Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2020;45(1):63-70
DOI: 10.17826/cumj.637127

ARAŞTIRMA / RESEARCH

Novel oxidative stress biomarker in sepsis: dynamic thiol-disulfide homeostasis

Sepsis için yeni bir oksidatif stres biyobelirteci: dinamik tiyol-disülfür homeostazisi

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Cukurova Medical Journal 2020;45(1):63-70

Abstract

Purpose: The aim of this study was to investigate the relationship between serum dynamic thiol-disulfide homeostasis (TDH) and sepsis, in adult patients.
Materials and Methods: In this observational, prospective, case-control study patients (n=44) diagnosed with sepsis compared with ages and gender-matched healthy controls (n=44). Patients were divided into two subgroups (survivors and non-survivors) dependent variable being remaining alive on the 28th day after ICU admission at the follow-up period. TDH parameters measured using a novel automatic and spectrophotometric method and compared statistically.
Results: In patients with sepsis, native thiol, total thiol, and disulfide levels were lower than the control group, and this difference was statistically significant. TDH parameters between surviving and non-surviving patients were similar at the sepsis diagnosis. However, significant differences found in native and total thiol levels at follow-up via repeated measurement analyses on days 3 and 7 in surviving and non-surviving sub-groups.
Conclusion: This study showed that impairment in dynamic TDH in adults with sepsis may be related to negative outcomes.
Keywords:. Dynamic thiol-disulfide homeostasis, sepsis, oxidative stress.

Öz

Amaç: Bu gözlemsel, prospektif, vaka kontrol çalışmasında erişkin hastalarda serum dinamik Tiyol-Disülfir Homeostazisi (TDH) ile sepsis arasındaki ilişkiyi araştırmayı amaçladık.
Gereç ve Yöntem: Sepsis tanısı ile alan 44 hasta, yaş ve cinsiyet uyumlu 44 sağlıklı kontrol ile karşılaştırıldı. Hastalar yoğun bakım ünitesi yatışından sonraki 28. günde sağ-kalan ve hayatını kaybedenler olarak 2 gruba ayrıldı. TDH parametreleri yeni, otomatik ve spektrofotometrik bir yöntem kullanılarak ölçüldü ve sonuçlar istatistiksel olarak karşılaştırıldı.
Bulgular: Sepsisli hastalarda, nativ tiyol, total tiyol ve disülfit seviyeleri kontrol grubuna göre daha düşük olarak bulundu ve bu fark istatistiksel olarak anlamlı idi. Başlangıç TDH parametreleri sağ-kalan ve hayatını kaybeden hastalar arasındaki benzer bulundu. Ancak sağ kalan ve ex olan hastaların 3 ve 7. günde tekrarlanan TDH ölçümleri (nativ ve toplam tiyol seviyeleri) arasında anlamlı farklar bulundu.
Sonuç: Bu çalışma, erişkin sepsis hastalarında dinamik TDH'deki bozulmanın negatif sonuçlara ilişkili olabileceğini göstermiştir.

Anahtar kelimeler: Dinamik tiyol-disülfir homeostazisi; sepsis, oksidatif stres.

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Geliş tarihi/Received: 23.10.2019 Kabul tarihi/Accepted: 03.12.2019 Published online: 03.01.2020

INTRODUCTION

Sepsis is the body's overwhelming and life-threatening response to infection, which can lead to tissue deterioration, organ dysfunction, and death caused via a dysregulated organism feedback [1]. Despite significant improvements in the hemodynamic monitoring devices and resuscitation measures, sepsis continues as one of the main reasons for morbidity and mortality in intensive care units (ICUs) even in developed countries [2,3]. It was reported that sepsis afflicts >1 million cases yearly in the USA[4], and the mortality rate is 25% for uncomplicated sepsis and 80% in cases who coexistence of with multiple organ failure in those afflicted.

The pathogenesis of sepsis is complicated and multifaceted. During sepsis, along with excessive creation of pro-inflammatory cytokines, there is additionally an overproduction of free radicals and reactive oxygen species (ROS) generated by different cells and multiple sources [5]. Despite the essential roles of free radicals as antimicrobial agents, enhanced levels promote a plurality of redox reactions in all tissues and finally changes protein and enzymatic function, modulates variations in microcirculatory hemodynamics, and incites vascular structural alterations. Control of overproduction of free radical levels in the body through an endogenous antioxidant system is necessary to maintain optimal physiological conditions in organisms.

Thiol, is an organic compound that includes a sulfhydryl (R-SH) group, have a significant role in the elimination of free radicals through enzymatic and non-enzymatic pathways [6-8]. Plasma levels of protein thiols are the signs of antioxidant status in the body. In case of oxidative stress, thiols release the hydrogen and form disulfide bonds to deactivation of ROS and protect the tissue from oxidative injury. Under normal states, these disulfide bonds can repeatedly be reduced into thiol groups; thereby, the dynamic thiol-disulfide homeostasis (TDH) is preserved [9]. Typically, there is an equilibrium between the thiols and the disulfides which perform a protective function during cellular redox homeostasis.

Classic tecniques measures only one side of the thiol-disulfide balance. Recently a new spectrophotometric method developed by Erel and Neselioğlu made it possible to determine the dynamic TDH, as well as the native thiol, total thiol, and disulfide levels separately in the body [10]. Little known, however, regarding the role of dynamic TDH in sepsis. Therefore, in the present study we aimed to investigate the relationship between serum TDH and the outcome of sepsis in adult patients with this newly developed method.

MATERIALS AND METHODS

Approvals for this study obtained from the Selcuk University Ethical Committee (Approval number: 2016/217). The study protocol conforms to the ethics guidelines of the 2013 Declaration of Helsinki. Written informed consent obtained from all participants or relatives. The present prospective and observational study was conducted in the intensive care unit (ICU) of the tertiary level education and research hospital.

Adults patients diagnosed with sepsis and septic shock recruited over three months from May 2018 to July 2018. Inclusion criteria were the diagnosis of sepsis according to the third international consensus definitions criteria set by Singer, Mervyn et al. [1] depend on patients clinical and laboratory findings. Exclusion criteria were: age < 18 years, being pregnant or lactating, end-stage cancer, liver cirrhosis, human immunodeficiency virus (HIV), solid or hematological tumor, or immunosuppressive, steroid or radiation therapy. Participants in the control group consisted of age- and sex-matched healthy adults absence of acute or chronic disease determined by medical history and physical examination.

Procedures

Subjects screened on days 0 (on the day of ICU admission), 3 and 7. Patients demographic characteristics (age, gender, BMI), comorbidities (chronic obstructive pulmonary disease, chronic renal failure, chronic heart failure, and diabetes) and the following clinical and biological variables; microbiological findings (primary infection source and the identified microorganisms), laboratory parameters (complete blood count, biochemical tests, blood gas analyses, PCT, CRP) were obtained. The severity of illness evaluated with APACHE-II and Sepsis-related Organ Failure Assessment (SOFA) score[11, 12].

SOFA score is used to track a person's status during the stay in an intensive care unit (ICU) to determine

the extent of a person's organ function or rate of failure[12]. The score based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems[12]. Patients divided into two sub-groups: survivors; cases who overcame the septic episode and remained alive at the 28th day after ICU admission; non-survivors; cases who died within the follow-up period.

Measurement of dynamic thiol/disulfide homeostasis

Venous blood samples collected into tubes containing ethylenediaminetetraacetic acid. With centrifugation at 1500 g for 10 min serum were separated from the cells. Dynamic TDH comprise native thiol (SH), total thiol (SH+SS), disulfide (SS) parameters and the disulfide/native thiol percent ratios (SS/SH %). Serum native thiol (SH) and total thiol (SH+SS) parameters were determined by a new automated method developed by Erel & Neselioglu with utilizing an automatic clinical chemistry analyzer (Roche, cobas 501, Mannheim, Germany)[10]. Briefly, the principle of the new assay is to reduce disulfide bonds (S=S) to reactive thiol groups with the help of sodium borohydride. The unused reductant NaBH4 is removed by formaldehyde. Both reduced and native thiol groups detected upon reaction with 5,50 -dithiobis-(2-nitrobenzoic) acid. Subtracting native thiol content from total thiol gives twice the disulfide (SS) amount. Disulfide/total thiol ratio, disulfide/native thiol ratio, and native thiol/total thiol ratio can be calculated by using percentages (%).

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences version 22 software (SPSS, Chicago, IL, USA). Categorical variables were presented as number (n) and percentage (%). In the comparison of categorical variables, the Chi-square and Fisher's Exact tests were used. Numerical variables were presented as means ± standard deviations or median and interquartile range (IQR) values.

Normal distribution of the data was checked using the Shapiro-Wilk test. Independent samples t-test or Mann–Whitney U test used for the analysis of continuous variables. Analysis of variance (ANOVA) with repeated measurements was used as a statistical approach to test the significance of differences ($p < 0.05$) between TDH parameter among sub-groups in day 0, 3 and 7. The relations among the TDH parameters and SOFA score in septic patients were analyzed with Pearson and Spearman correlation. A value of $p<0,05$ was considered as statistically significant.

RESULTS

A total of 44 patients with sepsis: 26 males (%) and 18 females (%), and 44 healthy controls; 20 males (%) and 24 females (36%) were evaluated in the study. Mean ages of the patients with sepsis and healthy controls was 63,8±16,9 and 60,2±7,3, respectively. There were no significant differences between groups regarding age and gender ($p=0,19$ and $p=0,20$, respectively).

The dynamic TDH measurements are depicted in Table 1. Native thiol, total thiol, and disulfide levels were determined to be significantly lower in patients with sepsis in comparison with the control group ($p < 0.01$ for each). The disulfide / total thiol ratio was lower in the sepsis group than the control group, whereas the disulfide / native thiol and native thiol / total thiol ratios were higher. However, these differences were not statistically significant (Table 1).

The mortality rate of the cases with sepsis on the 28th day after sepsis initiation was 40.9 % (18 out of 44). There were no significant differences among the survivors and non-survivors with regards to age, gender, and laboratory values. (Table 2).

Table 1. Serum dynamic thiol-disulfide homeostasis parameters

Variable	Sepsis (n=44)	Control (n=44)	p value
Native thiol (μmol/L)*	200[132-230]	275[232-290]	0.01
Total thiol (μmol/L)**	206±69	312±50	0.01
Disulfide (μmol/L)**	11.4±4.01	21.6 ±10.76	0.01
Disulfide/Native thiol*	6.5[4.4-10.05]	7.55[5.50-11.00]	0.75
Disulfide/Total thiol**	6.52 ±3.81	7.09 ±3.70	0.48
Native thiol/Total thiol**	86.9±7.65	85.8±7.41	0.47

Data are presented as median [interquartile range] or mean (standard deviation) as indicated;
* Mann–Whitney U test, ** Independent samples t-test

Table 2. Clinical and laboratory characteristics of survivors and non-survivors in the sepsis group

Variable	Survivor (n=26)	Non-Survivor (n=18)	p value
Gender (M/F)	13/13	13/5	0.14
Age (yr)**	64±17	63±17	0.79
BMI (kg/m2)*	26[22.8-28.4]	26.3[25.1-28.4]	0.25
WBC×10³/μL*	11[9-18]	12.5[10-22]	0.72
Hematocrit*	31.6[27-36.6]	29[25-38]	0.68
Platelet, ×10³/μL**	200.70±86.63	196.91±125.28	0.91
Lactate, mmol/L*	2[1-3]	3[2-5]	0.03
Creatinine (mg/dl)*	0.99[0.63-1.20]	1.67[0.80-2.84]	0.13
Glucose (mg/dl)**	162.43±75.14	150.61±46.03	0.56
APACHE II score**	17.73±6.58	20.72±8.78	0.20
SOFA score**	7.81±3.35	9.78±3.12	0.06
Direct Bilirubin (mg/dl)*	1.2[0.6-2.49]	0.9[0.5-2.38]	0.75
Indirect Bilirubin (mg/dl)*	0.815[0.315-1.425]	0.693[0.215-1.397]	0.73
Albumin(gr/dl)**	2.46±0.56	2.35±0.43	0.5
Comorbidity (Yes/No)	20/6	16/2	0.31
Site of infection			
Pneumonia (Yes/No)	16/10	11/7	0.98
Abdominal infection (Yes/No)	10/16	9/9	0.45
Urinary tract infection (Yes/No)	2/24	3/15	0.36
Skin infection (Yes/No)	1/25	0/18	0.40
Blood stream infection (Yes/No)	0/26	3/15	0.03
Non-infection (Yes/No)	5/21	2/16	0.47

BMI = body mass index

Data are presented as median [interquartile range] or mean (standard deviation) as indicated. In the comparison of categorical variables, the Chi-square and Fisher's Exact tests were used. * Mann–Whitney U test, ** Independent samples t-test

Table 3. Serum dynamic thiol-disulfide homeostasis parameters at the sepsis diagnosis between surviving and non-surviving patients

Variable	Survivor (n=26)	Non-survivor (n=18)	p value
Native thiol (μmol/L)*	202[166-215]	165[100-240]	0.37
Total thiol (μmol/L)**	214±57	193±83	0.31
Disulfide (μmol/L)**	11.6±4.5	11.1±3.1	0.60
Disulfide/Native thiol*	5.55[4.10-8.60]	7.25[5.50-15.30]	0.10
Disulfide/Total thiol*	5.20[3.70-7.30]	5.90[3.50-10.10]	0.28
Native thiol/Total thiol**	88.1±6.3	85.5±9.2	0.27

Data are presented as median [interquartile range] or mean (standard deviation) as indicated. * Mann–Whitney U test, ** Independent samples t-test

Comparison of dynamic TDH parameters at the sepsis diagnosis between surviving (n= 26) and non-surviving (n= 18) patients shown in Table 3. There was no significant difference between the survivor and non-survivor septic patients regarding dynamic TDH parameters (p>0.05 for each).

Repeated measurement analyses of the sub-groups (surviving and non-surviving) for each TDH parameters at days 0, 3 and and seven showed in figure 1 to 6 The native thiol levels were found similar among sub-groups on day 0 and 3 (p = 0.54 and p = 0.18, respectively), but there was a significant difference on day seven (p = 0.02). For native thiol; the time effect was significant (p= 0.04); however, the group effect and group-time interaction were not statistically significant (p=0,5 and p=0,12 respectively). When the sub-groups examined for total thiol levels at day 0, 3 and 7 the total thiol levels of sub-groups were found similar on day 0 and 3 (p = 0,38 and p = 0.17, respectively), but there was a significant difference on the seventh day (p = 0.04). For total thiol; the group effect was significant (p < 0.04); however, the time effect and group-time interaction were not statistically significant (p=0,26 and p=0,12 respectively). When the sub-groups examined disulfide level, and disulfide/native thiol,

disulfide/total thiol and native thiol/total thiol ratios at day 0, 3 and 7; the disulfide level, and disulfide/native thiol, disulfide/total thiol and native thiol/total thiol ratios among sub-groups were not statistically significant on day 0, 3 and 7 ($p>0.05$ for each). For disulfide level, and disulfide/native thiol, disulfide/total thiol and native thiol/total thiol ratios; the group effect, the time effect, and group-time interaction were not statistically significant ($p>0.05$ for each).

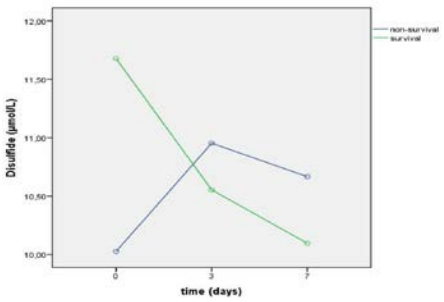

Figure 1. Disulfide levels of non-survival and survival groups among day 0, 3 and 7.

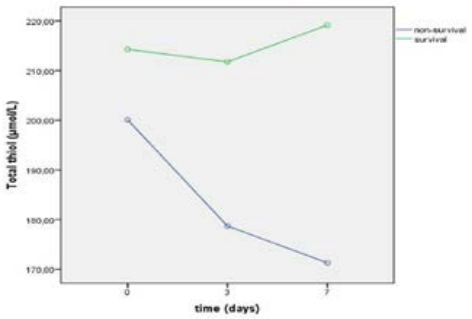

Figure 2. Total thiol levels of non-survival and survival groups among day 0, 3 and 7.

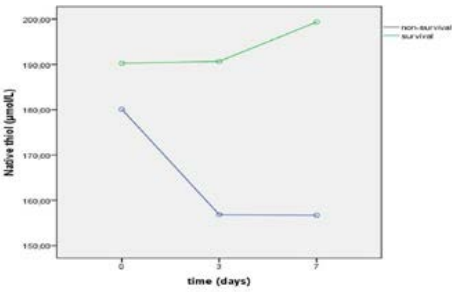

Figure 3. Native thiol levels of non-survival and survival groups among day 0, 3 and 7.

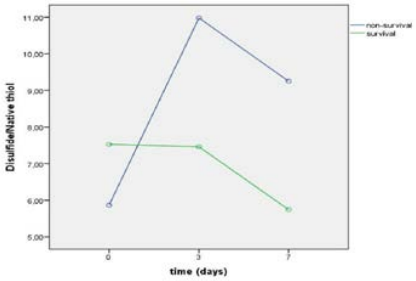

Figure 4. Disulfide/native thiol ratios of non-survival and survival groups among day 0, 3 and 7.

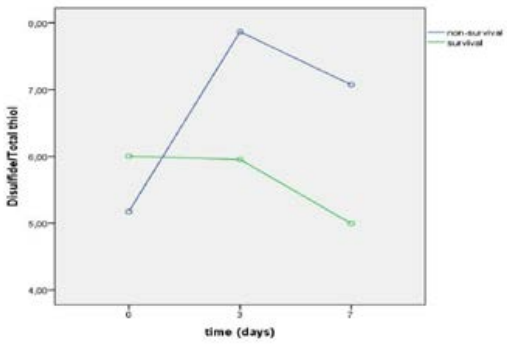

Figure 5. Disulfide/total thiol ratios of non-survival and survival groups among day 0, 3 and 7.

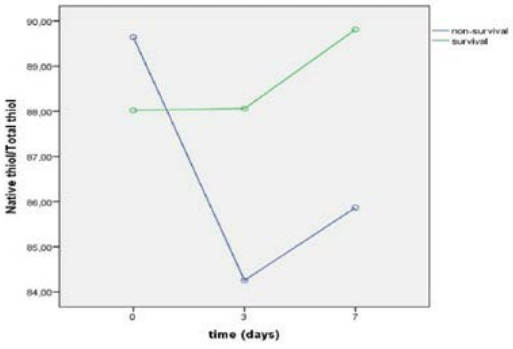

Figure 6. Native thiol/total thiol ratios of non-survival and survival groups among day 0, 3 and 7.

The correlations of parameters related to SOFA score are presented in Table 4. Accordingly, total thiol and native thiol levels and native thiol/total thiol ratio were negatively correlated with SOFA score (r=-0,388; p=0,01, r=-0,352; p=0,02, and r=-0,320; p=0,04 respectively). In addition, there was a positive correlation between disulfide/total thiol ratio and SOFA score (r=0,316; p=0,04).

Table 4. Correlations of TDH parameters with SOFA score in day 0 among septic patients.

Variable	SOFA Score	
	r	p value
Native thiol (μmol/L)	-0.388	0.01
Total thiol (μmol/L)	-0.352	0.02
Disulfide/Total thiol	0.316	0.04
Native thiol/Total thiol	-0.320	0.04

Pearson and Spearman correlation was used.

DISCUSSION

In the present study, we prospectively assessed the TDH in septic adult patients using a novel method which identifies both sides of homeostasis, including native thiol and disulfide, developed by Erel and Neselioglu [10]. Significant differences were determined in native thiol, disulfides, and total thiol levels between the septic patients and the control group. Also we found a negative correlation between the SOFA score and total thiol and native thiol levels and native thiol/total thiol ratio. To the best of our knowledge, this is the first study that provided data concerning the relationship between TDH and outcome of the sepsis in adult patients.

TDH has assessed in several infectious diseases, and the investigation generally indicates that thiol levels decrease due to inflammation [13-15]. Kara et al. reported that native and total thiol levels were found to be lower in acute tonsillopharyngitis patients than in healthy controls [15]. Also, thiol levels negatively correlated with the C-reactive protein values and white blood cell counts in tonsillopharyngitis patients [15]. Similarly, Kolgelier et al. showed depletion in native and total thiol levels with oxidative stress due to brucellosis infections [13]. Recently, Ayar and colleagues have demonstrated that plasma thiol levels were significantly lower than the control group in children with sepsis [16]. They reported that the non–survivors exhibited lower thiol levels than survivors suggesting that oxidative stress mediated by impaired cellular redox homeostasis, contributes to sepsis pathophysiology [17]. Similarly, we observed lower thiol levels in adult patients with sepsis when compared to controls.

Even though the pathophysiology of sepsis is still poorly understood, clinical investigations suggested an imbalance between oxidants and antioxidants; a situation defined as oxidative stress [18]. It has shown that the deficiency of antioxidants in non-survivors in severe sepsis patients correlates with mortality while the level of antioxidants was increased in survivors [19, 20]. The thiol levels are indicative of antioxidant status in the cohort [10]. The present study demonstrates that plasma thiol levels were significantly lower in patients with sepsis compared with healthy volunteers. Further more thiol levels, in non-survivors were lower than in survivors.

In oxidative stress conditions, low thiol levels and high disulfide levels were expected, in contrast, to our investigation. Probably disulfide products transform into S-nitrosothiol and sulfenic acid modifications reversibly [21]. Moreover, irreversible transformation sulfonic acids arise when oxidative stress is pervasive, causing permanent loss of protein activity [22]. For prediction, as mentioned above to be approved, further investigations are required regarding measuring reversible and irreversible modifications.

Various studies reported the importance of an abnormal TDH in acute pathological conditions. Topuz et al. have demonstrated that TDH altered during acute pulmonary embolism and may use as a prognostic marker for hospital mortality [23]. Bektaş et al. observed that thiol levels were lower in the acute ischemic stroke patients when compared to healthy controls [24]. They also showed a negative, significant correlation between the infarct volumes and native thiol levels. There fore authors suggested that a change in the thiol levels under oxidative stress may be associated with the severity of the stroke. In the present study, we observed significant differences in native and total thiol levels at follow-up in patients with sepsis via repeated measurement analyses of the sub-groups (surviving and non-surviving). Based on these findings, we speculate that determining of TDH during follow-up can serve as a novel biomarker to clinicians.

During sepsis caused by an uncontrolled inflammatory response to a pathogen, persistent oxidative stress and impaired O_2 utilization due to the

mitochondrial dysfunction may induce organ failure [25]. In addition, organ failure worsens the outcome of sepsis, three or more dysfunctional organs failure is associated with 70% ICU mortality [26]. Charan et al., showed that both the mean and highest SOFA scores were particularly useful predictors of outcome [27]. In the present study, non-surviving septic patients had higher SOFA scores than surviving septic patients. We also found a negative correlation between SOFA score and thiol levels in septic patients. It is noticeable that lower thiol levels imply the more significant oxidative burden in the non-survivors group. A relatively limited number of sample size and absence of comparison of dynamic TDH parameters with different enzymatic and non-enzymatic biomarkers of oxidative stress are the other limitations that should be stated in the present study.

To our knowledge, this is the first study demonstrating the TDH in adult septic patients. Our research presents clear evidence for altered redox balance through TDH with a new technique in septic adult patients. Further studies with larger cohorts are required to confirm the current findings and as well as comprehend the pathophysiology of oxidative stress widely in the process of sepsis.

Yazar Katkıları: Çalışma konsepti/Tasarımı: BK, GE; Veri toplama: MST; Veri analizi ve yorumlama: BTÇ; Yazı taslağı: BK; İçeriğin eleştirel incelenmesi: ÖE, GE; Son onay ve sorumluluk: BK, AİE, BTÇ, MST, GE, DBÖ, ÇDD, SN, ÖE; Teknik ve malzeme desteği: DBÖ; Süpervizyon: ÖE; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma Selçuk Üniversitesi Etik Kurulu tarafından onaylanmıştır (Onay numarası: 2016/217).
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

Author Contributions: Concept/Design : BK, GE; Data acquisition: MST; Data analysis and interpretation: BTÇ; Drafting manuscript: BK; Critical revision of manuscript: ÖE, GE; Final approval and accountability: BK, AİE, BTÇ, MST, GE, DBÖ, ÇDD, SN, ÖE; Technical or material support: DBÖ; Supervision: ÖE; Securing funding (if available): n/a.
Ethical Approval: This study has been approved by Selcuk University Ethical Committee (Approval number: 2016/217).
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

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