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Relationship Between Shelterin Proteins and Agmatine in Urinary Tract Infections

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ABSTRACT

The presence of microorganisms in the kidney, collecting system, or bladder with pyuria and clinical symptoms is called Urinary Tract Infection (UTI). UTI is one of the most common diseases in both nosocomial and community-acquired infections and affects millions of people every year. The clinical types of UTI range from cystitis to sepsis. In this study, we aimed to contribute to the literature by comparing the levels of shelterin proteins and agmatine in the serum of patients diagnosed with cystitis, one of the types of UTI, and healthy volunteers without any known medical disorder, and to bring new approaches to issues such as clinical severity, response to treatment, and pathogenesis of the disease.

In this study, 30 individuals diagnosed with cystitis from UTIs and 30 individuals without any systemic disease participated. Serum agmatine levels were measured by a fluorescence detector using the ultra-high-performance liquid chromatography method (UHPLC). Serum shelterin proteins levels were measured using the ELISA method.

When the patients and healthy controls were compared, serum Telomeric Repeat Binding Factor2 (TRF2) levels of the patients were found to be lower than the controls; serum agmatine levels were found to be higher than the controls and statistically significant ($p<0.05$). Other shelterin protein levels were not statistically different between patients and controls ($p>0.05$).

In conclusion, the statistically significant difference in serum TRF2 and agmatine levels between the groups may be associated with oxidative stress. It is thought that the shortening of telomere lengths may be associated with decreased TRF2 levels.

Keywords: Urinary Tract Infections, Cystitis, Telomeres, Shelterin Proteins, TRF2, Agmatine

İdrar Yolu Enfeksiyonlarında Shelterin Proteinleri ve Agmatin Arasındaki İlişki

Süreç

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ÖZ

Piyüri ve klinik semptomlarla birlikte böbrekte, toplayıcı sistemde veya mesanede mikroorganizmanın bulunması, Üriner Sistem Enfeksiyonu (ÜSE) olarak adlandırılır. ÜSE, hem nozokomyal enfeksiyonlar hem de toplumdan kazanılmış enfeksiyonlarda en sık rastlanan hastalıklar arasında yer almaktadır ve her yıl milyonlarca insanı etkilemektedir. ÜSE'nin klinik tipleri, sistitten sepsise kadar değişmektedir. Bu çalışmada, ÜSE türlerinden biri olan sistit tanısı almış hastalar ile bilinen herhangi bir tıbbi bozukluğu olmayan sağlıklı gönüllü kişilerin serumlarında, shelterin proteinleri ve agmatin düzeylerinin karşılaştırılarak hastalığın klinik ciddiyeti, tedaviye yanıt takibi, patogenezi gibi hususlara yeni yaklaşımlar getirilmesi ve literarüre katkı sağlanması amaçlanmıştır.

Bu çalışmada, ÜSE'lerden sistit tanısı almış 30 birey ve herhangi bir sistemik hastalığı olmayan 30 birey katılmıştır. Serum agmatin seviyeleri ultra yüksek performanslı sıvı kromatografi yöntemi (UHPLC) kullanılarak floresans dedektörle ölçülmüştür. Serum shelterin protein düzeyleri ise ELISA yöntemi kullanılarak ölçülmüştür.

Hastalar ile sağlıklı kontroller karşılaştırıldığında, hastaların serum Telomerik Tekrar Bağlanma Faktörü2 (TRF2) düzeyleri kontrollere göre düşük; serum agmatin düzeyleri kontrolllere göre yüksek bulunmuştur ve istatistiksel olarak anlamlıdır ($p<0,05$). Diğer shelterin protein düzeyleri ise hasta ve kontrol arasında istatistiksel farklı hesaplanmamıştır ($p>0,05$).

Sonuç olarak, serum TRF2 ve agmatin düzeylerinin gruplar arasında istatistiksel olarak anlamlı farklılık göstermesi oksidatif stres ile ilişkilendirilebilir. TRF2 düzeylerindeki azalma ile telomer uzunluklarında kısalma olabileceği düşünülmektedir.

Anahtar sözcükler: Üriner Sistem Enfeksiyonları, Sistit, Telomer, Shelterin Proteinleri, TRF2, Agmatin

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Introduction

The presence of microorganisms in the kidney, collecting system, or bladder with pyuria and clinical symptoms is called Urinary Tract Infection (UTI)[1]. UTI is one of the most common diseases in both nosocomial and community-acquired infections and affects millions of people every year[2]. UTI is the most common cause of bacterial infections in adults and can be seen in both sexes and all age groups. Globally, 150 million cases of UTI develop annually and the cost of treating these patients is estimated to be more than 6 billion dollars[3]. These infections are more common in women than in men, and approximately half of the women are reported to have UTI at some point in their lives. The clinical types of UTI range from cystitis to sepsis[4].

Agmatine is an aminoguanidine molecule formed by the release of a CO2 molecule as a result of the decarboxylation of the amino acid L-arginine by the enzyme arginine decarboxylase. Their metabolism has been shown by studies in neurons, kidney cells, and vascular endothelial cells. Agmatine is released into the synaptic gap and provides metabolic activation by binding to various receptors such as N-methyl-D-aspartate (NMDA), imidazoline, and α2-adrenergic. Due to all these aspects, it is considered a novel neurotransmitter in the brain[5,6]. Agmatine is taken back into presynaptic cells by the reuptake system. It participates in the polyamine pathway via putrescine with the enzyme agmatinase. In the kidneys, it is metabolized to guanidino butanoic acid with the help of the diamine hydroxylase enzyme[7].

Telomeres are composed of a large number of non-coding TTAGGG hexanucleotide DNA repeat sequences that cover the end regions of eukaryotic linear chromosomes. These specialized structures are essential for the maintenance of genomic integrity[8,9]. Telomeres protect chromosomes against unwanted end fusion or nucleolytic degradation. In addition to this physical protection of linear chromosome ends, eukaryotic telomeres play important roles in cellular processes such as chromatin organization and control of cell proliferation10. Human telomere DNA consists of 2-15 kb of tandem (TTAGGG)n sequence oriented in the 5'-3' direction at the ends of chromosomes. The telomere region at the ends of chromosomes is divided into a sub-telomeric region composed of heterogeneous DNA repeats and a main telomeric region composed of homogeneous DNA repeats11. The D-loop structure of telomeres, which terminate in large loops, is formed by the insertion of a single strand known as the guanine-rich G-tail into the double-stranded telomere. The T-loop structure is formed by the backward circular curling of the DNA double strand[12,13]. Telomeres protect chromosomes with this T-loop structure. Specialized proteins attached to various regions of telomeres play a role in shaping the telomere ends and maintaining telomere length[14].

The Shelterin complex contains six main proteins composed of different polypeptides. These are Telomeric Repeat Binding Factor1 (TERF1/TRF1), Telomeric Repeat Binding Factor2 (TERF2/TRF2), Repressor/Activator protein1 (TERF2IP/RAP1), TRF1-Interacting Nuclear protein2 (TINF2/TIN2), Telomere Protection1 (POT1) and Tripeptidylpeptidase1 (TPP1)15. In the Shelterin complex, TRF1 and TRF2 are two telomeric DNA-specific homodimeric proteins that bind to double-stranded TTAGGG repeats with Myb/SANT-type DNA-binding domains[16]. RAP1 is a sequence-specific DNA binding protein. It regulates telomere function and is essential for cell growth. RAP1 also provides a mechanism to regulate the signaling cascade in mammals17,18. The TIN2 shelterin subunit causes telomeric shortening by blocking the recruitment of telomerase. It also forms a link between TRF1 and the TRF2/RAP1 complex by incorporating the TPP1/POT1 heterodimer into the complex[15,19]. POT1 is associated with the 3' end of the single helix of telomeric DNA. It suppresses DNA damage through inhibition of the Ataxia-Telangiectasia Mutated (ATM) and Ataxia-Telangiectasia Related (ATR) signaling pathways20. TPP1, encoded by the ACD gene region, plays an important role in the maintenance of telomere integrity, telomerase migration to telomeres, and telomerase function[21].

The aim of this study was to compare the levels of shelterin proteins and agmatine in the serum of patients diagnosed with cystitis, one of the types of UTI, and healthy volunteers without any known medical disorder, to bring new approaches to issues such as clinical severity, response to treatment, the pathogenesis of the disease and to contribute to the literature.

Material Method

In this study, the patients group consisted of 30 individuals without any systemic disease who were

diagnosed with cystitis from UTIs followed up by the Department of Infectious Diseases, Sivas Cumhuriyet University Health Services Application and Research Hospital. Patients were randomly selected without any discrimination in terms of age and gender. Patients with any chronic disease such as cancer, diabetes, or renal failure were excluded from the study. Thirty healthy individuals who were admitted to Sivas Cumhuriyet University Health Services Application and Research Hospital, who was not diagnosed with cystitis from UTIs, who did not have any systemic disease, who had a similar age distribution to the patients group, and who did not discriminate gender, were determined as the control group. All individuals in the control group were included in the study voluntarily.

Approval for the study permission was obtained by Sivas Cumhuriyet University Clinical Research Ethics Committee (2018-06/07). In addition, written informed consent was obtained from all participants.

Collection of blood samples

After the individuals included in the patients and control groups were informed and the informed consent form was read and signed, 5-10 mL blood samples were collected from each of them into sterile biochemistry tubes. These blood samples were centrifuged at 4000 rpm for 15 minutes. The sera obtained were portioned into eppendorf tubes and stored at -80 0C until the study was performed to determine TRF1, TRF2, RAP1, TIN2, POT1, TPP1, and Agmatine levels. In our study, agmatine levels were determined by Ultra high-performance liquid chromatography (UHPLC)6. TRF1, TRF2, RAP1, TIN2, POT1, TPP1 levels were measured through the ELISA method.

Statistical Method

Frequency tables were created to understand the data obtained from our study. Chi-Square tests were performed for the analysis of patients-control groups and categorical variables and difference tests were performed for the analysis of continuous variables. The Shapiro-Wilk normality test was applied to continuous variables to select the correct test. It was understood that the continuous variables in the study were not suitable for normal distribution ($p<0.05$). Since the variables were not parametric, the Mann-Whitney U test was used to reveal the difference between categorical variables. Similarly, the relationship between continuous variables was analyzed with the Spearman correlation test. In the final stage of the analysis, a binomial logistic regression test was performed to reveal the effect of the variables obtained in the study on the patients-control change. Cox-Snell R2 and Nagelkerke R2 statistics were used as critical values to demonstrate causality. The statistics were interpreted at a 95% confidence level. Analyses were conducted using the SPSS 23.0 package program.

Results

In this study, 30 individuals who were diagnosed with cystitis from UTIs followed up by the Infectious Diseases Clinic of Sivas Cumhuriyet University Health Services Application and Research Hospital constituted the patients group. Another 30 individuals without any systemic disease were included in the control group. As a result of the difference analysis performed on the ages of the individuals included in the study, it was determined that there was no statistically significant difference between the study groups (Patients=33.63±14.24; Control=34.27±12.33) ($p=0.584$). The number of men and women in the patients (%Male=66.7; %Female=33.3) and control (%Male=56.7; %Female=43.3) groups were not statistically significantly different ($p=0.426$). The difference in the results of shelterin proteins and agmatine levels according to the study groups is analyzed in (Table 1).

Table 1. Descriptive Statistics of Variables by Groups and Results of Difference Analysis

Variables	Control (n=30) Mean ± SD. (min.-max.)	Patients (n=30) Mean ± SD. (min.-max.)	p (M-W U)
POT1 (pg/mL)	425,63±412,27 (193,0-1962,0)	280,27±72,95 (183,0-458,0)	0,274 (376,0)
TRF1 (pg/mL)	89,27±13,89 (67,68-130,15)	100,36±22 (68,84-135,41)	0,088 (334,50)
TRF2 (pg/mL)	150,18±45,22 (109,14-278,21)	116,11±28,39 (85,32-165,12)	**0,001 (217,50)**
RAP1 (ng/mL)	15,73±9,67 (8,73-53,61)	15,36±11,24 (8,31-61,17)	0,701 (424,0)
TIN2 (ng/mL)	2,65±1,48 (1,13-6,74)	2,59±1,61 (1,16-6,15)	0,712 (425,0)
TPP1 (pg/mL)	3,41±1,34 (2,54-7,47)	3,25±0,95 (2,43-5,90)	0,836 (436,0)
Agmatine (ng/mL)	7,63±4,77 (0,80-17,02)	28,95±12,60 (9,57-59,49)	**$1,62x10^{-9}$ (42,0)**

M-W U: Mann-Whitney U Test Statistics

According to the difference analysis results, TRF2 (pg/mL) (Control=150.18±45.22; Patients=116.11±28.39) and Agmatine (ng/mL) mean levels were statistically significantly different (p<0.05) (Table 1). Other shelterin protein levels were not statistically different between patients and controls (p>0.05). On the contrary, Agmatine (ng/mL) level in the control group was calculated as 7.63±4.77 ng/mL, while the same value was calculated as 28.95±12.60 ng/mL in the patients group. For a better understanding of the numerically obtained and explained results, a visualization is presented in (Figure 1).

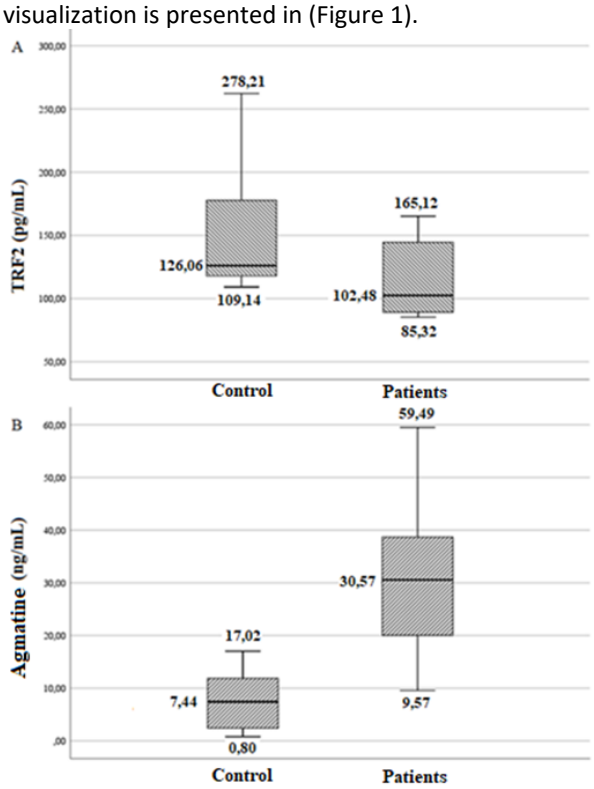

Figure 1. Patients-Control Box Plot for RF2 (pg/mL) and Agmatine (ng/mL)

Table 2. Correlation Analysis Results

Variable	Statistics	TRF1 (pg/mL)	TRF2 (pg/mL)	RAP1 (ng/mL)	TIN2 (ng/mL)	TPP1 (pg/mL)	Agmatine (ng/mL)	Age
TRF1 (pg/mL)	r (p)	-0,051 (0,349)						
TRF2 (pg/mL)	r (p)	**0,483 ($4,7x10^{-5}$)**	-0,131 (0,159)					
RAP1 (ng/mL)	r (p)	**0,394 (0,001)**	0,079 (0,274)	**0,551 ($3,0x10^{-6}$)**				
TIN2 (ng/mL)	r (p)	**0,305 (0,009)**	-0,148 (0,13)	**0,586 ($4,3x10^{-7}$)**	**0,475 ($6,4x10^{-5}$)**			
TPP1 (pg/mL)	r (p)	**0,54 ($4,0x10^{-6}$)**	-0,015 (0,456)	**0,609 ($1,2x10^{-7}$)**	**0,785 ($5,7x10^{-14}$)**	**0,669 ($2,6x10^{-9}$)**		
Agmatine (ng/mL)	r (p)	-0,135 (0,151)	**0,22 (0,04)**	**-0,413 (0,001)**	-0,081 (0,27)	-0,01 (0,47)	-0,085 (0,26)	
Age	r (p)	-0,153 (0,122)	0,15 (0,127)	-0,115 (0,191)	-0,202 (0,061)	-0,025 (0,424)	-0,127 (0,167)	-0,012 (0,46)

Correlation analysis was performed to reveal the relationship between shelterin proteins and agmatine levels (Table 2). The statistically significant results obtained are listed below:

•A moderate positive correlation (r=0.483) was calculated between TRF2 (pg/mL) and POT1 (pg/mL) levels.

•There was a weak positive correlation between RAP1 (ng/mL) and POT1 (pg/mL) levels (r=0.394) and a moderate positive correlation between TRF2 (pg/mL) levels (r=0.551).

•There was a weak positive correlation between TIN2 (ng/mL) and POT1 (pg/mL) levels (r=0.305) and a moderate positive correlation between TRF2 (ng/mL) (r=0.586) and RAP1 (pg/mL) (r=0.475) levels.

•A moderate positive correlation was calculated between TPP1 (pg/mL) and POT1 (pg/mL) levels (r=0.540); a strong positive correlation was calculated between TRF2 (ng/mL) (r=0.609), RAP1 (ng/mL) (r=0.785) and TIN2 (pg/mL) (r=0.669) levels.

•A weak positive correlation between agmatine (ng/mL) and TRF1 (pg/mL) levels (r=0.220) and a moderate negative correlation between TRF2 (pg/mL) levels (r=-0.413) were calculated.

Table 3. Logistic Regression Analysis Results

Variable	B	Std. Err.	Wald	p	Exp.(B)	95% Confidence Interval	
						Lower Limit	Upper Limit
TRF2 (pg/mL)	-0,037	0,11	11,709	0,001	0,963	0,943	0,984
Agmatine (ng/mL)	0,323	0,099	10,719	0,001	1,382	1,139	1,677

Due to the presence of a dependent variable consisting of two groups, it was decided to apply the binomial logistic regression technique. It is possible to examine the values calculated as a result of the analysis in (Table 3). To calculate the significance of the model with all variables (shelterin proteins and agmatine), the back-feedback Wald technique was used. The mathematical model obtained as a result of the model was determined as.

$$[\mathbf{0},\mathbf{1}] = \mathbf{0},\mathbf{323} \cdot (\boldsymbol{Agmatine}) - \mathbf{0},\mathbf{037} \cdot (\boldsymbol{TRF2}) \quad \rightarrow \quad \mathbf{0} = \boldsymbol{Control}; \mathbf{1} = \boldsymbol{Patients}$$

As a result of the analysis, it was calculated that the risk of high agmatine level was 1.382 times higher in the patients group. Similarly, the risk of having a low TRF2 (pg/mL) level was 0.963 times higher in the patients group. The agmatine (ng/mL) level calculated as a risk factor is effective between 1.139-1.677 times, while TRF2 (pg/mL) level is effective between 0.943-0.984 times.

It is understood that the results obtained by calculating the Cox-Snell R2 value of 0.603 will be similar in 60.3% of the population and the results obtained by calculating the Nagelkerke R2 value of 0.805 will be similar in 80.5% of the population. The following crosstabulation table (Table 4.) shows the classification results including the actual and LR (Logistic Regression) prediction values.

Table 4. Cross Table of Actual and Estimated Values

	Group	Estimate	
		Control	Patients
serve	Control	28	2
	Patients	4	26

As can be seen from (Table 4), it is clear that the effect of TRF2 (pg/mL) and Agmatine (ng/mL) levels is quite successful in separating the patients and control groups. The control group can be correctly predicted at 93.3% and the patients group 86.7% of the time. Overall, 90.0% of successful classification is achieved. Based on this, a person who is not sick can be predicted not to be sick according to the variables in the model with a 93.3% Positive Predictive Value (PPV), and a person who is sick can be predicted to be sick with 86.7% Negative Predictive Value (NPV).

Discussion

In this study; shelterin proteins and agmatine levels were evaluated in the sera of patients diagnosed with cystitis, one of the types of UTI, and healthy volunteers without any known medical disorder.

UTI is an infection of any part of the urinary system, such as the kidneys, ureters, bladder, and urethra. Most infections involve the lower urinary tract. UTI is a common transmission between men and women but is more common in women due to their physiology. UTI, whose clinical types vary from cystitis to sepsis, is one of the most common infectious diseases in outpatients and inpatients with high social costs [4,22].

The Shelterin complex is found in the telomeric end structures of DNA as tandem repeats of the TTAGGG sequence. Six members, consisting of TRF1, TRF2, TIN2, POT1, TPP1, and RAP1, associate with double-stranded and single-stranded DNA repeats at telomeres to form this complex25. Shelterin proteins contribute to telomere protection from unwanted DNA damage control-specific responses by aiding telomere stability[29]. Alterations in the structure or function of any of these proteins trigger unwanted DNA damage responses and trigger cellular aging and death processes[24]. It has been stated that telomeres can be protected against the DNA damage response that may occur at chromosome ends through the specific functions of each subunit of the shelterin complex, therefore, changes in the structure/function of the shelterin complex during the development and aging processes are an area of intense research30. In this context, when the relevant literature studies on shelterin proteins, which is a very important research topic, are examined; it is observed that the proteins are explained by associating them with different variables[24,25,30]. The ultimate role of the shelterin complex is to maintain telomere homeostasis, which is critical for genome stability and cell fate. Furthermore, the shelterin complex is described as multifunctional due to its involvement in various cellular processes such as replication, mitosis, meiosis, heterochromatin stability, immunity, and oxidative stress[24,30]. These functional processes of the shelterin complex led us to question the contributions of the whole complex versus its subunits and the individual functions of the six main proteins that make up the complex in order to bring new approaches to the pathogenesis of UTI.

Within the scope of the findings obtained in our study, it was concluded that TRF2 (pg/mL) and Agmatine (ng/mL) levels were statistically significantly different between the patients and control groups ($p<0.05$), while other shelterin proteins POT1 (pg/mL), TRF1 (pg/mL), RAP1 (ng/mL), TIN2 (ng/mL) and TPP1 (pg/mL) levels were not statistically significantly different ($p>0.05$). It is evaluated that TRF2 (pg/mL) level may be expected to decrease in case of cystitis from UTIs. When the literature is examined, there is no study showing shelterin proteins in the sera of patients with cystitis from UTIs. This situation reveals the scientific contribution and importance of the study in terms of Shelterin Proteins. On the other hand, there are a number of studies on shelterin proteins in cancer, viral infections, molecular and animal modeling.

TRF2 expression, one of the subunits of the Shelterin complex, has been reported to be

increased in various human cancers[31,32]. In a study, it was reported that protein expressions of TRF1, TRF2, and TIN2 were significantly higher in gastric cancer tissues at different stages compared to normal gastric mucosal tissues[26]. In another study involving acute myeloid leukemia patients, increased TRF2 expression correlated with poor prognosis and suggested that TRF2 has prognostic significance[33]. Despite these studies, there are studies in the literature in which TRF2 levels are decreased. In one study, it was revealed that TRF2 depletion cannot suppress ATM pathway activation and may initiate genomic instability by causing chromosome fusions mediated by the non-homologous end joining (NHEJ) pathway[35]. In a study investigating the ability of SARS-CoV-2 infection to affect DNA damage response and telomere stability, it was reported that TRF2 expression was decreased in infected Vero E6 kidney cells. In the study, it was shown that decreased TRF2 expression destabilizes telomeres, initiates telomere fusions, and causes genomic instability[36]. The contributions of the Shelterin proteins TRF1 and TRF2 to the maintenance of genomic integrity and telomeric stability are invaluable. This complex is known to play a regulatory role in telomere length and telomerase enzyme. Depending on the presence of TRF1 or TRF2 in the environment, the stimulatory and restrictive functions of the shelterin complex are shaped in opposite directions. If TRF1 protein is present in the medium, the complex can be found as TRF1-TIN2-TPP1-POT1 form, while in the presence of TRF2, it can be found in TRF2-RAP1-TIN2-TPP1-POT1 subunits. In this context, the significantly lower TRF2 levels in our study compared to the control group can be interpreted in light of the literature. TRF2 is a shelterin subunit required for chromosomal end protection, DNA damage response suppression and to prevent topological stress. Low levels of TRF2 can fail to inactivate enzymes that lead to the DNA damage response when bound to the telomere. This can trigger genomic instability by initiating end-to-end fusions. Failure to maintain telomere structure can shorten telomere lengths. In addition, when TRF2 levels are evaluated in relation to the complex members that affect each other in terms of structure and function; RAP1 failed to strengthen its effect by binding to TRF2, TPP1 failed to increase the activity of the telomerase enzyme, and TIN2 protein failed to ensure that the telomere is firmly attached to the nuclear matrix, as a result, it can be mentioned that these factors may affect the telomere length of cystitis patients in a shortening direction. One of the causes of telomere length shortening is oxidative stress. The failure of TRF2 to inactivate the enzymes that cause the DNA damage response may have led to severe oxidative stress in telomeres due to ROS, as it may cause an increase in reactive oxygen species (ROS) due to UTI. Severe oxidative stress may deplete a homodimeric binding protein, such as TRF2, as it may cause an accumulation of oxidized DNA base products in guanine-rich telomeres. This may lead to the shortening of telomere length[34,37-47]. All these results are consistent with the hypothesis of our study.

Agmatine, a biogenic polyamine, is a metabolite of the amino acid L-arginine produced by decarboxylation with the ADC enzyme and hydrolyzed to putrescine by the enzyme agmatinase[27]. The metabolism of agmatine in neurons, kidney cells, and vascular endothelial cells has been elucidated by studies. In cell culture studies, it has been reported that agmatine suppresses apoptotic signaling pathways and shows a protective effect against oxidative damage[5,6,56]. Studies showing differences in the levels of agmatine in various diseases have been reported[28,48]. In light of the given information, it was aimed to evaluate agmatine in terms of bringing new approaches to the pathogenesis of UTI. According to these results, it is expected that the level of agmatine (ng/mL) will be increased in case of cystitis from UTIs. When the literature is reviewed, there is no study measuring Agmatine levels in the serum of patients with cystitis from UTIs. As with Shelterin proteins, the lack of any evaluation of Agmatine levels in the literature reveals the importance of this study. In contrast, there are a number of comprehensive studies on agmatine under various titles. In a study evaluating the effects of agmatine supplementation against nephrotoxicity in rats, it was noted that agmatine supplementation reduced oxidative stress-related kidney damage by reducing free oxygen radicals and lipid peroxidation, restoring NO levels to normal levels and inhibiting inflammatory mediators such as TNF-α[49]. In another study in which enzymatic activities of NOS isoforms were determined in the brain, macrophage, and endothelial cells, agmatine was shown to be a potent NOS inhibitor with an antioxidant effect[50]. In a metabolomics study conducted for the diagnosis of UTI, it was observed that infections caused by Enterobacterales, Klebsiella, and Citrobacter species, such as E. coli, which cause the most prominent UTI, were associated with high agmatine levels[51]. In another urine analysis study, a significant increase in agmatine levels was observed in patients infected with at least one of Escherichia coli, Proteus mirabilis, and Citrobacter species. Therefore, it has

been reported that the presence of agmatine is considered a strong indicator of UTI[52]. In this context, the fact that agmatine levels were significantly higher in our study compared to the control group can be interpreted in light of the literature. Agmatine is known to be a NOS inhibitor and has a strong antioxidant effect. When the uncompensated increase in the number of reactive nitrogen species (RNS) and reactive oxygen species (ROS) in metabolism exceeds the capacity of antioxidant defense mechanisms, it disrupts the oxidative balance and causes oxidative/nitrosative stress. ROS can cause tissue destruction by damaging various macromolecules and may act as signaling molecules in the activation and induction stages of some genes. Agmatine may play a protective role against oxidative damage. In our study, it can be suggested that high agmatine levels may eliminate the oxidant damage caused by severe oxidative stress that may occur with the virulence effect of a uropathogenic in the lower urinary tract caused by UTI with its antioxidant effect. The failure of the decreased TRF2 level in our study to inactivate the enzymes leading to DNA damage response may have led to severe oxidative stress since it may cause an increase in ROS due to UTI. Severe oxidative stress may cause an accumulation of oxidized DNA base products in telomeres, which may shorten telomere length. It can be said that the high agmatine finding in our study may prevent oxidant damage caused by the aforementioned reasons. In addition, the increased activity of the arginine decarboxylase (ADC) enzyme, which is responsible for agmatine synthesis, and the effects of UTI on polyamine metabolism may have caused the high agmatine finding[23,38,39,53-57]. The results of the studies in the given literature support our findings.

According to the data we obtained as a result of the comparison, there are different correlations between cystitis and serum levels of shelterin proteins and agmatine molecules. While positive correlation is noteworthy in the significant correlations between shelterin proteins, TRF1 and TRF2 show positive and negative correlations with agmatine.

In studies examining the relationship between POT1 and TRF2, it has been reported that POT1 and TRF2 interact to form a complex with telomeric DNA and that this interaction is important for telomere length and homeostasis58. In a study similar to our study, TRF1, TRF2, POT1, TPP1, TIN2, and RAP1 levels were compared. As a result of the study, it was shown that there was a strong positive correlation between TRF2, TIN2, TPP1, and RAP1 levels, and TRF2 levels decreased as the disease duration increased. In light of these findings, they reported that the shelterin complex is not present as a whole under telomere length control, but may be formed as different sub-complexes according to the presence of TRF1 or TRF2 homodimeric proteins37. When these results obtained by the researchers are evaluated, it is seen that results compatible with our study were obtained.

Conclusion

In conclusion, there has not been a study similar to our study in patients diagnosed with cystitis from UTIs. Thanks to this study, the serum levels of shelterin proteins and agmatine molecules were determined together for the first time in the sera of patients diagnosed with cystitis from UTIs and healthy volunteers without any medical disorder.

Our study has some limitations. These include the absence of urine analyses, the measurement of telomere length using different methods and with larger sample groups, and the limitation of the scope of the universe. Considering these limitations, if the results of this study are supported by future research, it is believed that, in addition to evaluating whether these molecules can be a factor or biomarker affecting the pathogenesis of the disease, they may also provide guidance on the clinical severity of the disease and the response to treatment.

Conflict of interest

The authors declare no conflict of interest.

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