}_0 & A_0 \end{bmatrix}\right) + \rho^H\left(\begin{bmatrix} R_A & R_{\tilde{A}} \\ R_{\tilde{A}} & R_A \end{bmatrix}\right) = 4.845511 > 0.$$

Theorem 4. *Let the family* $\mathcal{A}$ *(1) be given, the inequalities (6) are satisfied and* $0 \in [a_2^-, a_2^+]$ *and* $0 \in [\tilde{a}_2^-, \tilde{a}_2^+]$. *Define*

$$\tilde{x}_1(a) = \frac{2G}{F + \sqrt{\Delta}}, \qquad \tilde{x}_2(a) = \begin{cases} \dfrac{F + \sqrt{\Delta}}{2E} & , \quad \text{if } E > 0 \\ \infty & , \quad \text{if } E = 0 \end{cases}. \tag{15}$$

Then there exists a common diagonal solution if and only if

$$\tilde{\alpha} := \max_{a \in Q} \tilde{x}_1(a) < \tilde{\beta} := \inf_{a \in Q} \tilde{x}_2(a). \tag{16}$$

If (16) is satisfied, for every $t \in (\tilde{\alpha}, \tilde{\beta})$ *the matrix* $D = \mathrm{diag}(t, 1)$ *is a common solution.*

Proof. $\Rightarrow$: Assume that there exists a common diagonal solution and $\mathrm{diag} = (t_*, 1)$ is a such solution. Then for any $a \in Q$

$$Et_*^2 - Ft_* + Q < 0. \tag{17}$$

If $a \in Q_1$, then $\dfrac{F - \sqrt{\Delta}}{2E} = \dfrac{2G}{F + \sqrt{\Delta}} < t_* < \dfrac{F + \sqrt{\Delta}}{2E}$,

If $a \in Q_2$, then $E(a) = 0$ and $\dfrac{G}{F} = \dfrac{2G}{F + \sqrt{\Delta}} < t_* < \infty$. Recall that the sets Q_1 and Q_2 are defined by (11).

Consequently, for any $a \in Q$

$$\tilde{x}_1(a) < t_* < \tilde{x}_2(a),$$

where $\tilde{x}_1(a)$ and $\tilde{x}_2(a)$ are defined by (15).

The function $\tilde{x}_1(a)$ is continuous in $a \in Q$. Therefore

$$\max_{a \in Q} \tilde{x}_1(a) < t_* \le \inf_{a \in Q} \tilde{x}_2(a),$$

and (16) is satisfied.

$\Leftarrow$: Conversely, assume that (16) is satisfied and $t_* \in (\tilde{\alpha}, \tilde{\beta})$. Then for all $a \in Q$ we have $\tilde{x}_1(a) < t_* < \tilde{x}_2(a)$ or

$$\frac{2G}{F + \sqrt{\Delta}} < t_* < \tilde{x}_2(a). \tag{18}$$

If $a \in Q_1$, then $E > 0$ and from (18) we obtain

$$\frac{F - \sqrt{\Delta}}{2E} < t_* < \frac{F + \sqrt{\Delta}}{2E} \quad \text{or} \quad Et_*^2 - Ft_* + G < 0.$$

If $a \in Q_2$, then $E = 0$ and (18) gives

$$\frac{2G}{F + \sqrt{\Delta}} = \frac{G}{F} < t_* < \infty,$$

or $Et_*^2 - Ft_* + G = -Ft_* + G < 0$. Consequently for all $a \in Q$

$$Et_*^2 - Ft_* + Q < 0$$

and $\mathrm{diag}(t_*, 1)$ is a common solution. □

Example 3. *Consider the following family*

$$\mathcal{A} = \begin{bmatrix} [-3,-2] + j\,[1,2] & [-1,2] + j\,[-3,1] \\ [-2,-1] + j\,[0.5,1] & [-5,-4] + j\,[1,2] \end{bmatrix}.$$

Here $0 \in [a_2^-, a_2^+] = [-1,2]$, $0 \in [\tilde{a}_2^-, \tilde{a}_2^+] = [-3,1]$ *and the family is robust diagonally stable, since* $a_1^+ = -2 < 0$, $a_4^+ = -4 < 0$ *and* $\min_a(F^2 - 4EG) = 284 > 0$, $\min_a F = 22 > 0$. *Define* $\tilde{x}_1(a)$ *and* $\tilde{x}_2(a)$ *as in Theorem 4. Then*

$$\tilde{\alpha} = \max_{a\in Q} \tilde{x}_1(a) = 0.257385,$$
$$\tilde{\beta} = \inf_{a\in Q} \tilde{x}_2(a) = 2.238979$$

and $\tilde{\alpha} < \tilde{\beta}$ *and by Theorem 4 for any* $t \in (\tilde{\alpha}, \tilde{\beta})$ *the matrix* $D = \mathrm{diag}(t, 1)$ *is common solution.*

In this example, both sufficient conditions from [15] are not satisfied. The matrix U *from Theorem 1 is* $U = \begin{bmatrix} -2 & \sqrt{13} \\ \sqrt{5} & -4 \end{bmatrix}$ *with* $\rho^H(U) = \max\{\mathrm{Re}(\lambda_i(U)) : \ i = 1,2\} = 0.010358 > 0$. *The inequality (5) is satisfied as well, indeed*

$$A_0 = \begin{bmatrix} -\frac{5}{2} & \frac{1}{2} \\ -\frac{3}{2} & -\frac{9}{2} \end{bmatrix}, \qquad \tilde{A}_0 = \begin{bmatrix} \frac{3}{2} & -1 \\ \frac{3}{4} & \frac{3}{2} \end{bmatrix},$$

$$R_A = \begin{bmatrix} \frac{1}{2} & \frac{3}{2} \\ \frac{1}{2} & \frac{1}{2} \end{bmatrix}, \qquad R_{\tilde{A}} = \begin{bmatrix} \frac{1}{2} & 2 \\ \frac{1}{4} & \frac{1}{2} \end{bmatrix},$$

$$\rho^H\left(\begin{bmatrix} A_0 & \tilde{A}_0 \\ -\tilde{A}_0 & A_0 \end{bmatrix}\right) + \rho^H\left(\begin{bmatrix} R_A & R_{\tilde{A}} \\ R_{\tilde{A}} & R_A \end{bmatrix}\right) = -2.25 + 2.620185 = 0.370185 > 0.$$

4. Conclusion

In this paper for a second order complex interval family we consider the problem of existence and evaluation of common diagonal solutions to the Lyapunov matrix inequalities. This problem is very important in the stability theory, since it gives more simple Lyapunov functions of the diagonal types. Firstly, a criterion for the robust diagonal stability is given. The existence problems of a diagonal solutions are reduced to the simple smooth optimization problems.

Obtaining smiliar conditions for a third order complex interval family and for a general complex interval familiy may serve the topics of the future investigations.

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