Acta Medica Alanya

e-ISSN: 2587-0319

Volume 4 Issue 3
September-December 2020

Cilt 4 Sayı 3
Eylül-Aralık 2020

http://dergipark.gov.tr/medalanya

actamedica@alanya.edu.tr

e-ISSN: 2587-0319

DERGİNİN KÜNYESİ/ *JOURNAL INFO*:

Derginin Adı/ *Journal Name*: Acta Medica Alanya

Kısa Adı/ *Short Name*: Acta Med. Alanya

e-ISSN: 2587-0319

doi prefix: 10.30565/medalanya.

Yayın Dili/ *Publication Language* : İngilizce /*English*

Yayın peryodu/ *Publication period*: Yılda üç kez (Nisan, Ağustos ve Aralık) / *Three times a year (April, August and December)*

Sahibi/ *Owner*: Prof.Dr. Ekrem Kalan (Rektör/ *Rector*)

Sorumlu Yazı İşleri Müdürü/*Publishing Manager*: Prof.Dr.Ahmet Aslan

Kuruluş/ *Establishment* : Alanya Alaaddin Keykubat Üniversitesi, Tıp Fakültesi bilimsel yayım organı olarak, Üniversitemiz Senatosunun 2016-95 sayılı kararıyla kurulmuştur. Yasal prosedürleri tamamlanmış ve Ekim 2016 tarihinde TÜBİTAK ULAKBİM Dergipark sistemine kabul edilerek online (çevrimiçi) olarak yayım hayatına başlamıştır. / *The scientific publishing journal of the Faculty of Medicine of Alanya Alaaddin Keykubat University. It was founded by the decision of the University Senate of 2016-95. The legal procedures have been completed and on October, 2016, on TÜBİTAK ULAKBİM Dergipark system was accepted and started publishing online.*

Dizinler ve Platformlar/ *İndexing and Platforms*: TUBITAK-ULAKBİM TR Dizin, Türkiye Atıf Dizini , Sobiad ,Türk Medline, DOAJ, CAS Source Index, J-Gate, Index Copernicus, EuroPub, Ulrich's ProQuest, CrossRef, Google Scholar, ResearchBib

Kurucular/ *Founders* : Prof. Dr. Ahmet Pınarbaşı, Prof. Dr. Fatih Gültekin, Doç. Dr. Ahmet Aslan

Web Adresi/ *Web address* : http://dergipark.gov.tr/medalanya

Yayınlayan Kuruluş/ *Publisher* : Alanya Alaaddin Keykubat Üniversitesi http://www.alanya.edu.tr/

Makale gönderim ve takip sistemi/ *Article submission and tracking system*: ULAKBİM Dergi Sistemleri http://dergipark.gov.tr/

Web barındırma ve teknik destek/ *Web hosting and technical support*: Dergipark Akademik http://dergipark.gov.tr/

İletişim/ *Contact*: Alanya Alaaddin Keykubat Üniversitesi Tıp Fakültesi Temel Tıp Bilimleri Binası Kestel Kampüsü, Alanya / Antalya. mail: actamedica@alanya.edu.tr Tel/Phone: +905056462411

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EDITORIAL

EDİTÖRYAL

Acta Medica Alanya

2020;4(3):207-208

DOI:10.30565/medalanya.809103

Trend topics in prestigious and popular medical journals: The effect of Covid-19.

"Saygın ve bilinir tıp dergilerindeki trend konular: Covid-19'un etkisi"

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ABSTRACT

Medical journals keep a critical role for reflecting on how clinical trials have evolved while researchers manage to overcome many challenges. 2020 will be the year of coronavirus for medical publications. As of 2020, The Covid-19 has announced as a pandemic viral disease by the World Health Organization. As of october 2020, when you search for ''Coronavirus-Covid-19'' in pubmed; more than 40,000 results have already found their place in literature. Now we will try to review how relevant are the prestigious and popular medical journals on this topic.

Keywords: medical journal, Covid-19, coronavirus

ÖZ

Tıp dergileri, araştırmacılar birçok zorluğun üstesinden gelmeyi başarırken, klinik araştırmaların nasıl geliştiğini yansıtmak için kritik bir rol oynamaktadır. 2020 tıbbi yayınlar için koronavirüs yılı olacak. Covid-19, 2020 yılı itibarıyla Dünya Sağlık Örgütü tarafından salgın viral hastalık olarak ilan edildi. Ekim 2020 itibarıyla, pubmed'de "Coronavirus-Covid-19" araması yaptığınızda; 40.000'den fazla sonuç literatürde yerini buldu. Şimdi bu konuda, prestijli ve popüler tıp dergilerinin ne kadar ilgili olduğunu gözden geçirmeye çalışacağız.

Anahtar sözcükler: tıp dergisi, Covid-19, koronavirüs

Received: 22.03.2019 Accepted: 20.04.2020 Published(Online):29.10.2020

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To cited: Öncel CR, Aslan A. Trend topics in prestigious and popular medical journals: The effect of Covid-19. Acta Med. Alanya 2020;4(3):207-208. doi:10.30565/medalanya.809103

Since over 2000 years, physicians and researchers continue to find solutions for eradicating diseases and improving community health. Every year medical researchers in all areas conduct new clinical studies for this purpose. At this stage medical journals keep a critical role for reflecting on how clinical trials have evolved while researchers manage to overcome many challenges. But what did recent clinical studies in medical field show us in 2020 ? what were trend topics in medical journals ? Where do we go ? If we should answer the question, there is no doubt that 2020 will be the year of coronavirus for medical publications. As of 2020, The Covid-19 viral disease that has swept into at least 213 countries with more than 34,000,000 confirmed cases and caused deaths of more than 1,000,000 people and is announced a pandemic by the

World Health Organization. As of october 2020, when you search for ''Covid-19'' in pubmed; more than 40,000 results have already found their place in literature. But how relevant are the prestigious medical journals on this topic?

The New England Journal of Medicine (NEJM) is accepted as the world's one of the leading medical journal and website. It has been published continuously for over 200 years, and now there is a separate section for Covid-19 on the journal's website. A collection of articles and other resources on the coronavirus outbreak, including clinical reports, management guidelines, and commentary can be followed from this section in NEJM.

JAMA Internal Medicine is an international peer-reviewed journal which publish articles that are stimulating to read, educate and inform readers with the most up-to-date research, and lead to positive change in general health care systems. As being one of the prestigious medical journals, it can not be expected for the journal to remain indifferent to Covid-19. For instance, almost all of the most read articles of last month were about Covid-19. Similarly when we look at the most cited article published in the journal in last three years, it is about risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China [1].

2019 has also been a great year for basic medical sciences. 2019 Nobel prize in medicine was awarded for discovering oxygen sensing mechanisms. It was really important for us to understand cellular, tissue and organismal responses in the body. CELL; is the one of the leading journals in cell and molecular biology. The journal remains at the forefront of exciting developments in this area. When the latest issues in the journal are reviewed, articles related to Covid-19 stand out. The article published by Au et al., contribute to our understanding of immunopathology and immune protection in cancer patients, placed in the context of an evolving pandemic Covid-19 [2].

The Lancet is a world leading medical journal. It is an independent, international weekly general medical journal founded in 1823 and since its first issue (October 5, 1823), the journal has worked hard to make science widely available. As expected, to assist clinicians and researchers working under challenging conditions to bring this coronavirus disease to a close, The Lancet had a Coronavirus Resource Centre. This resource brings together new 2019 novel coronavirus disease content from across The Lancet journals as it is published. One of the latest articles published online ahead of print in the journal is about extracorporeal membrane oxygenation support in Covid-19 [3].

Nature is a weekly international journal publishing the finest peer-reviewed research in all fields of science and technology. The first issue of Nature was published in November 1869. Befitting a prestigious scientific journal, the journal has tried to fulfill its duty on studies on Covid-19 disease and its treatment. As humans do not have pre-existing immunity to SARS-CoV-2, there is an immediate need to develop therapeutic agents and vaccines to ease the current pandemic and to prevent the re-emergence of Covid-19. The latest two articles of the journal are about Covid-19 vaccine and preclinical testing of vaccine candidates and therapeutic agents for this disease [4-5].

While researchers manage to overcome many challenges against this global health emergency, we are going to follow the results of the studies closely.

Conflict of interests: The authors declare that there is no conflict of interests.

Funding sources: There is no source of funding or financial interest in this study.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):209-215

DOI:10.30565/medalanya.690410

Effects of endocannabinoids in pentylenetetrazole induced seizures in mice

Farelerde pentilentetrazol ile oluşturulan epilepsiye endokannabinoidlerin etkisi

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ABSTRACT

Aim: Pentylenetetrazole (PTZ) is an agent widely used for the assessment of putative anticonvulsant drugs and is supposed to induce repetitive firing of nerve fibers as well as shorten the refractory period. Anandamide is an endocannabinoid synthesized in neurons, excreted by depolarization and inactivated very quickly. Ethanol is a psychoactive substance which has an anticonvulsive effect after acute application, although repeated administrations of high doses lead to proconvulsant actions. In order to explore whether endocannabinoids are effective in the treatment of epilepsy or not, we aimed to study the effect of anandamide on PTZ induced epileptic seizures in mice by determining which types of cannabinoid receptors are.

Materials and methods: In our small animal experimental model, thirty-two Swiss albino male mice weighing 25-35 g were used. During the study, the experimental animals were randomly divided into four groups as the control, anandamide, synthetic analogue of anandamide (WIN 55.212-2), and ethanol and the number of epileptic attacks, duration of the first epileptic attack, the total duration of the epileptic attacks and the latency time to the first attack after PTZ injection, mortality and the day of kindling development were compared in each group.

Results: The mortality rate and seizure duration were significantly lower in all of the anandamide, WIN 55.212-2 and ethanol groups. After pre-PTZ injection of ethanol and anandamide, latency periods were significantly higher, without any difference between the groups. However, a similar relationship was not present between WIN 55.212-2 and ethanol.

Conclusion: Our data showed that the antiepileptic effect of endocannabinoid anandamide observed was due to the its solvent, ethanol; however this effect was not found with its analogue WIN 55.212-2. Although both endocannabinoids resulted in interaction in the cannabinoid receptors, this difference may be the result of their different pharmacokinetics, metabolisms or degradation products and active metabolites.

Keywords: Endocannabinoids, Pentylenetetrazole, Seizure, Mice, Ethanol, Anandamide

ÖZ

Amaç: Pentilentetrazol (PTZ) yaygın antikonvülzan olduğu varsayılan ilaçların değerlendirilmesi için kullanılan bir maddedir ve sinir liflerinin tekrarlayan şekilde uyarılmasını ve refrakter süresinin kısaltılmasını sağlar. Anandamid, nöronlarda sentezlenen ve depolarizasyon sırasında atılan bir endokannabinoiddir ve çok hızlı bir şekilde inaktive olur. Epilepsi gibi sinirsel bozukluklarda, yüksek anandamid düzeyleri gözlenmiştir. Etanol akut bir uygulama sonrası, antikonvülsif etkisi olan bir psikoaktif maddedir, ancak yüksek dozlarda tekrar tekrar verilmesi prokonvülsan etkiye yol açar. Bu çalışmada amacımız endokannabinoidlerin farelerde PTZ kaynaklı epileptik nöbetleri indükleyen anandamidin etkisini, kannabinoid reseptörlerinin tiplerini belirleyerek araştırmaktı.

Materyal ve metod: Küçük hayvan deney modelimizde, 25-35 gr ağırlığında otuz iki İsviçre albino erkek fare kullanıldı. Çalışma sırasında, deney hayvanları; kontrol, anandamid, anandamidin sentetik analoğu (WIN 55.212-2) ve etanol olarak rastgele dört gruba ayrıldı. Epileptik atakların sayısı, ilk epileptik atak süresi, epileptik atakların toplam süresi ve PTZ enjeksiyonundan sonraki ilk ataktaki bekleme, mortalite ve alevlenme gelişim süresi her grupla karşılaştırıldı.

Bulgular: WIN 55.212-2, anandamid ve etanol gruplarının tümünde mortalite oranı ve nöbet süresi anlamlı olarak daha düşüktü. PTZ enjeksiyonu öncesi etanol ve anandamid uygulandığında gecikme süreleri anlamlı olarak yüksek bulundu; ancak gruplar arasında bir fark yoktu. Bununla birlikte, benzer bir ilişki WIN 55.212-2 ve etanol arasında da mevcut değildi.

Sonuç: Endokannabinoid anandamid'in gözlenen antiepileptik etkisinin, çözücüsü olan etanol'ün etkisine bağlı olduğu söylenebilir; fakat bu etki de analoğu olan WIN 55.212-2 ile gözlenmemiştir. Her iki endokannabinoid de kannabinoid reseptörlerini etkilese de, bunların etkileri arasındaki söz konusu fark, onların farklı farmakokinetik, metabolizma ya da yıkım ürünlerine veya aktif metabolitlerine de bağlı olabilir.

Anahtar Kelimeler: Endokannabinoidler, Pentilentetrazol, Epilepsi, Fare, Ethanol, Anandamid

Received: 17.02.2020 Accepted:30.05.2020 Published (Online):29.10.2020

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To cited: Yücel E, Aktuna Z, Keskil IS. Effects of endocannabinoids in pentylenetetrazole induced seizures in mice. Acta Med. Alanya 2020;4(3):209-215. doi:10.30565/medalanya.690410

INTRODUCTION

Epilepsy is a neurological disorder characterized by chronic seizures in repeating patterns of sudden episodes of loss of consciousness, convulsions and other abnormal motor activities. It is known that excitatory neurotransmitters, such as glutamate and aspartate, are effective in the emergence of epileptic seizures in the mammalian brain. Some information suggests that seizures may be related to an increase in the extracellular concentrations of excitatory amino acids; however, some experimental studies have shown a decrease in the levels of glutamate and aspartate during epileptic seizures [1]. The imbalance between the excitatory and inhibitory neurotransmitters during PTZ-induced seizures is evident in the early stage of the epileptogenic process [2]. Glutamate is metabolized to glutamine in glial cells and transported to the synaptic terminal for subsequent glutamate resynthesis [1]. This observation may explain the high levels of glutamine after PTZ injection. Glutamate facilitates calcium (Ca) influx into the cell by interacting with the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors and increases the Neuronal Nitric Oxide Synthase (nNOS) activity, consequently elevating the Nitric Oxide (NO) levels. NO has a major impact on learning but it may be toxic at high levels [3]. Depending on the stimulating agent and the NO concentration administered, it may show anticonvulsant or proconvulsant effects [4]. Reductions in NO levels produce anticonvulsant effects [5]. On the other hand, inhibitory neurotransmitters such as gamma-aminobutyric acid (GABA) and glycine affect the neuronal stimulatory mechanisms in a reverse way. GABA increases the entry of chloride (Cl-) into the cell via GABA-A subtype receptors, causes hyperpolarization of the cell membrane and prevents epileptic seizures [5].

Anandamide, isolated from the pig brain in 1992, is an endogenous cannabinoid ligand that shares many of the biochemical and physiological effects of herbal or synthetic cannabinoids [6,7]. Anandamide has been detected in the human, rat, porcine, and bovine brains, in the skin and spleen of rats, as well as in the human spleen and heart [8].

The aim of this study was to investigate the effects of endocannabinoids on epileptic seizures and to demonstrate how endocannabinoids would affect epileptic seizures and provide a new perspective for the physiopathology of epilepsy, as well as approaches used in its treatment.

MATERIALS AND METHODS

The experimental procedure was approved by the Institutional Animal Care and Use Committee of Kırıkkale University (22.02.2005, 2005/37). Every step of the experiment was planned according to animal welfare guidelines.

For the purpose of this study, Pentylenetetrazole (Sigma), Anandamide (Sigma), WIN 55.212-2 (RBI, Tocris), and Ethanol (Sigma-Aldrich) chemicals were obtained. In this study, thirty-two Swiss albino male mice weighing 25-35 g were used. The animals were housed in groups of eight per cage and were maintained at a temperature of 20-30˚C and a humidity level of 50-55% in a natural light and dark cycle, with free access to food and water. Only active and apparently healthy animals were selected for the experiments. During the study, experimental animals were randomly divided into four groups identified as the control, the anandamide, the WIN 55.212-2, and the ethanol group.

Study groups

Control group (n=11) received only intraperitoneal injections (ip) of PTZ at a dose of 60 mg/kg.

Anandamide group (n=10) received 5 mg/kg anandamide ip followed by the PTZ injection of 60 mg/kg ip 10 minutes later.

WIN 55.212-2 group (n=5) received WIN 55.212-2 at a dose of 5 mg/kg ip followed by the PTZ injection of 60 mg/kg ip 10 minutes later.

Ethanol group (n=6) received 0.10-0.12 ml of ethanol ip followed by the PTZ injection of 60 mg/kg ip 10 minutes later.

PTZ was diluted in saline to a concentration of 6 mg/ml. Anandamide was initially diluted in 10 mg/ml ethanol, followed by a series of dilutions with saline. WIN 55.212-2 was diluted with 2.5 mg/2 ml ethanol, followed by a series of dilutions with

saline.

PTZ was administered ip at a dose of 60 mg/ kg in the control group. The animals included in the experiment were observed for the first 30 minutes, to record the length of time until the occurrence of the first epileptic seizure (latency period), the length of the first epileptic seizure, the number of seizures and the total duration of the seizures. Following the observing period in the first 30 minutes following the injection, the animals were observed for another 30 minutes to monitor mortality. In the experimental groups other than the control group, anandamide, WIN 55.212-2 and ethanol injections were administered 10 minutes before the PTZ injections, after which the same observation procedure as the one applied to the control group was conducted with these other experimental groups.

The seizure stages were defined as follows:

Stage 0: No evidence of convulsive activity, normal exploratory activity.

Stage 1: Ear and facial twitching, head nodding, loss of muscle tone and reduced activity.

Stage 2: Head clonus, chronic forelimb convulsions lasting less than 3 seconds (sec).

Stage 3: Chronic forelimb convulsions lasting more than 3 seconds.

Stage 4: Generalized convulsions with tonic extension episodes; full status epilepticus.

Stage 5: Death.

Statistical analysis

Statistical analysis of the data was expressed as the arithmetic mean of the number of experimental data and ± the standard error of the mean (SE). In all groups, the number of epileptic attacks, duration of the first epileptic attack, the total duration of the epileptic attacks and the latency time to the first attack after PTZ injection, mortality and the day of kindling development, were all compared. Data was assessed by nonparametric (Kruskal-Wallis) analysis of variance and by post hoc Dunn's test. Mortality was evaluated by the Fisher Exact test. $p<0.05$ was considered as statistically significant difference. During the study, "Instat" (Graph Pad, U.S.A.), Excell-2000 (Windows Edition) (Microsoft, U.S.A.) and Microsoft Word-2000 (Windows Edition) (Microsoft, U.S.A.) programs were used.

RESULTS

The latency period, defined as the time elapsed until the first epileptic seizure, was 297.8 ± 67.2 seconds (sec) (mean ± standard error) in the anandamide (n:10) group and 262 ± 28.6 sec in the ethanol (n:6) group. The latency period was significantly different in the control, anandamide, WIN 55.212-2 and ethanol experimental groups (Kruskal Wallis, $p=0.0038$). It was 155.8 ± 43.1 sec and statistically significantly lower in the control group (n: 11) compared to the experimental groups (Anandamide vs. control: $p<0.01$, ethanol vs control: $p<0.05$) (Figure 1). The latency period of 155.8 ± 43.1 sec in the control group was not statistically significantly different from the latency period of 171.4 ± 25.3 sec in the WIN 55.212-2 (n:5) group ($p>0.05$) (Figure 1). The latency periods of anandamide (297.8 ± 67.2 sec) and ethanol (262 ± 28.6 sec) groups were not statistically significantly different ($p>0.05$).

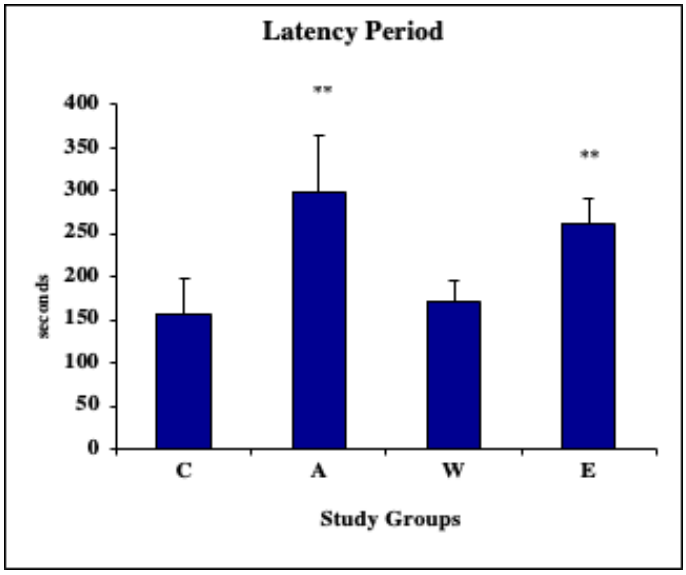

Figure 1: Comparison of the groups for the latency period. C=Control/ PTZ group, n=11; A=Anandamide group, n=10; W=WIN group, n=5; E=Ethanol group, n=6

The total duration of epileptic seizures was not statistically significantly different between the control and experimental groups. It was 32.5 ± 12.1 sec in the control group (n:11), 34.71 ± 17.5 sec in the ethanol group (n:6), 23.3 ± 4.4 sec in the anandamide group (n:10) and 48.4 ± 17.3 sec

in the WIN 55.212-2 (n:5) group (Kruskal Wallis, p=0.4) (Figure 2).

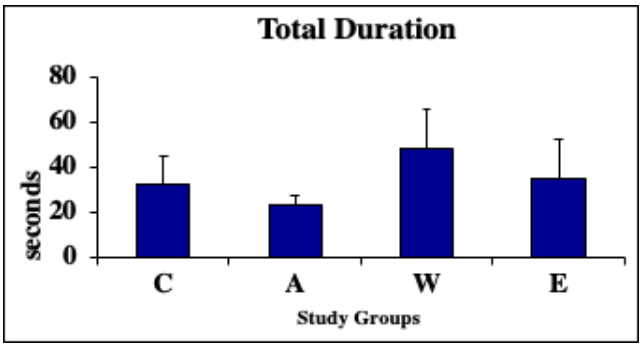

Figure 2: Comparison of the groups for the total duration of seizures. C=Control/PTZ group, n=11; A=Anandamide group, n=10; W=WIN group, n=5; E=Ethanol group, n=6

The length of the first seizure in the experimental animals was statistically significantly different among all groups in the study (Kruskal Wallis, p=0.0327) (Figure 3). However, the post-hoc binary comparisons between the groups revealed no statistically significant differences between the paired groups. There was no statistically significant difference between the control group (n:11) and the ethanol group (n:6) with 16.4 ± 5.3 sec and 16.0 ± 2.1 sec, respectively. The length of the first seizure was 18.8 ± 0.8 sec in the anandamide group (n:10) and 20.8 ± 2.3 sec in the WIN 55.212-2 (n:5) group with no statistically significant differences (p>0.05).

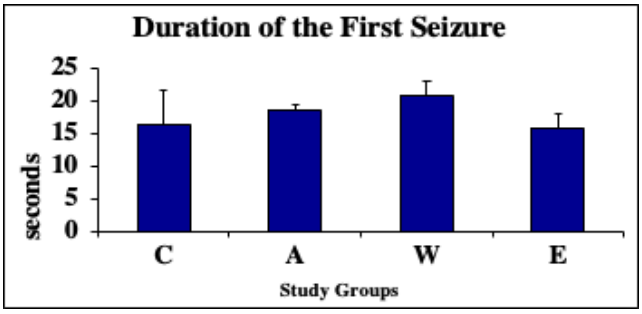

Figure 3: Comparison of the length of the first seizure between the groups. C=Control/PTZ group, n=11; A=Anandamide group, n=10; W=WIN group, n=5; E=Ethanol group, n=6

When the total number of seizures was compared between the groups, no statistically significant differences were observed. The total number of seizures was 1.3 ± 0.2 in the control (n:11) group, 1.6 ± 0.4 in the ethanol (n:6) group, 1.2 ± 0.2 in the anandamide (n:10) group, and 2.2 ± 0.6 in the WIN 55.212-2 group (n:5) (p=0.2715) (Figure 4).

Regarding mortality resulting from the epileptic seizures, the mortality rate was 45.50% in the control group (n:5/11), 0% in the ethanol (n:0/6) group, 0% in the anandamide (n:0/10) group, and 20% in the WIN 55.212-2 (n:1/5) group. The mortality rate was found to be statistically significantly ($p<0.05$) higher in the control group compared to the other experimental groups. However, there were no statistically significant differences among the mortality rates of 0% in the ethanol group, 0% in the anandamide group and 20% in the WIN 55.212-2 group ($p>0.05$) (Table 1).

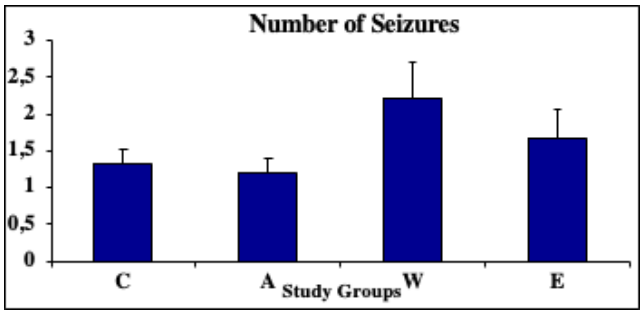

Figure 4: Comparison of the total number of epileptic seizures between the groups. C=Control/PTZ group, n=11; A=Anandamide group, n=10; W=WIN group, n=5; E=Ethanol group, n=6

Table 1. Mortality Rates in the Study Groups

	Control (n=11)	Ethanol (n=6)	Anandamide (n =10)	WIN (n=5)
Mortality	5 (45.50%)	0 (0.00%)	0 (0.00%)	1 (20.0%)

DISCUSSION

The results of our study demonstrated that the latency period was significantly higher in the ethanol group after 0.1-0.12 ml ip ethanol injection before the PTZ administration, but no significant differences were found in the duration of epileptic seizures. In the anandamide group, receiving 5 mg/kg/ml anandamide ip followed by the PTZ injection, the latency period was significantly higher compared to the PTZ group. Although there was a numerical difference between the anandamide and ethanol groups in favor of the anandamide group, the results were not statistically significant. The difference merely suggests that the anti-convulsive effect of ethanol is enhanced by anandamide, however a similar relationship was not observed between the ethanol group and the group receiving WIN 55.212-2, a synthetic analogue of anandamide.

The intracellular effects of anandamide occur through two different mechanisms. The first is the inhibition of N or P/Q type calcium (Ca++) channels via cannabinoid-1 (CB1) and/or cannabinoid-2 (CB2) receptors and the activation of potassium (K+) influx channels and mitogen activated protein kinase; this mode of action was studied by using selective receptor antagonists specific for both receptors. The second type of effect does not occur through the two known receptor subtypes but interaction occurs via the G proteins. Stimulation of arachidonic acid release and inhibition of the nexus-mediated Ca++ signaling pathway in astrocytes are examples of such effects [9].

Anandamide binds to the CB1 receptors in humans and rats with relatively high affinity [10], decreases cyclic adenosine monophosphate (cAMP) synthesis [11, 12] and reduces N-type Ca++ influx [13]. CB2 receptors are synthesized mainly in immune cells and partly in B lymphocytes (B-cells) and natural killer cells [14]. They are limited to the periphery and are not seen in the central nervous system (CNS).

Anandamide has been shown to protect cerebral cortical neurons in rats during in vitro models of ischemia [15]. Nagayama et al. found that synthetic cannabinoid WIN 55.212-2 reduced ischemic damage in the rat brain [16]. In vitro and in vivo results suggest that endogenous cannabinoids may have a neuroprotective role. Alleviation of the neuroprotective effect of 2-arachidonoylglycerol (2-AG) by SR 141617A, a CB1 receptor antagonist, clearly indicates that the mechanism is mediated through the cannabinoid receptors [17]. Anandamide and 2-AG may act as neurotransmitters or neuromodulators. There is evidence that they are synthesized in neurons when needed: they can be released from the neurons in association with depolarization and, once released, they are removed from the extracellular space via the membrane transport process [18, 19 , 20, 21]. Anandamide is suggested to be hydrolyzed to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH) [18, 20, 22, 23].

Increased levels of arachidonoyl ethanolamide anandamide (AEA) have been found in neurological disorders, such as epilepsy. The hydrolysis of AEA was measured by the rate of synthesis of ethanolamine, the product of the AEA (separated from the substrate using activated charcoal). FAAH activity was found to be similar in the epileptic and nonepileptic human cortex (0.29 and 0.37 nmol ethanolamine/ mgr protein/ min, respectively) [24].

The results of the Steffens et al. study suggest that elevated AEA levels during epilepsy resulted from increased synthesis and did not appear to be associated with reduced hydrolysis. The administration of FAAH inhibitors to further enhance the endocannabinoid activity, may be therapeutically useful in treating neuronal hyperexcitability [25]. The results of the Keith A. Kwan Cheung et al. study suggest that, elevated in particular, the cannabinoid cannabidiol (CBD) appears to have anti convulsant and anti-inflammatory properties, and it shows promise for epilepsy treatment. There are a multitude of signaling pathways that involve endocannabinoids, eicosanoids and associated receptors, by which cannabinoids could potentially exert their therapeutic effects. Further research is needed to better characterize these pathways, and consequently, improve the application and regulation of medicinal cannabis [26].

Cannabidivarin (CBDV), the propyl analogue of CBD, showed anti-convulsant properties in pre-clinical studies, however a plant-derived, purified proprietary formulation of CBDV, recently failed the Phase II randomized clinical trial (RCT) in patients with uncontrolled focal seizures [27].

CBD anticonvulsant effects are associated with a great variety of mechanisms of action such as endocannabinoid and calcium signaling. CBD has shown effectiveness in the clinical scenario for epilepsies but its effects on epilepsy-related comorbidities are scarce, even in basic research. More detailed and complex behavioral evaluation about CBD effects on seizures and epilepsy-related comorbidities are required [28].

CBD has a clear interaction with clobazam, significantly increasing the levels of its active metabolite N-desmethylclobazam in several studies: this is felt to be due to CBD's inhibition of CYP2C19. Expanded access program (EAP) data demonstrates other possible interactions

with rufinamide, zonisamide, topiramate, and eslicarbazepine. Additionally, there is one case report demonstrating the need for warfarin dose adjustment with concomitant CBD. Our understanding of CBD's efficacy and safety in the treatment of temporal region epilepsy (TRE) has expanded significantly in the last few years. Future controlled studies of various ratios of CBD and Δ9-tetrahydrocannabinol (THC) are needed however, as there could be further therapeutic potential of these compounds for patients with epilepsy [29].

A single dose of WIN 55.212.-2 administered soon after status epilepticus (SE) improved survival of animals and reduced cell loss in the dentate hilus but did not prevent appearance of spontaneous recurrent seizures in the chronic period. Thus, a brief pharmacological stimulation of the endocannabinoid system soon after a brain insult exerts beneficial effects on its pathological outcome though does not prevent epileptogenesis [30].

Limitations of Study:

This study was designed to evaluate the effect of anandamide on pentylenetetrazole induced epileptic seizures in small animals, by determining which types of cannabinoid receptors are. The result of this small experimental study was obtained with a limited numbers of experiments and experimental groups, principally as a result of economic constraints. Therefore, we concluded that in order to clarify the relationship between endocannabinoids and epilepsy, it would be necessary to add the WIN 55.212-2 group, which we did not do. In addition, studies should be conducted with other analogues, specific receptor agonists-antagonists and in particular, concentrations of ethanol in order to clarify the ethanol relationship.

CONCLUSION

As an interpretation of our results, it can be said that the observed antiepileptic effect of endocannabinoid anandamide is due to the effect of its solvent, ethanol, however this effect was not found with its analogue WIN 55.212-2. Although both endocannabinoids affect the CB1 and CB2 receptors, this difference between their effects may be the result of their different pharmacokinetics, metabolisms, or perhaps their degradation products and active metabolites.

Finally, we acknowledge that the findings from our current study require further validation. Future studies will build further on our existing pilot data, involve increased numbers of mice, use different or specific receptor agonist of endocannabinoids, as well as further explore the interaction of ethanol in various concentrations.

Conflict of Interest: The authors have no relevant conflicts of interest and received no financial advantage for this experimental study.

Funding Sources: The authors received no financial support.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):216-219

DOI:10.30565/medalanya.706592

Does iron-deficiency anemia affect M1 macrophage activation and inflammation?

Demir eksikliği anemisi M1 makrofaj aktivasyonu ve inflamasyonu etkiler mi?

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ABSTRACT

Aim: Iron deficiency anemia (IDA) is a prevalent disorder and may be a problem for various systems. Anemia of inflammation has been extensively investigated before, but there is still a lack of knowledge about macrophage activation in IDA. Hence, the aim of this study was to investigate the relationship between IDA and macrophage activation.

Patients and Methods: The present study included 88 female subjects. The participants were divided into two groups: 48 IDA patients in the patient group and 40 healthy subjects in the control group. M1 macrophage activation was measured with the triggering receptor expressed on myeloid cells-1 (TREM-1). TREM-1 levels and C-reactive protein were compared between patient and control groups. The relationship between TREM-1 levels and hemogram parameters and iron status was investigated.

Results: TREM-1 levels of the patient group were significantly higher than of the control group [124.5 (6.8-770.5) pg/ml vs 48.5 (0.66-401.1) pg/ml, p=0.02], while CRP levels remained similar between the groups. There was no correlation between TREM-1 levels and hemoglobin, mean erythrocyte volume, ferritin, transferrin saturation and serum iron (p =0.96, 0.14, 0.21, 0.16, and 0.26, respectively) in IDA patients.

Conclusion: The present study showed that IDA might increase TREM-1 levels and this condition might be a clue of macrophage activation. IDA patients should be considered in terms of pro-inflammatory conditions and further investigations are needed to clarify the association mentioned above.

Keywords: Anemia, iron deficiency, triggering receptor expressed on myeloid cells-1

ÖZ

Amaç: Demir eksikliği anemisi (DEA) farklı sistemler için sorun olabilen yaygın bir bozukluktur. İnflamasyonun oluşturduğu anemi çalışılmakla birlikte, DEA'nın yol açtığı makrofaj aktivasyonu hakkında bilgi eksikliği bulunmaktadır. Bu çalışmada DEA ile makrofaj aktivasyonu arasındaki ilişkinin araştırılması amaçlanmıştır.

Hastalar ve Yöntem: Çalışmaya 88 kadın katılımcı alındı. Katılımcılar; 48 DEA hastası ve 40 sağlıklı kontroller olmak üzere iki gruba ayrıldı. M1 makrofaj aktivasyonu, triggering receptor expressed on myeloid cells-1 (TREM-1) düzeyleri ölçülerek değerlendirildi. CRP ve TREM-1 düzeyleri iki grupta karşılaştırıldı. TREM-1 düzeyleri ile vücudun demir durumu ve hemogram parametreleri arasındaki ilişki araştırıldı.

Bulgular: TREM-1 düzeyi, hasta grubunda kontrol grubuna göre daha yüksek saptandı [124.5 (6.8-770.5) pg/ml vs 48.5 (0.66-401.1) pg/ml, p=0.02]. CRP düzeyi her iki grupta benzerdi. Hasta grubunda TREM düzeyi ile hemoglobin, ortalama eritrosit hacmi, ferritin, transferrin saturasyonu, serum demiri arasında korelasyon saptanmadı (sırasıyla p değerleri 0.96, 0.14, 0.21, 0.16, 0.26).

Sonuç: Bu çalışmada DEA'da TREM-1 düzeylerinin artışının gösterilmesi, makrofaj aktivasyonunun göstergesi olabilir. DEA hastaları proinflamatuvar durumlar açısından göz önünde bulundurulabilir. Bahsedilen ilişki için daha kapsamlı çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: Anemi, demir eksikliği, triggering receptor expressed on myeloid

Received: 19.03.2020 Accepted: 12.06.2020 Published (Online):29.10.2020

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To cited: Karahan İ, Çifci A, Dindar Badem N. Does iron-deficiency anemia affect M1 macrophage activation and inflammation? Acta Med. Alanya 2020;4(3): 216-219. doi:10.30565/medalanya.706592

Introduction

Iron deficiency anemia (IDA) is a frequent and global healthcare problem, in particular for young women in developed countries. Iron is necessary for biologic functions, such as cell cycles, energy production, respiration and DNA synthesis [1]. Impairing oxygenation at tissue level, IDA has adverse effects on the cardiovascular system [2] and while anemia is well-known in inflammatory conditions, the effects of anemia on immunity, especially on macrophages, require clarification. Macrophages are the main elements of the innate immune system and they play a critical role in the stimulation of the adaptive immune system. They can polarize to M1 and M2 macrophages according to the type of stimulation, wherein M1 macrophages produce pro-inflammatory proteins while M2 macrophages produce anti-inflammatory factors [3]. The triggering receptor expressed on myeloid cells-1 (TREM-1) is a glycoprotein that weighs 30 kDa and owns an extracellular V-type Ig-like domain from the immunoglobulin superfamily. It is expressed from cell membranes of neutrophil, monocyte, and macrophages. TREM-1 induces various pro-inflammatory chemokines and cytokines and also reflects M1 macrophage activation [4]. It has a crucial role in initiating the inflammatory process by cross-talking with Toll-like receptors and/or nucleotide-binding oligomerization domain-like receptors (NLRs). It was defined in infectious diseases and considered as a response to infections. There are soluble and membrane-bounded TREM-1 forms [5] and although TREM-1 is a main potential biomarker of infectious diseases [6], the negative results of high TREM-1 levels were studied in inflammatory but non-infectious diseases, such as irritable bowel syndrome [7], inflammatory bowel diseases [8], obstructive sleep apnea [9], and coronary artery disease [10].

It is still not known whether IDA may direct macrophages to M1 polarization and cause pro-inflammatory status. Therefore, the present study aimed to investigate the effects of IDA on M1 macrophage activation, via TREM-1 levels.

Materials and methods

Participants

The present study included 88 female subjects who were between 18 and 45 years of age and in the reproductive period. The subjects were selected among people who admitted to Kırıkkale University, Department of Internal Medicine Outpatient Clinic between August and October 2019. The participants were divided into two groups: while the patient group was composed of patients with IDA, healthy participants constituted the control group. For this purpose, hemoglobin (Hb) level <12 g/dl, transferrin saturation <16%, and ferritin <10 µg/l were accepted as the criteria of the diagnosis of IDA [1]. TREM-1 levels were compared between the two groups and the relationship between these, iron parameters and hemogram parameters of the patients were sought. The alanine aminotransferase (ALT), as a liver function test, and serum creatinine, as a kidney function test, were noted for the evaluation of organ failures. C-reactive protein (CRP) levels were compared between the two groups regarding inflammation. The patients with inflammatory conditions, such as polycystic ovary syndrome, anemia resulting from secondary causes, menopause, smoking, any organ failure, chronic illnesses as well as any infectious disease, were excluded from the study. Informed consent was obtained from all the participants.

Biochemical analysis

All blood samples were collected from the antecubital vein after a 12-hour fasting period. The samples were then centrifuged at 3500 rpm for 10 minutes and their sera were separated. The serum was divided into groups and stored at -80°C until the analysis. Serum human TREM-1 levels were measured using the micro ELISA method with the help of the HUMAN TREM-1 (Cusabio® code: CSB-E04836h, uniprot: Q9NP99). Blood counts, iron parameters, and liver and kidney function tests were studied at Kırıkkale University, Biochemistry Laboratory. The results were exported as pg/ml.

Statistical analyses

The IBM SPSS version 25.0 was used for all the statistical analysis. Normally distributed data was summarized as mean and standard deviation, while the median (minimum-maximum) was given for non-normally distributed values. The normality was checked with the Kolmogorov-

Smirnov test. The two-group comparisons were performed using the Mann-Whitney U test or the T-test. Spearman's Correlation Coefficient was calculated to determine the correlation between TREM-1 levels and Hb, ferritin, and transferrin saturation. The significance level was taken as $p< 0.05$ in all statistical analysis.

Ethical consideration: Kırıkkale University, Institutional Review Board granted ethical approval for the study (Date: 07.08.2019, Number: 17/04).

Results

All the participants were divided into two groups: 48 IDA patients in the patient group and 40 healthy participants in the control group. The median age of the patient group was 35 years while it was 32 years in the control group. Hemoglobin, transferrin saturation, ferritin, and serum iron levels were significantly lower in the IDA group, as expected ($p<0.001$ for each parameter). Thrombocyte counts were higher in the patient group, which was evaluated as reactive thrombocytosis. CRP levels were similar between the groups, while TREM- 1 levels were significantly higher in the patient group than in the control group [124.5 (6.8-770.5) pg/ml vs 48.5 (0.66-401.1) pg/ml, p=0.02] (Table 1). There was no correlation between TREM levels and Hb, ferritin, transferrin saturation and serum iron ($p = 0.96, 0.21, 0.16, 0.26$, respectively)

Discussion

The present study concluded that IDA might raise plasma TREM-1 levels and this result may be a clue for the direction of pro-inflammatory macrophages by IDA. TREM-1 levels may also not be related to the levels of iron parameters. The difference of platelet counts in the two groups can be explained by reactive thrombocytosis of IDA. TREM-1 elevation may be about anemia but iron status.

A recent study showed that cell iron status might influence macrophage polarization. Low iron diet exacerbated pro-inflammatory response via M1 macrophages, while intracellular iron increase reduced inflammation and M2 macrophage polarization in vivo and in vitro [11].

One study on an animal model concluded that neonatal piglets with dietary iron deficiency had impaired peripheral immunity [12]. Another animal study showed that iron supplementation in mice induced innate cellular defenses and made the organism stronger to malaria infection [13].

Table 1. Two-group comparisons about subject and laboratory values.

	Patients with IDA (n=48)	Control group (n=40)	Significance
Age, years	35 (19-45)	32 (20-45)	p=0.14
BMI, kg/m2	21.9 (19.3-24.8)	23.6 (19.4-24.8)	p=0.37
Hemoglobin (g/dl)	9.6(5.5-11.6)	13.1(12.0-15.5)	p<0.001
Platelet counts ($x10^3$/µl)	304 (176-556)	256 (156-347)	p<0.001
Leukocyte counts ($x10^3$/µl)	6.5(4.2-9.4)	7.4(4-11.2)	p=0.18
Ferritin (µg/L)	4.6 (2.1-8.8)	46 (16-203)	p<0.001
Transferrin saturation (%)	5 (0.8-17)	26 (16-128)	p<0.001
Serum iron (µg/dl)	21.5 (2-58)	66 (28-186)	p<0.001
Creatinine (mg/dl)	0.6 (0.4-0.8)	0.7(0.5-0.8)	p=0.11
ALT	12.7(5.6-20.7)	12 (5-21)	p=0.78
C-reactive protein (mg/l)	2.43 (0.3-4.6)	2.26 (0.8-3.6)	p=0.61
TREM-1 (pg/ml)	124.5 (6.8-770.5)	48.5 (0.66-401.1)	p=0.02

Macrophages have a critical role in iron homeostasis by recycling iron through phagocytosis of old erythrocytes and making it suitable for erythropoiesis. Macrophage polarization is closely related to differential regulation of iron metabolism due to the molecules involved in iron uptake, storage, and release [14,15]. Low intracellular iron in macrophages may prevent the expression of pro-inflammatory cytokines, such as IL-6 and TNF-α, and it may also decrease the expression of inducible nitric oxide synthase [16,17]. Iron deficiency in M2 macrophages also prevent the formation of functional iron-containing enzymes in arachidonic acid metabolism. In addition, Iron may affect the biosynthesis of the iron-containing enzyme, tyrosine hydroxylase, which catalyzes the rate-limiting step in catecholamine biosynthesis and, consequently, the inflammatory response [18]. Nevertheless, the present study showed that TREM-1 levels were higher in the patient group, which is not consistent with the findings above.

Iron retention in macrophages stimulates the expression of pro-inflammatory cytokines and the innate immune response in vivo [19]. The iron accumulation and the induction of the M1 phenotype are characterized by the production of reactive oxygen and nitrogen species, which may result in impaired capacity for tissue repair [20]. Therefore, it can be asserted that iron has diverse effects on macrophage function via multiple pathways.

Askar et al. [21] demonstrated that pro-inflammatory cytokines, such as TNF-α and interleukin-6, antimicrobial proteins, such as hepcidin, defensin, and chemerin, acute phase reactants, such as C-reactive protein, were elevated in older patients with IDA. The investigators noted that both aging and IDA might affect pro-inflammatory cytokines. Their findings support ours and may be a clue for the inflammation occurring in IDA.

On the other hand, the present study had several limitations. It was a case-control study with a relatively small sample size. Moreover, only female patients were included in the study: male participants were excluded from the study because IDA in male patients was a result of other conditions, which could have affected the standardization among patients with IDA. Additionally, TREM-1 levels were evaluated only in the plasma, while the other parameters about macrophage polarization and inflammatory cytokines were not assessed. Moreover, we did not evaluate other types of anemia in this study. To the best of our knowledge, ours is the first research study about TREM-1 levels of IDA patients, hence more extensive and comprehensive studies are required to explain macrophage polarization and IDA.

The present study concluded that iron deficiency anemia, which is a prevalent disorder, may cause elevations in TREM-1 levels. This condition may imply that IDA induces macrophage polarization to M1 macrophages which are pro-inflammatory cells. IDA patients should be considered for both inflammation and response to inflammation, and further investigations are needed to clarify the mechanism of inflammatory processes in IDA patients.

Financial support and conflict of interest

The authors declare that there is no financial support and conflict of interest regarding the publication of this article.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3): 220-225

DOI:10.30565/medalanya.725992

Predictors of Early Mortality in Geriatric Patients after Hemiarthroplasty for Femoral Neck Fracture

Femur Boyun Kırığı Nedeniyle Hemartroplasti Uygulanan Yaşlı Hastalarda, Erken Mortalitenin Öngörücüleri

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ABSTRACT

Aim: Hemiarthroplasty is an appropriate treatment option for hip fractures that provides early mobilization, and good functional results. In the available literature, there are few studies that particulary investigate the risk factors affecting the 30-day mortality after hemiarthroplasty in geriatric patients with hip fractures. This study aimed to determine patient-related factors and biochemical predictors, which were easily accessible, inexpensive, and routinely examined in the perioperative period of 30-day mortality in geriatric patients after hemiarthroplasty for femoral neck fractures.
Materials and methods: We enrolled 169 patients in our study, retrospectively. Age, body mass index, hospitalization time from admission to surgery, total hospitalization time, comorbidities, American Society of Anesthesiologists scores, blood transfusions, anticoagulant medications, albumin and plasma replacements, preoperative and postoperative hemogram, and biochemical tests were analyzed to determine the major predictors of 30-day mortality.
Results: The 30-day mortality rate was 14.2%. Multivariate analysis indicated increased age (>80 years of age) (1.095 odds ratio, p = 0.029), American Society of Anesthesiologists score (3.584 odds ratio, p = 0.007), and postoperative creatinine level (2.845 odds ratio, p = 0.001) as the major predictors of 30-day mortality after hemiarthroplasty for femoral neck fractures in geriatric patients.
Conclusion: Older age (>80 years of age), higher American Society of Anesthesiologists scores (ASA score 3 or 4) and increased postoperative creatinine levels were associated with an increased risk of 30-day mortality.

Key words: femoral neck fractures; hip fractures; creatinine; hip replacement arthroplasty; osteoporotic fractures; mortality

ÖZ

Amaç: Kalça kırıkları tedavisinde uygulanan hemiartroplasti ile erken mobilizasyon ve iyi fonksiyonel sonuçlar elde edilmektedir. Güncel literatürde, kalça kırığı olan geriatrik hastalarda hemiartroplasti sonrası 30 günlük mortaliteye etki eden risk faktörlerini araştıran az sayıda çalışma bulunmaktadır. Bu çalışmamızda, geriatrik femur boyun kırıklarında hemiartroplasti uygulaması sonrası 30 günlük mortaliteye etki eden hasta ile ilişkili faktörler, ucuz, rutin olarak kullanılan ve kolayca ulaşılabilen biyokimyasal belirteçlerin incelenmesi amaçlanmıştır.
Yöntemler: Çalışmamızda 169 hasta retrospektif olarak incelenmiştir. Yaş, vücut kitle indeksi, başvuru - ameliyat arası geçen süre, toplam yatış süreleri, komorbid hastalıklar, Amerikan Anestezi Derneği Skorları, kan transfüzyonu, antikoagülan ilaç kullanımı, albumin ve plazma replasmanları, ameliyat öncesi ve sonrası hemogram ve biyokimya testlerinin 30 günlük mortaliteye etkisi incelenmiştir.
Bulgular: 30 günlük mortalite oranı %14,2 olarak bulunmuştur. Çoklu değişkenler analizi sonucunda; yaş (>80 yaş) (1.095 odds ratio, p = 0.029), Amerikan Anestezi Derneği Skoru (3.584 odds ratio, p = 0.007) ve ameliyat sonrası kreatinin değerleri (2.845 odds ratio, p = 0.001), geriatrik femur boyun kırıkları için uygulanan hemiartroplasti sonrası 30 günlük mortalitenin ana belirleyicileri olarak bulunmuştur.
Sonuç: İleri yaş (>80 yaş), yüksek Amerikan Anestezi Derneği skorları (ASA skoru 3 veya 4) ve artmış ameliyat sonrası kreatinin değerleri, artmış 30 günlük mortalite riski ile ilişkili bulunmuştur.

Anahtar kelimeler: femur boynu kırıkları; kalça kırıkları; kreatinin; kalça replasman artroplasti; osteoporotik kırıklar; mortalite

Received:23.04.2020 Accepted:07.07.2020 Published (Online):29.10.2020

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To cited: Tanoglu O, Arican G, Ozmeric A, Sahin O, Iltar S, Alemdaroglu BK. Predictors of Early Mortality in Geriatric Patients after Hemiarthroplasty for Femoral Neck Fracture. Acta Med. Alanya 2020;4(3): 220-225 doi:10.30565/medalanya.725992

Introduction

Proximal femur fractures are commonly seen in geriatric patients and are as a significant cause of mortality and morbidity [1-7]. An increased life expectancy increases the incidence of future geriatric proximal femur fractures [1, 2]. These fractures necessitates consideration of patient related factors for determining the surgical method [8, 9]. Hemiarthroplasty is an appropriate treatment option for a certain number of patients as it provides early mobilization and good functional results for unstable intertrochanteric and femoral neck fractures [10, 11].

Many studies have focused on the factors affecting 30-day mortality after varied treatment modalities for proximal femur fractures [1, 4, 5, 7-9, 12-19]. Most of these studies investigated the effects of different predicting factors on mortality, including the American Society of Anesthesiologists (ASA) score, sex, age, comorbidities, medications, troponin-T levels, surgeon experience, alanine transaminase (ALT) levels, hemoglobin (Hb) count, creatinine (Cre) levels, renal insufficiency, serum lactate levels, lymphocyte count, serum urea levels, type of hip fracture, and particularly hospitalization time between admission and operation [1-10, 12-23]. Routine blood tests, which are inexpensive, reliable, and accessible, that were frequently used in these studies can predict mortality risk after proximal femur fractures in the geriatric population [1, 2, 6, 8, 23].

The majority of studies examined the impact of hospitalization time between admission and surgery on the mortality rate; however, the results of these studies still remain controversial [4, 7, 9, 12-15, 21]. A majority of these studies demonstrated statistically significant, lower mortality rates with early surgery [4, 7, 9, 14, 15]. However, some other studies found no association between the preoperative hospitalization time and mortality [12, 13, 21].

There are several studies on the routine biochemical parameters that affect the 30-day mortality following cemented or cementless hemiarthroplasties for proximal femur fractures in the geriatric population [5, 17, 22].

Our study aimed to determine the patient-related factors and biochemical predictors, which were easily accessible, inexpensive, and routinely examined during the pre- and postoperative periods, of 30-day mortality in geriatric patients after hemiarthroplasty for the femoral neck fractures.

Material and methods

This retrospective study was conducted after obtaining the approval of the institutional review board of Ankara Research and Training Hospital (Approval Number: 07.06.2017/103). Between January 2015 and December 2018, 169 geriatric patients with femoral neck fractures who were treated with cemented or cementless hemiarthroplasty at Ankara Research and Training Hospital, Orthopaedics and Traumatology Department were enrolled in the study.

Patients younger than 65 years, those who were lost to follow-up, and who exhibited pathological fractures, those with polytrauma with concomitant fractures, and those with incomplete medical records were excluded from our study.

A total of 169 patients, 63 (37.2%) males and 106 (62.7%) females, who met these criteria were enrolled in our study.

Blood samples from all patients were taken at admission and 6 hours postoperatively. Blood samples of patients with pre-existing chronic renal failure were collected after preoperative hemodialysis.

Thromboembolic prophylaxis with 4000 IU low molecular weight heparin was subcutaneously administered at admission and was continued until the end of the postoperative first month. The hemiarthroplasties were performed by two surgeons using a modified Gibson approach in the lateral decubitus position. Preoperative intravenous prophylaxis with 1000 mg first-generation cephalosporin was administered to all patients 30 minutes before the surgery and continued for 24 hours postoperatively. Cemented or cementless standard bipolar prosthesis (Platin Medikal, Ankara, Turkey) was utilized for the fractures (Figure 1). A Hemovac drain was placed under the fascia for 24 hours postoperatively. There were no intraoperative deaths or complications

related to anesthesia or orthopedic intervention. All patients were followed up at the postoperative second and fourth week.

Patient medical records, hospital registry system, and radiological images were retrospectively investigated. Age, body mass index (BMI), hospitalization time from admission to surgery, total hospitalization time, comorbidities, ASA scores, blood transfusions, anticoagulant medications, albumin (Alb) and fresh frozen plasma replacements, preoperative and postoperative Hb level, platelet, white blood cells (WBCs), neutrophil and lymphocyte counts, Cre, blood urea nitrogen (BUN), sodium (Na), potassium (K), calcium (Ca), Alb, ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase, lactate dehydrogenase (LDH), international normalized ratio, bilirubin levels, hospitalization in the intensive care unit, and enzyme-linked immunosorbent assay markers were analyzed for all patients.

Statistical analysis was performed using the IBM SPSS Version 19 program (IBM Software, New York, United States). The results were presented as numbers and percentages for categorical variables and as mean ± standard deviation, median, and minimum- maximum for continuous variables. The distribution normality for continuous variables was confirmed using the Kolmogorov–Smirnov test. Comparison of the categorical variables between groups was performed using Chi-square or Fisher exact test. Independent continuous variables between the two groups were compared using the Student's t-test or Mann–Whitney U test depending on whether the statistical hypotheses were fulfilled or not. Multivariate logistic regression analysis was performed to determine the effects of possible risk factors on mortality risk. Thus, on the basis of the univariate analysis, variables significantly related to mortality risk and those with a significance of $p < 0.25$ were included in the analysis. Age and sex were included in the model as a biological factor. Post-hoc power analysis was performed with the use of G-Power Version 3.1.9.2 (Düsseldorf University, Düsseldorf, Germany), and the power of the study was found to be 98.8%. Power analysis was performed using the odds ratio (OR) = 2.845, R-square = 0.04 (between postoperative Cre and all other covariates in the model), and probability of mortality = 0.10 when the postoperative Cre is one standard deviation above its mean and all other covariates are set to their mean values. The statistical level of significance for all tests was considered as 0.05.

Results

In our study, 24 patients (14.2%) died within 30 days postoperatively, of whom 12 were males and 12 were females ($p = 0.16$).

Cemented hemiarthroplasty was performed in 88 patients (52%), whereas cementless hemiarthroplasty was performed in 81 patients (48%). There was no statistically significant difference in the mortality rates between the cemented and cementless hemiarthroplasty groups ($p = 0.5$). A total of 162 patients (95.8%) were operated under spinal anesthesia, and seven patients (4.1%) were operated under general anesthesia. In the preoperative period, 67 patients (39.6%) were prescribed anticoagulant medication (acetyl salicylic acid, clopidogrel, or warfarin). The mean BMI of the patients was 27.1 ± 5 kg/m2 (range, 17.5–42 kg/m2). The comorbidities of the patients are shown in Table 1.

Table 1 Comorbidities of the patients

Diseases	Number of patients
Hypertension	126 (74.5%)
Diabetes Mellitus	55 (32.5%)
Coronary artery disease	83 (49.1%)
Respiratory system disease	66 (39%)
Chronic renal insufficiency	30 (17.7%)
Neurological disease	55 (32.5%)
Hepatitis B	5 (2.9%)
Hepatitis C	2 (1.1%)

In our study, a total of thirty patients with chronic renal insufficiency (ten patients in the deceased group and twenty patients in the surviving group) were evaluated. The main causes of chronic renal insufficiency were hypertensive nephropathy in the deceased group (70%) and diabetic nephropathy in the surviving group (60%). Although the difference between groups in terms of pre-existing chronic renal insufficiency was statistically significant according to the univariate analysis ($p= 0.001$), multivariate analysis results revealed no statistically significant difference ($p= 0.276$).

The mean hospitalization time from admission to surgery was 3.3 ± 2.9 days (range, 1–28 days). The patients in this study were divided into three groups based on the hospitalization time from admission to surgery: group 1, operation in 48 h; group 2, operation between 48–96 h; and group 3, operation after 96 h. The group distribution of patients is indicated in Table 2. Multivariate analysis revealed that there was no statistically significant difference for the mean hospitalization time from admission to surgery between the surviving (3.3 ± 3.1 days) and deceased (3.5 ± 1.9 days) patient groups in the 30-day follow-up period (p = 0.3). The mean total hospitalization time was 9.2 days (range, 3–37 days).

Table 2 Distribution of the patients based on the hospitalization time from admission to surgery

		Alive	Mortality	Total
<48 hours	n	61	10	71
	%	85.9%	14.1%	100%
48–96 hours	n	55	5	60
	%	91.7%	8.3%	100%
>96 hours	n	29	9	38
	%	76.3%	23.7%	100%
Total	n	145	24	169
	%	85.8%	14.2%	100%

Univariate analysis revealed that sex, coronary artery disease, chronic renal insufficiency, neurological disease, ASA score, age, pre- and postoperative WBC count, preoperative K level, pre- and postoperative BUN, postoperative Alb, pre- and postoperative Cre, pre- and postoperative LDH, postoperative AST, and postoperative neutrophil count were statistically significant (p < 0.025) between the two groups.

These parameters were included in multivariate logistic regression analysis to determine the major predictors of 30-day mortality after hemiarthroplasty. Age, ASA score, and postoperative Cre parameters were found to have an effect on early mortality risk (Table 3).

The mean age of our study group was 81.3 ± 6.8 (range, 65–96 years). According to the results of the multivariate analysis, there was a statistically significant difference between the age of the surviving (80.8 ± 6.8 years) and deceased (84.4 ± 5.6 years) patient groups (p = 0.029).

The results of the multivariate analysis showed that there was a statistically significant difference in the postoperative Cre levels between the surviving (1 ± 0.5 mg/dL) and deceased (1.9 ± 1.5 mg/dL) patient groups (p = 0.001). A 1 mg increase in the postoperative Cre level increases the risk of mortality by 2.8 times. OR and 95% confidence intervals for variables are presented in Table 3.

Table 3. Results of multivariate logistic regression analysis (major predictors of 30-day mortality after hemiarthroplasty for femoral neck fractures in the elderly)

	Odds Ratio	95% Confidence Interval)		P
		Lower	Upper	
Age	1.095	1.009	1.189	0.029
Postoperative Creati-nine	2.845	1.569	5.165	0.001
ASA score	3.584	1.411	9.105	0.007

The median ASA score was 3 (range, 1–4). All deaths were seen in patients with ASA scores of 3 and 4. According to the multivariate analysis results, there was a statistically significant difference in the ASA scores between the surviving and deceased patient groups (p = 0.007). An increase in the ASA score increases the risk of mortality by 3.5 times (Table 3).

Discussion

In our study, increased postoperative Cre levels, increased ASA scores (ASA scores of 3 or 4), and older age (> 80 years of age) were found to be the major predictors of 30-day mortality after hemiarthroplasty for femoral neck fractures among geriatric patients.

The strongest predictor of mortality in this study was increased postoperative Cre levels. To the best of our knowledge, there is no study suggesting that increased postoperative Cre levels are associated with increased 30-day mortality in patients who were treated with only hemiarthroplasty due to femoral neck fractures. Pre-existing chronic renal insufficiency is a known risk factor for the increased 30-days and one year mortality after proximal femur fracture surgery in geriatric population [3, 20]. There are many studies in the current literature showing that an increase in pre- or postoperative parameters indicative of renal dysfunction is associated with early and late mortality after proximal femur fracture surgery [3, 5, 7, 16-20, 22, 23]. An increased postoperative

Cre level, which can only be preventable and correctable within the parameters determined in the multivariate analysis of our study, is a major predictor of 30-day mortality. The incidence of acute renal insufficiency (ARI) after proximal femur fracture in the geriatric population is about 24% [20]. Older age, male sex, and having more than one comorbidity were found to be risk factors for developing postoperative ARI [20]. The patients who developed postoperative ARI have a longer hospital stay and an increased requirement of intensive care unit services, increased medical expenses, and increased mortality and morbidity [17, 18, 20]. Limitation of long starvation times, suitable fluid replacement, better control of intraoperative hemorrhage, correction of hypotension, avoidance of nephrotoxic medications, and prompt treatment of sepsis may prevent postoperative ARI and associated potential problems [18, 20].

The ASA score is the main comorbidity index widely used in clinical practice [24]. It is validated, universal, and easily reproducible for different patient cohorts [25]. In our study, 30-day mortality was observed only in patients with ASA scores of 3 and 4. Our results support other studies in the literature emphasizing that high ASA scores are associated with increased 30-day mortality [9, 14].

According to the results of our study, older age (>80 years of age) is a major predictor of 30-day mortality after hemiarthroplasty. Our results support other studies in the literature that have emphasized increased mortality rates for the geriatric patients after proximal femur fracture surgery in the short- and long-term follow-up [2, 7-9, 14, 16].

The mean hospitalization time from admission to surgery in our study was 3.3 ± 2.9 days (range, 1–28 days). The most important reasons for the unavoidable delays in our study were the difficulties of finding an operative theater, electrolyte imbalance, and the use of anticoagulant medication in patients [12, 15, 17]. It was noted that there was no statistically significant difference in the mortality rates between the groups when the patients were categorized according to the hospitalization time from admission to surgery. The lack of significant differences between the groups may be attributed to the heterogeneity in the distribution of patient numbers between groups and the small number of patients in our study.

In our study, 24 patients (14.2%) died within the 30-day follow-up. The 30-day mortality rates of reported studies after surgical treatment of proximal femur fractures vary between 4.3 and 11%; the mortality rate in this study was higher than that of the other studies [1, 4, 7-9, 12-16, 19]. In many studies, different treatment modalities (internal fixation methods, total hip arthroplasty, or hemiarthroplasty) have been used for treating proximal femur fractures, and the effects of these methods on mortality rates have been evaluated together [4, 8, 9, 13-16, 19]. These studies have shown that the mortality rate and risk in patients undergoing arthroplasty are higher than in those undergoing other treatment modalities [9, 13]. In these studies, the number of patients undergoing different treatment modalities, were heterogeneous with frequent application of internal fixation methods [8, 9, 13, 15, 16]. This heterogeneity may have resulted in lower mortality rates.

Another reason for the high mortality rate in our study is the inclusion of patients treated with hemiarthroplasty alone, which is the typically preferred method in, older age patients with limited mobility and low-demand [10, 11].

Our study has some limitations. The major limitation of our study was the inclusion of only the patients, who were treated with hemiarthroplasty for the femoral neck fractures. The different types of proximal femur fracture (intertrochanteric, subtrochanteric) or the different treatment modalities (total hip arthroplasty, cannulated screws, intramedullary nail, dynamic hip screw or plates) could alter the early mortality rates. Another limitation of our study may be the short follow-up period, however the one of the main goals of our study was to determine the preventable and correctable parameters, that could be used to prevent early mortality in the perioperative period. The different results could be obtained with a longer follow up period. Although our study includes the small number of samples, the power of our study was found 98.8%. A large study population may reveal different outcomes. Moreover, our study was retrospectively designed. A prospectively

designed study with a larger number of patients may yield different results.

In conclusion, hemiarthroplasty is still an appropriate and frequently used treatment option for elderly patients with femoral neck fractures, as it provides early mobilization and good functional results. The patients older than 80 years of age with ASA scores of 3 or 4, who are scheduled for hemiarthroplasty due to femoral neck fractures, should be evaluated carefully during the perioperative period in terms of increased early mortality risk. We suggest that geriatric patients treated with hemiarthroplasty for femoral neck fractures should be monitored closely with regard to renal functions during the postoperative period, and thus, appropriate treatments may be beneficial in increasing survival rates.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

Acknowledgement: Authors, thanks to Yusuf Kemal Arslan, PhD, Erzincan University Faculty of Medicine, Department of Biostatistics for their contribution.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):226-229

DOI:10.30565/medalanya.728239

Comparison of 21 G and 22 G EBUS TBNA Needles Diagnostic Value in Mediastinal and Hiler Lymph Nodes

21 ve 22 G EBUS TBNA İğnelerinin Mediastinal ve Hiler LENF nodları Tanısal Değerinin karşılaştırması

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ABSTRACT

Aim: EBUS TBNA is an important diagnostic procedure for the intrathoracic lymph nodes. 21 G, 22 G and 25 G needles are used for sampling. Better samples can be expected to be taken via 21 G needle, as the inner diameter of 21 G needle is larger. However, the results of the studies comparing 21 G and 22 G needles are controversial.
Methods: The study population consists of patients with EBUS TBNA performed via 21 G needles (Group 1; n=40) and the patients for whom 22 G needles used (Group 2; n=40). The data of patients were retrospectively analyzed. ROSE was performed for all samples.
Results: The sensitivity, specificity and diagnostic accuracy of the procedure with 21 G needle was 95%, 85%, 93%, respectively. The diagnostic accuracy of 21 G needle was found to be higher than that of 22 G needle (93% versus 80%). In the procedure performed with 21 G needle, fewer samples were sufficient for the diagnosis than 22 G needle ($r = 0.03$, $p < 0.05$).
Conclusion: The diagnostic accuracy rate of 21 G needle was higher than 22 G needle. According to that result, it is better to prefer 21 G needle. With a 21 G needle, a smaller number of sample was sufficient for diagnosis than a 22 G needle. Diagnostic opportunity with less sample obtained with 21 G needle may provide time advantage to the cytopathologist who performs ROSE. Due to this advantage, in EBUS TBNA with ROSE, 21 G needles can be prioritized.

Key words: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), Rapid On Site Evaluation (ROSE), 21 Gauge Needle, 22 Gauge Needle

ÖZ

Amaç: EBUS TBNA intratorasik lenf nodları için önemli bir tanısal işlemdir. Örnekleme için 21, 22 ve 25 G iğneler kullanılır. 21 G iğnenin iç çapı daha geniş olduğu için daha iyi örneklerin alınması beklenir. Halbu ki, 21 ve 22 G iğneleri kıyaslayan çalışmaların sonuçları çelişkilidir.
Yöntem: Çalışma grubu, 21 G (Grup 1; n=40) ve 22 G (Grup 2; n=40) iğne kullanılarak EBUS TBNA yapılmış hastalardan oluşmuştur. Hasta verileri retrospektif olarak analiz edilmiştir. Tüm örneklemlerde ROSE uygulanmıştır.
Bulgular: 21 G iğnenin sensitivite, spesifite ve tanısal doğruluğu sırasıyla %95, % 85 %93 idi. 21 G iğnenin tanısal doğruluğu 22 G iğneye göre daha yüksek idi (93% karşı %80). 22 G göre 21 G iğne ile yapılan işlemde, tanı için daha az örnek yeterli oldu ($r = 0.03$, $p < 0.05$).
Sonuç: 21 G iğnenin tanısal doğruluk oranı, 22 G iğneden daha yüksekti. Bu sonuca göre, 21 G iğneyi tercih etmek daha iyidir. 21 G iğne ile 22 G iğneye göre, daha az sayıda örnek tanı için yeterlidir. 21 G iğne ile elde edilen daha az örnekle teşhis imkanı, ROSE yapan sitopatoloğa zaman avantajı sağlayabilir. Bu avantajdan dolayı ROSE yapılan EBUS TBNA'da 21 G iğneye öncelik verilebilir.

Anahtar kelimeler: EBUS TBNA, ROSE, 21 Gauge İğne, 22 Gauge İğne

Received: 28.04.2020 Accepted: 13.07.2020 Published (Online):

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To cited: Dirol H, Uzun R, Sadullahoglu C. Comparison of 21 G and 22 G EBUS TBNA Needles Diagnostic Value in Medias-tinal and Hiler Lymph Nodes. Acta Med. Alanya 2020;4(3):226-229. doi:10.30565/medalanya.728239

INTRODUCTION

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is an important initial tool for the diagnosis of both benign and malignant pathologies [1]. It has been reported that the rate of diagnosis is higher than the conventional TBNA [2]. The diagnostic rate of the process may vary between 69% and 97% depending on various factors [3- 6]. One of these factors is that the selection of appropriate TBNA needle [7,8].

MATERİAL AND METHODS

In the EBUS TBNA process, it is recommended to use 21-22-25 G needles for cytological evaluation, whereas 19 G needles with a larger inner diameter are preferred for histological evaluation [9,10]. In this study,40 patients for whom 21 G needles were used and 40 patients for whom 22 G needles were used during EBUS TBNA, in respect to age, gender, presence of an endobronchial lesion, mass presence in thorax CT, lymph node diameter assessed by EBUS, lymph node diameter measured in thorax CT, sampled lymph node number, stations and biopsy results, were analyzed retrospectively.

Study population: The data of patients with convex probe EBUS (CP-EBUS) for diagnosis or staging between 01 January 2019 and 30 September 2019 at the Antalya Training and Research Hospital Chest Diseases clinic were retrospectively analyzed. 22G needles had been used regularly in our hospital before. But for some period, 22G needles were not supplied. At that time, 21G needles (40 in total) were used instead of 22G needle. Therefore, the study population consists of patients with EBUS TBNA performed via 21G needles (Group 1) and the first forty patients for whom 22G needles used (Group 2), immediately after the 21G needles run out.

EBUS-TBNA procedure: All EBUS-TBNA procedures were performed in the operating theatre under conscious sedation (midazolam + propofol), using a Fujinon EBUS device (7.5mhz EB-530US/Sonart SU-1, Tokyo, Japan). For the sampling, 21G needles (NA-201SX-4021; Olympus) were used in the first 40 patients, and 22G (NA-201SX-4022; Olympus) needles were used in the next 40 patients. Identification of mediastinal lymph nodes was made according to the International Association for the Study of Lung Cancer (IASLC) criteria [11]. In the patients, with suspicion of benign disease, at least two lymph node stations were sampled and at least three sampling was performed for each lymph node. In patients, suspicious for malignancy, all stations were scanned, starting from the N3 lymph node, and at least three sampling was performed for each lymph node. If there were more than one lymph node, N3-N2-N1, respectively, lymph nodes were sampled. In patients reported as benign lymph nodes, the final benign outcome was decided at least 6 months after clinical and radiological findings or surgical pathological confirmation.

Radiological evaluation: Contrast-enhanced thoracic CT and/or Positron Emission Tomography (PET-CT) was performed in all patients. The EBUS-TBNA procedure was applied to the patients with a short axis of ≥10 mm and SUV max ≥ 2,5 in mediastinal-hilar lymph nodes on CT and/or PET CT.

Pathological evaluation: Rapid On Site Evaluation (ROSE) was performed for all samples. Cytological samples were stained with Diff-Quik during the procedure and evaluated by the cytopathologist in the operating room. The remaining materials were placed in 10% formaldehyde for the cell block and sent to the pathology laboratory for histological evaluation. Samples were evaluated and reported by the same pathologist.

Statistical Analysis: Statistical analysis was performed using the SPSS (Statistical Package for Social Science, Chicago, Il, USA) 19.0 Windows packet program. Descriptive data were expressed in mean ± standard deviation, median (min-max), or number and percentage. For the comparison of definitive diagnostic rates of the groups with different needles (21G-22G), the chi-square test was used. ROC (Receiver-Operating Characteristic) analysis was performed and ROC curves were drawn to examine the consistency of the diagnostic efficacy evaluations of the study groups with the actual mortality. AUROC (Area Under the Receiver Operating Characteristic) values were calculated to compare the ROC fields. A p value of <0.05 was considered statistically

significant.

RESULTS

33 (82.5%) of 40 patients for whom 21 G needles used (Group 1) and 28 (70%) of 40 patients for whom 22 G needles used (Group 2), were male. The mean age of the patients in Group 1 was 63,23±10,51 while 62,8±12,25 in Group 2. The groups were similar in respect to age and gender. The groups were similar also in respect to the presence of endobronchial lesion, mass presence in thorax CT, lymph node diameter assessed by EBUS, lymph node diameter measured in thorax CT, sampled lymph node number and stations. The patient characteristic in Group 1 and Group 2, is summarized in table 1.

Table 1. The characteristics of the patients with 21 G and 22 G.

		21 G	22 G	p
Patient number (n)		40 (%50)	40 (%50)	-
Gender (M/F)		33(%82,5)/7 (%17,5)	28(%70)/ 12(%30)	0,16
Age		63,23±10,51	62,8±12,25	0,39
Endobronchial lesion		7 (%17,5)	3(%7,5)	0,08
Mass in CT		24 (%60)	23 (%57,5)	0,86
EBUS Lymph node diameter		20,39±4,39	20,25±4,87	0,82
BT Lymph node diameter		21,57±3,87	21,01±2,82	0,85
Stations	4	23 (%43)	22 (%35)	0,08
	7	20 (%38)	23 (%37)	
	10	4 (%8)	2 (%3)	
	11	6 (%11)	15 (%24)	

In Group 1, 30 (75%) patients were diagnosed as malignant, while in Group 2, 24 (60%) patients were diagnosed as malign. Although the diagnosis of malignancy was higher in group 1, the difference was not statistically significant (p = 0.09). On the other hand, the diagnosis of benign disease was significantly higher in Group 2.

The average number of sampling was 3,52±0,41 in Group 1, while the average number of sampling was 3,94±0,45 in Group 2 (Table 2). In the procedure performed with 21 G needle, fewer samples were sufficient for diagnosis than 22 G needle (r = 0.03, p <0.05).

While the sensitivity, specificity and diagnostic accuracy of the procedure with 21 G needle were 95%, 85%, 93%, respectively, it was 89%, 80% and 80% in the procedure with 22 G needle (Table 3). Both needles were found to have high diagnostic sensitivity and specificity (Fig.1). The diagnostic accuracy of 21 G needle was found to be higher than that of 22 G needle (93% versus 80%). This value was statistically significant (p = 0.01).

Table 2. 21 G- 22 G Sampling number

	21 gauge (n=53)	22 gauge (n=62)	p
Sampling number	3,52±0,41	3,95±0,45	0,03*

Table 3:.21 G and 22 G needle diagnostic evaluation

ROC	21G (n=40)	22G (n=40)
Sensitivity	95%	89%
Specificity	85%	80%
Diagnostic Accuracy	93% (p=0,01)	80% (p=0,01)

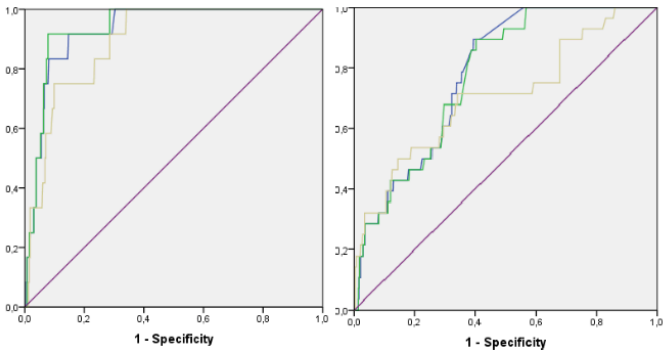

Figure 1: Diagnostic evaluation of 21G and 22G needles.

DISCUSSION

EBUS TBNA is a minimally invasive method, with a proven effectiveness for the diagnosis of mediastinal lymph nodes. 21 G, 22 G and 25 G needles are the needle types, used for cytological sampling in EBUS TBNA. Biopsies can be taken from submucosal, peribronchial, mediastinal and hilar lymph nodes with these cytological needles, which are more capable of curling than histological needles. Initially, EBUS TBNA was performed via 22G needles. Later, the 21G needle with a larger inner diameter began to take part in daily practice.

The inner diameter of the 21 G needle is 20% wider than the 22 G needle. Therefore, better samples can be expected to be taken via 21 G needle. However, the results of the studies comparing 21 G and 22 G needles are controversial. In a study,

that evaluated the EBUS TBNA results in 60 patients, no difference was found between the 21G and 22G needles in terms of diagnostic efficacy [12]. In another study, the diagnostic value of 21 G needle was found to be significantly higher than that of 22 G needle and it was suggested that 21 G needle can be prioritized especially in cases where benign pathologies such as sarcoidosis and tuberculosis are considered [13]. The study with the largest case series comparing 21 G to 22 G needles was performed by Ioony et al. In this study, according to the results of 1235 patients, it was reported that there was no difference between 21G and 22G needles in terms of sample adequacy or diagnostic efficiency [14]. In our study, the diagnostic accuracy rate of 21 G needle was higher than 22 G needle.

ROSE has a positive effect on the duration and accuracy of diagnostic procedures [15,16]. However, ROSE is a laborious and time-consuming procedure for the cytopathologist. We think that ROSE has contributed to the high efficiency of the EBUS TBNA procedure with both needles in our study. However, the data of this study is not suitable for evaluating the effect of ROSE on diagnostic efficiency and commenting on this subject. For this, it is necessary to design a separate study and compare the cases with ROSE and without. However, we can state that lesser sample was sufficient for the diagnosis via 21G needle. The average number of sampling was 3,52±0,41 via 21 G needle, while the average number of sampling was 3,94±0,45 via 22 G needle. In the Lonny et al study, similarly, lesser number of sampling was enough in 21 G group also [14]. Based on this, if you will do ROSE, it may be better to perform TBNA with a 21G needle because it allows diagnosis with less sampling, so it takes less time for your cytopathologist.

Our study has some limitations. One of them is that the study population is small. For a more reliable interpretation of whether the 21G needle provides time advantage for ROSE, new randomized controlled, double-blinded studies with a larger population is required.

In this study, it was observed that both needles were reliable in establishing the correct diagnosis of malignancy. Malignancy was diagnosed effectively with both needles and there was a high level of agreement between the malignancy accuracy rates of both needles. In comparison, the diagnostic accuracy rate of 21 G needle was higher than 22 G needle. With a 21 G needle, a smaller number of samples was sufficient for diagnosis than a 22 G needle. Diagnosis opportunity with less sample obtained with 21 G needle may provide time advantage to the cytopathologist who performs ROSE. Due to this advantage, in EBUS TBNA with ROSE, 21 G needles can be prioritized.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):230-235

DOI:10.30565/medalanya.731636

Development of De Novo Chronic Total Occlusion in Native Coronary Arteries of Coronary Artery Bypass Grafting Surgery Patients

Koroner Arter Bypass Greft Cerrahisi Hastalarının Nativ Koroner Arterlerinde Yeni Kronik Total Oklüzyon Gelişimi

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ABSTRACT

Aim: Postoperative de novo chronic total occlusions (CTOs) of preoperatively non-occluded native coronary arteries are commonly seen in coronary artery bypass grafting (CABG) surgery patients in the clinical follow-up; however, data about this course is limited. The aim of this study was to investigate the prevalence of new CTO development in native coronary arteries postoperatively and the clinical factors which may play role in this context.

Materials and methods: A total of 492 CABG patients has been searched from the computer database at Başkent University Hospital Alanya Application and Research Center and patients with a recurrent coronary angiography (CAG) procedure after the first 6 months following surgery were involved in the study population. Recurrent CAG recordings were evaluated for the presence of new CTO development. Logistic regression analysis was used to search the role of demographical and angiographical characteristics in the development of de novo CTOs in native coronary arteries.

Results: Seventy-three CABG patients with recurrent CAG were involved in statistical analysis (Mean age was 65.2 ± 9.8 years; male gender 76.7%). Two hundred eighteen preoperatively non-occluded native coronary arteries were evaluated and 119 new CTOs were detected (54.5% of involved vessels). Preoperative proximal stenosis ≥90% is related to more than 3 times new CTO development (67.8% vs. 22.2%) (p<0.001). Dual antiplatelet therapy (DAPT) is found as a protective factor for the patency of native coronary arteries (HR:-0.259; 95% CI:-0.475 to -0.017; p=0.036).

Conclusion: De novo CTO development in native coronary arteries is commonly seen in CABG patients postoperatively. Significance of preoperative stenosis and absence of DAPT seem to be the essential factors in new CTO occurrence.

Key words: coronary artery bypass grafting; native coronary arteries; chronic total occlusion

ÖZ

Amaç: Koroner arter bypas greftleme (KABG) cerrahisi hastalarının postoperatif klinik takibinde preoperatif olarak tam tıkalı olmayan nativ koroner arterlerin kronik total oklüde (KTO) hale gelmesi sık görülse de, seyriyle ilgili veriler kısıtlıdır. Çalışmamızın amacı postoperatif dönemde nativ koroner arterlerde yeni KTO gelişim sıklığını ve bu konuda rol oynayan faktörleri araştırmaktır.

Yöntem: Başkent Üniversitesi Hastanesi Alanya Uygulama ve Araştırma Merkezi'nde gerçekleştirilen tüm KABG operasyonları bilgisayar sisteminden tarandı ve postoperatif ilk 6 aylık dönemden sonra rekürren koroner anjiyografi (KAG) prosedürü uygulanan hastalar çalışma grubuna alındı. Rekürren KAG görüntüleri yeni KTO gelişimi açısından değerlendirildi ve nativ koroner arterlerde yeni KTO gelişimine yol açan demografik ve anjiyografik özellikler lojistik regresyon analizi kullanılarak test edildi.

Bulgular: Rekürren KAG yapılmış olan 73 KABG hastası istatistiksel analize dahil edildi (Ortalama yaş 65.2 ± 9.8 yıl; erkek cinsiyet %76.7). Preoperatif olarak tam tıkalı olmayan ve greftlenmiş olan 218 nativ koroner arter incelendi ve 119'unda (%54.5) yeni KTO saptandı. Preoperatif proksimal darlığın ≥%90 olması 3 kat daha fazla yeni KTO gelişimi ile ilişkili bulundu (%67.8 vs. %22.2) (p<0.001). İkili antiplatelet tedavisinin nativ koroner arterlerin açıklığını koruyucu rolü olduğu tespit edildi (HR:-0.259; %95 CI:-0.475'den -0.017'ye; p=0.036).

Sonuç: Nativ koroner arterlerde postoperatif yeni KTO gelişimi CABG hastalarında sık görülmektedir. Preoperatif darlığın daha ciddi olması ve ikili antiplatelet tedavinin yokluğu yeni KTO gelişimi için temel faktörler olarak bulundu.

Anahtar kelimeler: koroner arter bypas greftleme; nativ koroner arterler; kronik total oklüzyon

Received: 04.05.2020 Accepted: 05.07.2020 Published (Online):

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To cited: Çoner A, Akıncı S, Akbay E, Budak AB, Saba T, Müderrisoğlu H. Development of De Novo Chronic Total Occlusion in Native Coronary Arteries of Coronary Artery Bypass Grafting Surgery Patients Acta Med. Alanya 2020;4(3):230-235. doi:10.30565/medalanya.731636

Introduction

Coronary artery bypass grafting (CABG) is a well-known surgical technique for the management of diffuse coronary artery disease (CAD). For over 50 years, CABG has been recognized as an effective and safe procedure for critical CAD [1] and in recent years, surgical techniques have evolve considerably: shorter cardio-pulmonary bypass times, global usage of arterial conduits and optimal medical treatment (OMT) strategies have increased the effectiveness of CABG in clinical practice. O n the other hand, CABG patients carry unique problems in their follow-up, such as graft failure and the need of recurrent coronary angiographies (CAG) for anginal attacks, and a substantial number of them undergo recurrent CAG procedures, whereas the majority of these cases have been recognized with patent surgical grafts [2].

Acceleration of CAD in preoperatively non-occluded native coronary arteries, was identified as the responsible factor for the development of recurrent anginal attacks in various studies. Progression of native coronary arterial disease from preoperative stenosis to postoperative total occlusion is an important feature, especially in cases of graft failure [3,4]. De novo total occlusion of a priorly non-occluded native coronary artery in the long-term postoperative period is a problematic situation, because total occlusion in CABG patients generally have a more challenging nature than chronic total occlusions (CTO) in native coronary anatomy without CABG operation [5]. The more diffuse, more calcific nature of total occlusions in native coronary arteries make percutaneous revascularization harder, even impossible, in CABG patients [6].

Clinical data about the postoperative progression of stenosis in native coronary arteries in CABG patients is limited. The main aim of the study was to search the prevalence of postoperative developments of de novo CTO in native coronary arteries in the CABG population, and to define the factors related to de novo CTO developments in native coronary arteries.

Material and methods

This study was approved by the Baskent University Institutional Review Board (Project no: 19/442) and supported by the Baskent University Research Fund.

Study population: We enrolled the patients who had undergone isolated CABG surgery for revascularization of CAD at the Başkent University Hospital Alanya Application and Research Center, in Alanya, Turkey. All CABG patients enrolled in the study population were recorded retrospectively and we searched the percentage of the patients who had a clinical indication of recurrent coronary angiography (CAG) in the first 6 months following the surgery for follow-ups. We specifically did not enroll the CABG patients within the first 6 months of the postoperative period, in order to prevent the effect of probable surgical complications on the statistical evaluation. We recorded the baseline characteristics and medical treatments of the patients who needed CAG, as well as how much time had passed from the CABG to the CAG. The study population was evaluated for the development of “malign graft failure”, defined as the occlusion of both of the vascular graft and the grafted native coronary artery. Non-occluded, but severely stenosed native coronary arteries before the surgical procedure, were selected and we aimed to search the prevalence of new chronic total occlusions (CTO) of native coronary arteries, in the CABG patients.

Exclusion criteria from the study population: The consort diagram of the study population is expressed in Figure-1. We searched all CABG procedures from the hospital computer database and we applied some exclusion criteria:

- Presence of additional cardiac surgery such as valvular interventions at the same time as the CABG procedure
- CABG patients without any recurrent CAG procedure in the postoperative period
- Our inability to access preoperative and/or postoperative CAG data
- Patients with recurrent CAG within the postoperative first 6 months
- Grafted native coronary arteries with a preoperative CTO segment

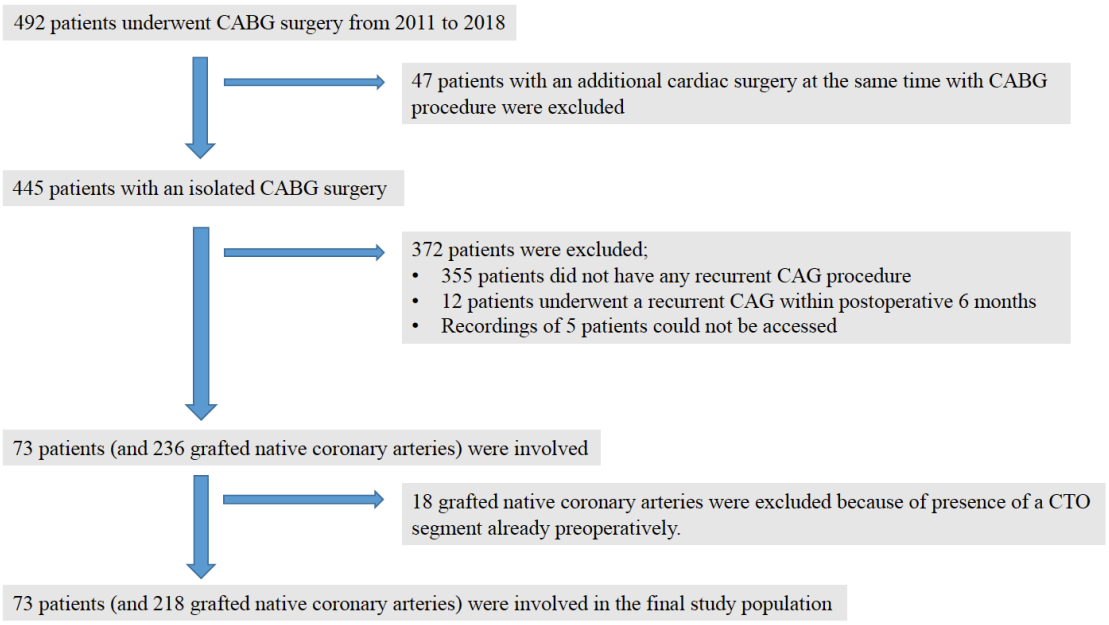

Figure-1: Derivation of the study cohort. CABG, coronary artery bypass grafting; CAG, coronary angiography; CTO, chronic total occlusion

Evaluation of coronary angiographies: All coronary angiography views were selected from the hospital's computer database and two independent cardiology specialists evaluated the views for patency of vascular grafts and native coronary arterial system. CTO were defined (preexisting or newly-developed) as the presumed occlusion of the native coronary artery, at least for 3 months prior to either preoperative or postoperative diagnostic CAGs. Severity of luminal narrowing has been classified as ≥90% or <90% in non-CTO native coronary arteries.

Statistical analysis: Continuous variables were expressed as mean ± SD and median (25th to 75th percentile) and categorical variables as percentages (%). Normal distribution was tested with the Kolmogorov-Smirnov test. Differences between the two groups were tested with the Student-t test and the Mann Whitney-U test for continuous variables, as well as the Chi-square test, for categorical variables. We evaluated the effects of demographical characteristics of the study population on clinical end points, via the multivariable logistic regression analysis. Intraclass correlation coefficient was used to test intraobserver and interobserver differences and their coefficients of variations were calculated as 2.8% and 3.3%, respectively. A p value<0.05 was accepted as significant for all statistical analysis.

Results

A total of 492 patients were found to have undergone a CABG procedure from 2011 to 2018. After the exclusion of patients whose status was in violation of the study protocol, 73 CABG patients were included in the final study population (14.8% of total CABG patients), with a mean age of 65.2 ± 9.8 years and a range of 43 to 87 years, with 56 males and 17 females (a male percentage of 76.7%). We found that a total of 236 native coronary arteries had been bypassed in these 73 patients (3.2 grafts per patient in average). Preoperative CTO of at least one native coronary artery were detected in 24.7% of patients (18 CTOs in 18 patients: there were 218 preoperatively non-CTO native coronary arteries). The time from CABG to recurrent CAG was determined to be a median of 20 months, with a minimum of 6 and a maximum of 85 months. Over 70% of study population had presented as an ACS clinic prior to CABG procedure (52 patients, 71.2% of study population). Most of the patients had undergone an urgent CABG (during the index hospitalization) performed following the basal coronary angiography (75.3%).

We evaluated 218 preoperatively non-CTO native coronary arteries for the development of new

CTO, in the postoperative follow-up. New onset CTO was found in 119 native coronary arteries (119 of 218 vessels: 54.5% of preoperatively patent native coronary bed). New onset CTO in at least one coronary artery was been seen in 72.6% of study population (53 patients). Graft failure were detected in 31 grafts (30 saphenous vein grafts, 1 radial artery graft) from a total of 236 grafts (13.1%). Malign graft failure was seen in 9 grafts from 9 patients (1 graft for each patient); its prevalence was 12.3% of patients (9/73) and 3.8% of total grafts used (9/236).

Approximately one fourth of all grafted coronary arteries revealed a preoperative stenosis of ≥90% (56 of 218 vessels, 25.6% of total non-occluded vessels included). In our study, preoperative severity of native coronary artery disease was found to be related to the development of new CTO. Coronary arteries which were narrowed ≥90% ended with a new CTO more commonly than the arterial stenosis by <90% (67.8% vs. 22.2%) (p<0.001) (Table-1).

Table-1: Effect of severity of preoperative native coronary artery stenosis on the development of new CTO (p<0.001).

		CTO development		
		New CTO (+) (# of vessels)	New CTO (-) (# of vessels)	Total # of vessels
Coronary stenosis (%)	≥90	38	18	56
	<90	36	126	162
	Total # of vessels	74	144	218

Abbreviations: CTO, chronic total occlusion

DAPT (acetylsalicylic acid plus either clopidogrel or ticagrelor) at least in the first year postoperatively was detected in 24 patients (32.9% of study population). DAPT therapy was more common in patients with ACS presentation prior to CABG (91.6% vs. 4.0%) (p<0.001). New onset CTO in native coronary arteries were lower in DAPT group (54.1% vs. 75.4%) (p=0.024). Saphenous vein graft failure was present in 27 patients (36.9%) and DAPT had a tendency to protect from saphenous vein graft failure, though this difference did not reach a statistical significance (45.8% vs. 32.6%) (p=0.310). Comparison of demographical characteristics and laboratory results regarding the presence or absence of new onset CTO, have been outlined in Table-2.

Table-2: Comparison of the demographical and laboratory characteristics of the study population regarding the development of new onset CTO in native coronary arteries.

Parameter	New CTO (+) 53 patients	New CTO (-) 20 patients	"
Age (mean±SD)	64.9±9.9	66.0±9.5	0.679
Male gender (%, N)	69.8 (37)	95.0 (19)	0.029
Time from CABG to control CAG (months) (median, 25th-75th percentile)	21.0 (11.5-42.5)	13.5 (7.0-37.5)	0.175
Diabetes mellitus (%, N)	50.9 (27)	40.0 (8)	0.404
Insulin needed diabetes mellitus (%, N)	22.6 (12)	20.0 (4)	0.808
Hypertension (%, N)	75.4 (40)	60.0 (12)	0.193
Hyperlipidemia (%, N)	35.8 (19)	50.0 (10)	0.270
İliofemoral atherosclerosis (%, N)	9.4 (5)	25.0 (5)	0.085
Carotid artery disease (%, N)	43.3 (23)	55.0 (11)	0.375
Prominent aortic calcification (%, N)	11.3 (6)	10.0 (2)	0.872
Cigarette smoking (%, N)	39.6 (21)	45.0 (9)	0.677
Preoperative glucose (mg/dL) (median, 25th-75th percentile)	111.0 (96.0-181.0)	101.5 (92.2-160.7)	0.390
Preoperative LDL-C (mg/dL) (median, 25th-75th percentile)	126.0 (103.5-156.0)	97.5 (92.0-117.5)	0.003
Preoperative HDL-C (mg/dL) (mean±SD)	39.3±11.5	38.3±9.3	0.733
Preoperative Hemoglobin (gr/dL) (mean±SD)	13.5±1.4	13.3±2.1	0.555
Preoperative Hematocrit (%) (median, 25th-75th percentile)	41.0 (38.3-44.7)	41.9 (38.0-43.4)	0.951
Preoperative Creatinine (mg/dL) (median, 25th-75th percen-tile)	0.9 (0.8-1.0)	0.9 (0.7-1.2)	0.975
Glomerular filtration rate (mL/min) (median, 25th-75th per-centile)	80.0 (65.0-93.5)	91.5 (58.7-95.0)	0.692
LV ejection fraction (%) (mean±SD)	52.0±11.8	54.8±11.8	0.372

Abbreviations: CABG, coronary artery bypass grafting; CAG, coronary angiography; LV, left ventricle

Factors which might have a role in the development of new CTO has been evaluated via a regression analysis. Age, gender, DM, smoking, LVEF, beta blocker or statin therapy were found to be unrelated to the development of new CTO. Only DAPT has shown to have a protective role from the development of new CTO in native coronary

arteries (HR: -0.259; 95% CI: -0.475 to -0.017; p= 0.036) (Table-3).

Table-3: Multivariate regression analysis of clinical factors for the prediction of the development of new CTO of native coronary arteries.

	B	Standardized Coefficients (Beta)	95 % C.I. for EXP (B)		P
			Lower	Upper	
Age (years)	-0.007	-0.147	-0.018	0.004	0.230
Gender (male)	-0.247	-0.234	-0.533	0.039	0.089
Diabetes mellitus	0.015	0.017	-0.200	0.230	0.890
Smoking	0.057	0.063	-0.180	0.294	0.634
Preoperative LVEF	-0.003	-0.081	-0.012	0.006	0.499
Beta blocker usage	-0.097	-0.128	-0.056	0.009	0.241
Statin usage	-0.028	-0.048	-0.165	0.110	0.687
DAPT usage	-0.246	-0.259	-0.475	-0.017	0.036
Constant	1.592		0.588	2.596	0.002

Abbreviations: LVEF, left ventricle ejection fraction; DAPT, dual antiplatelet therapy

Discussion

We found the prevalence of de novo CTO in native coronary arteries in 54.5% of the total grafted vessels. This prevalence is higher than the previous results reported in the literature [7,8]. A delayed latent period between CABG and recurrent CAG may be the reason for a higher de novo CTO percentage in native coronary arteries in our study: indeed, median time intervals between the surgery and the postoperative CAG was approximately 2 years in our CABG population. Additionally, the significance of proximal stenosis in native coronary arteries was found as an important predictor for the development of de novo CTO [8]. Similar to this finding, we found that a proximal stenosis ≥90% is related to more common development of new occlusion in native coronary arteries (67.8% vs. 22.2%) (p<0.001). Independent from the type of conduit used, the presence of a low resistant competitive flow seems to be the main cause of the acceleration of preexisting stenosis in native coronary arteries, in CABG patients [9]. In previous clinical studies, the competitive flow between non-occluded native coronary artery and conduit graft was thought of as the most probable mechanism of either graft failure, or new native coronary occlusion [10-12].

In the PREVENT-IV clinical trial, SVG failure was determined to be 43% at the end of first year postoperatively [13]; our result for SVG failure (13.1% of total SVGs) was therefore lower. Higher percentages of preoperative stenosis ≥90% than previous studies [14] may play a role for increased SVG patency in our CABG cohort. Cataldo et al. found that the SVG patency at the end of postoperative first year was related to several angiographic factors, such as target vessel diameter and coronary territory of right coronary artery, rather than demographical characteristics of the patients [14]. SVG failure with the possible acceleration of atherosclerosis in native coronary arteries, ends up as “malign graft failure”, an essential problem in the follow-up of CABG patients [4]. In our study, the presence of malign graft failures was low (3.8% of total grafted vessels). Otherwise, our population revealed a relatively high prevalence for native coronary arteries stenosed as ≥90% preoperatively (25.6% of total grafted vessels) and this might play a protective role from the development of malign graft failure, because of the absence of an important competitive native coronary artery blood flow.

Recent studies demonstrated the possible advantages of DAPT therapy in CABG patients, such as lower SVG failure at the end of first year postoperatively [15]. A meta-analysis of 22 clinical trials showed that DAPT therapy within the postoperative first year, has a protective role for the patency of SVGs [16]. We did not reach the same result (45.8% vs.32.6%) (p=0.310) but this might be related to our delayed median time interval between CABG and postoperative CAG procedure. Additionally, type II error could not be ignored in the failure of DAPT from protection of SVG disease. The effect of DAPT therapy on the de novo CTO development in native coronary arteries had not been studied before, and we found that DAPT may have a protective role for the patency of native coronary arteries. The new CTO percentage was lower in the DAPT group in our study (54.1% vs. 75.4%) (p=0.024). DAPT may protect the patency of native coronary artery by preventing coronary thrombosis, which can be triggered through decreased blood flow as a result of competition with anastomosed conduit graft. Decrement of blood flow rate in distal coronary bed

can be the main responsible factor in development of coronary thrombosis [15,16]. Beside DAPT therapy, beta blockage and statin treatments were also found in a tendency to prevent de novo CTO in native coronary arteries in CABG patients, but these findings did not reach statistical significance for both of these medications. In the previous randomized studies, optimal medical treatment (OMT) [17-19] was linked to better outcomes in revascularized patients, both in percutaneous coronary intervention and CABG patients. We did not find the similar effect of OMT, but insufficient patient compliance in Turkey to prescribed medical treatments such as beta blockers or statins, because of their possible side effect; this may be the most probable cause for statistical analysis not reaching significance.

In conclusion, de novo CTO development in native coronary arteries is commonly seen in the follow-up of CABG patients. Preoperative significance of proximal stenosis and absence of postoperative DAPT treatment, are the prominent factors related to the development of new total occlusion in native coronary arteries. We should define a sweet spot point for DAPT usage, somewhere between protectiveness from de novo CTO development and increased risk of bleeding. DAPT should be considered in the first year postoperatively, in particular in patients with low bleeding risk. Additionally, we need prospective, randomized clinical trials to search the compliance to medical treatment in CABG patients in Turkey.

Study limitations

Our study has some limitations, namely the fact that it was a single center evaluation with a retrospective design, and we need prospective studies in this context. Small population size is another limitation and may have a possible effects, such as type II error. Additionally, all of the surgeries were performed by the same surgeon, the atherosclerotic progression in the non-grafted native coronary arteries were not evaluated and were not compared with the grafted ones, and we did not examine the effect of vascular conduit type or grafted coronary territory on the development of endpoints. Finally, complications related to medical treatment such as bleeding were not examined in our clinical evaluation.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):236-241

DOI:10.30565/medalanya.745576

Investigation of the Endothelial Response of the Super Elastic Braided Stent: An Experimental Evaluation

Süper Elastik Örgülü Stentin Endotel Yanıtının İncelenmesi: Deneysel Bir Değerlendirme

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ABSTRACT

Aim: This study aimed to experimentally investigate the vascular biocompatibility of the new super elastic braided stents and ultrasonographically follow up the mid-term outcomes on the blood flow of stent-implanted vessels.
Method: After designation of the study steps, stents were implanted into the iliac arteries of two ewes. The preoperative and postoperative blood flow and local findings were noted. The ewes were inspected periodically for abnormal body changes, and the stent-implanted vessels were checked with Doppler ultrasonography. The subjects were sacrificed at six months, and the iliac arteries were examined histopathologically.
Results: During follow up an occlusive pattern was not observed in the stent-implanted vessels. The histopathological analysis revealed that both two stent area had no significant neointimal hyperplasia, despite sufficient re-endothelisation at the stent surface. Occlusive or stenotic patterns were not detected in both macroscopic and microscopic findings.
Conclusion: According to the mid-term findings, our results show that the new super elastic bare stents have good vascular biocompatibility with high patency rates.

Keywords: Super elastic bradied stent, vascular biocompatibility, neointimal hyperplasia, experimental model

ÖZ

Amaç: Bu çalışmada yeni süper elastik çıplak stentlerin vasküler biyoyararlanımının deneysel olarak araştırılması ve stent implante edilen damarların kan akışındaki orta dönem sonuçlarının ultrasonografik olarak izlenmesi amaçlandı.
Metot: Çalışma adımlarının belirlenmesinden sonra stentler iki koyun ilyak arterlerine implante edildi. Ameliyat öncesi ve sonrası kan akışı ve lokal bulgular kaydedildi. Denekler anormal vücut değişiklikleri açısından periyodik olarak takip edildi ve stent implante edilen damarlar Doppler Ultrasonografi ile kontrol edildi. Denekler 6. ayda sakrifiye edildi ve iliak arterler histopatolojik olarak incelendi.
Bulgular: Takip sırasında stent implante edilmiş damarlarda herhangi bir oklüzyon paterni gözlenmedi. Histopatolojik analiz, stent yüzeyinde yeterli yeniden endotelizasyona rağmen, her iki stent bölgesinin de belirgin neointimal hiperplazi olmadığını ortaya koydu. Makroskopik veya mikroskopik bulgularda oklüzif veya stenotik patern saptanmadı.
Sonuç: Sonuçlarımız, yeni süper elastik çıplak stentlerin, orta dönem bulgularına göre yüksek açıklık oranları ile iyi vasküler biyoyararlılığa sahip olduğunu göstermiştir.

Anahtar kelimeler: Super elastic çıplak stent, vasküler biyouyumluluk, neointimal hiperplazi, deneysel model

Received: 01.06.2020 Accepted: 11.08.2020 Published (Online)29.10.2020:

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To cited: Karahan O, Ocal N, Sumer T, Hafız E, Khalil E. Vascular compatibility of superelastic stent. Acta Med. Alanya 2020;4(3):236-241. doi:10.30565/medalanya.745576

Introduction

Peripheral vascular diseases can be controlled with either, or a combination of, medical management or lifestyle changes. Usually, medication and lifestyle changes are ineffective in providing enough distal blood flow when a lesion becomes a progressively occlusive degree. In this case, advanced techniques are required, such as interventional or surgical methods [1–3]. Recent advances in percutaneous techniques offer more advantages compared with surgery, such as less operation time, a small and single interventional point, a local anaesthesia solution and simultaneous visualisation. Foreign body reaction, re-occlusion rates and disrupted endothelial structure are the most common disadvantages of interventional techniques. Therefore, new tissue-friendly techniques and compatible products are tried to be developed [3–6].

Vascular stents, one of the important products in the application of percutaneous techniques, have been used for decades to maintain the patency of occluded vessels [6]. Scientists have focused on improving the basic features of metal stents, such as content, coating and braiding design, which can increase tissue compatibility [6–8]. It is thought that these artificial objects, which are well designed and adapted to tissue, may be the vascular technology of the future. All of these technological touches target minimal damage to tissue, sustainability in endothelial functions and reduced thrombogenicity [6–8].

The current study aimed to experimentally investigate the mid-term endothelial patency, six-month endothelial reaction and tissue biocompatibility of the new super elastic braided stents in sheep.

Material and Methods

After designation of the study steps, ethical approval was obtained from the local animal ethical committee (No. 2018/10–57) of the university. Two healthy female Akkaraman ewes (weighing 35–45 kg) were obtained from the laboratory production unit of the university. The animals were kept in 12-hour light/dark-cycle cages programmed with standard humidity (50 ± 5%) and temperature (22 ± 2°C). The animals were allowed free access to a standard diet and tap water until study protocols began. All regulations were met throughout the time the experiments were performed in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals.

The first step was applying a general anaesthesia, as described in previous reports [5, 6]. One of the ewes was called stent I and the other stent II (both stents were NoetriX®, Noegenix, Ankara, Turkey) for recording the findings separately. Due to the anatomical condition of iliac arteries in sheep, the external iliac arteries were selected for stent implantation. (The iliac artery does not exist in sheep, and abdominal aorta is bifurcated external and internal iliac arteries directly with a small trunk. External iliac arteries are progressing and is called the deep circumflex artery, which is divided into two branches known as cranial and caudal segments.)

The follow-up duration was determined to be six months for obtaining mid-term results. During this time, acute, sub-acute and late reactions were evaluated. Initially, acute, dermal, vascular and systemic reactions were observed after the stent application. Dermal and intradermal reactions were evaluated with inspections, and any changes were noted. The intravascular evaluation was done by a colour flow Doppler ultrasound (Mindray ® DP-20 Vet, Shenzhen, China). Systemic reactions were assessed with vital signs, and any changes were noted. After the six-month follow-up period, the ewes were sacrificed with an overdose intravenous barbiturate injection (Narcoren, Rhone Merieux, Laupheim, Germany).

Implantation of the stents:

The inguinal area of the ewes was shaved and disinfected, and the vascular structures were evaluated with a Doppler ultrasound (Figure 1A, B). An arterial puncture was then made with the Seldinger technique, and a 7 Fr vascular sheath was placed into the femoral arteries of the ewes (Figure 1C). The infusion catheter (GoraN®, Noegenix, Ankara, Turkey) was progressed to the abdominal aorta and a contrast agent infused from the catheter. During the contrast infusion, X-ray radiograms of the groin area were obtained for documenting the vascular anatomy of the ewes (Figure 2A). After determining the anatomy, the

stent upload was made with a WelaN® catheter (Noegenix, Ankara, Turkey), and the stents were placed into the external iliac artery with ultrasound guidance (Figure 2B).

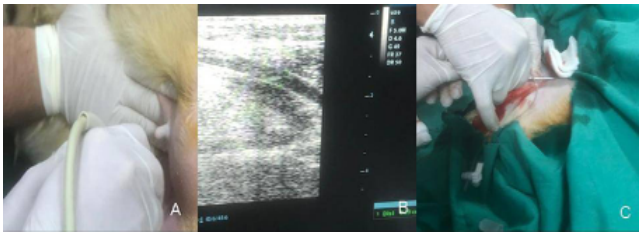

Figure 1 A,B. Ultrasonographically monitoring of sheep iliac artery, C. Puncture of sheep iliac artery with Seldinger technique.

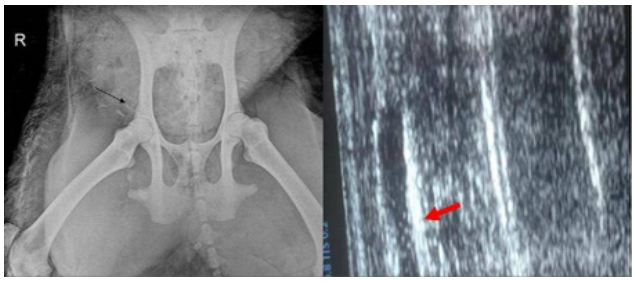

Figure 2 A. Radiograms of placed stent (black arrow), B. Intravascular visualization of stent (red arrow)

Ultrasonography monitoring:

After implantation of the stents, the vascular flows were periodically evaluated (every two hours on the first day, every six hours on the second day and twice a day thereafter) to determine the early occlusion or thrombosis. During the follow-up period, all ultrasound evaluations (Mindray® DP-20 Vet Ultrasonography device, Shenzhen, China) were made by the same practitioner. At each assessment, three areas were evaluated and recorded as follows: 1) An area of 5 mm on the upper side of the stents' proximal aperture; 2) The medial segment; and 3) An area of 5 mm on the lower side of the stents' distal aperture. Both the distal and proximal aperture areas and the total edge areas were calculated and compared with previous records.

Medication:

Anticoagulation was provided for the first three months with a daily 2x4000 IU subcutaneous enoxaparin injection (Oksapar 4000 IU, 4000IU/0,4 ml, Koçak Farma, Istanbul, Turkey). The anticoagulation protocol was stopped at the end of the third month, and the stent patency was evaluated without anticoagulation in the second three-month period.

Histopathological analysis:

After scarification of the animals, normal and stent-implanted vascular tissue biopsies were taken (Figure 3A, B, C). The tissue cytology tests were applied to all the taken samples in accordance with previous reports [5, 6]. The vascular intima-media thickness and inflammatory reactions were determined histopathologically.

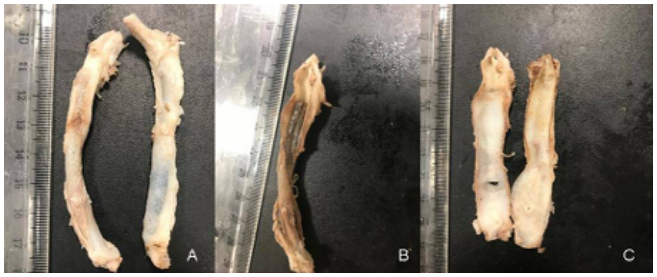

Figure 3 A. External macroscopic visualization of vessel biopsies, B. Internal visualization vessel and implanted stent together in biopsy samples. C. The visualization of endothelial layers in vessel biopsies.

Tissue samples were fixed in 10% buffered formalin from 48 to 72 hours and washed at topwater for 6 to 8 hours. The tissue samples were then processed into alcohol at different temperatures (70º, 80º, 90º, 96º and 99.5º) and xylol series for follow-up and embedded into paraffin blocks at 56 to 58 ºC. Coronal 4–5µm thick paraffin sections were obtained manually with a microtome. One slice was stained with hematoxylin-eosin (HE) and the other was stained with elastic stain kit (Sigma-Aldrich, USA). All sections were evaluated histopathologically with an Olympus BX51 trinocular light microscope (Olympus Optico Co. Ltd, Tokyo, Japan), and digital micrographs were taken with a DP25 digital camera (Olympus, Tokyo, Japan).

The average lengths of the proximal, medial and distal parts of the lamina elastica and lamina intimae (which contains an endothelial layer) were calculated with image analysis software (ImageJ v1.46r, National Institute of Health, Bethesda, MD, USA) using a 10X objective. The histopathological findings were evaluated according to endothelisation, existing thrombosis and endothelial and intimal thickening criteria,

and the obtained data were scored as follows, as described in the previous reports:

Endothelisation: Score 0: Luminal surface was covered 90%

Score 1: Luminal surface was covered 75% to 90%

Score 2: Luminal surface was covered <75%

Thrombosis: Score 0: No intraluminal thrombus formation

Score 1: <5% intraluminal thrombus formation

Score 2: <5% to intraluminal 50% thrombus formation

Score 3: >50% intraluminal thrombus formation

Leukocyte infiltration: Score 0: No infiltration

Score 1:Slightly

Score 2:Moderate

Results

During the early implantation period (the first 24 hours) of the stents, acute reactions, such as dermal or intradermal hyperaemia, necrosis, hematoma, bleeding, limping or leg swelling symptoms, were not observed. The systemic findings on the operated ewes were in the normal ranges, and any other finding in favour of systemic toxicity was not detected.

The location of the stents was periodically checked with direct radiograms, and during the observation period migration was not noted. The patency of the stents was evaluated with Doppler ultrasonography. While minimal insignificant intimal thickening (<0.1 mm) was ultrasonographically detected in stent 1, progressive neointimal hyperplasia was not detected in the three ultrasound plans. There was no stenosis or occlusion of any segments of the stent-implanted arteries of the experimental models during the six-month observation period.

Complete re-endothelisation was detected in vascular samples from stent II, and the whole stent surface was covered with an endothelial layer. However, endothelisation was was histologically detected as partially incomplete in vascular samples from stent I. Macro or micro thrombosis was not observed in either sheep. While a slight intimal thickening, related to mild neutrophil, leukocyte, and lymphocyte infiltration, was observed in the vascular structure of stent I (Figure 4A, B), intimal thickening and leukocyte infiltration (Table 1) was not observed in stent II (Figure 4C, D). Histological stenosis or occlusion was not detected in either vascular structure.

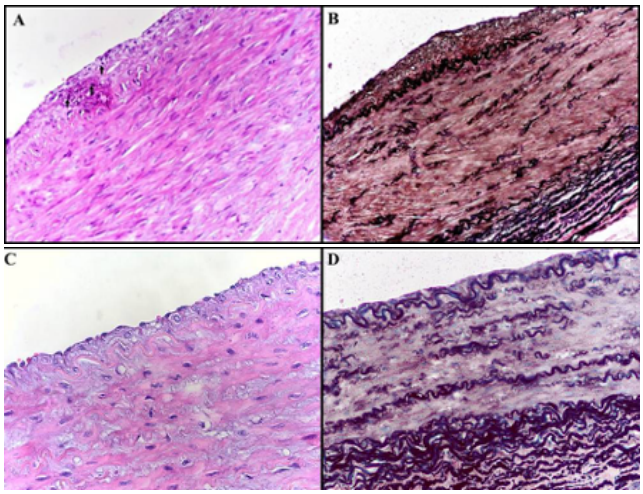

Figure 4: A. Complete endothelial formation, slightly thickened intimal region with mild leukocyte infiltration (arrow), Hematoxylin and Eosin staining, 20x magnifying of stent 1, B. Internal elastic lamina and elastic fibrils (black / blue-black staining), Elastin staining, 20x magnifying of stent 1, C. Normal intimal layer with competition of intimal layer, Hematoxylin and Eosin staining, 40x magnifying of stent 2, D. Normal internal elastic lamina and elastic fibrils, Elastin staining, 40x magnifying of stent 2.

Table 1: Histopathologic scoring of stent implanted vessel areas

	Re-endotelisation	Thrombosis	Leukocyte	Intimal Thickening (µm)
Stent1	1	0	1	2,59
Stent2	0	0	0	3,16

Discussion

According to our preliminary results, new super elastic stents seem to have good compatibility with the endothelial structure. During the six-month follow-up period, marked endothelial damage and associated neointimal hyperplasia were not observed histopathologically. Occlusive or stenotic patterns were also not detected at the

proximal or distal sites of both stents.

Preventing arterial occlusions is important to avoid disrupted blood flow and impaired end-organ circulation. Continuing blood flow can be provided with interventional or surgical procedures in stenotic or occlusive arterial lesions. Interventional techniques are the least invasive and easiest way to treat these disorders [9]. Balloon and stent angioplasty are the main application types of interventional techniques. The main problem is maintaining vessel patency after these applications. In particular, stent implantation leads to reactions in the vessel wall that can result in re-occlusion [9–11]. Stents have foreign body potential for vascular endothelium and circulating blood cells, and this foreign body triggers thrombosis formation with platelet activation. The other point that contributes to re-stenosis after stent replacement are endothelial reaction and neointimal hyperplasia [10–12]. Therefore, manufacturers are focused on producing bioavailability stents that offer the maximum as possible as it can. Despite the advanced technology, every strategy leads to reactions that can result in less or more vessel occlusion [13, 14]. The main tissue responses can be listed as endothelial hyper-reactivity, platelet adhesion, activation of thrombotic processes and inflammatory events, such as leucocyte recruitment, smooth muscle cell migration and proliferation. These reactions against stents are related to many stent related features, such as the structure, shape, produced material, knitting, size and radial force [13–15]. Initially stents were bare and simple products. Thus, manufacturers tried to find the best combination to obtain minimal tissue response and the longest patency rates. Thereafter, some stent options, such as bare, coated and drug-released stent variants, were produced. While every new development has offered promising results, compared to new derivatives bare stents have always yielded positive data [14–17]. Cejna et al. (2001) compared covered and bare stents in their experimental model and claimed that “the bare stent performed best in regard to neointimal formation and caused the least inflammatory response” [18]. They had a three-month follow-up period and suggested bare stent usage for iliac arteries, in accordance with intravenous ultrasonography and electron microscopy findings [18]. While our follow-up period was six months, we used the same experimental model as the Cejna et al. study [18]. We only observed the new super elastic braided stent and found a minimal inflammatory response and complete patency during the follow-up period. Ocke Reis et al. (2019) suggested that there are conflicting results on covered and bare stents in iliac artery lesions, and these results need to be clarified with further studies [17]. They added that exact subgroup analyses are insufficient or lesion characteristics can be different in both studies, and the results of this research can be different due to infrastructural differences and insufficient real-world data [17]. Recently, larger trials have presented balanced results. For example, a new study that compared VIABAHN and bare stents in human subjects concluded that both stent types have similar long term results[19]. This study also reported the same mid-term outcomes in patients with iliac artery disease, localisation of lesions and propensity scores [20]. We did not compare bare stents with other types of stent. However, our study involved the longest experimental follow-up period for bare stents implanted in the iliac arteries of sheep. We found minimal neointimal hyperplasia over six months, and there was no occlusive pattern histopathologically.

Limitations of the study: Firstly, this study presents preliminary results from animals. So, results should be confirmed with human subjects that have pathologically iliac occlusive disease required. Secondly, this study is not a comparative study and may offer one-sided results without comparison of another product.

We concluded that the new super elastic bare stent seems to be safer for iliac arteries with minimal tissue response in animals. However, these findings should be confirmed with human studies.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):242-246

DOI:10.30565/medalanya.755050

How do the effective therapeutics for hepatocellular carcinoma treatment change PIWI Interacting RNA expressions?

Hepatoselüler karsinom tedavisi için etkili terapötikler PIWI Interacting RNA ifadelerini nasıl değiştirir?

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ABSTRACT

Aim: PIWI interacting RNAs (piRNAs) are novel members of small non-coding RNAs that cannot produce proteins but are effective on transcription and post-transcriptional mechanisms of cells. Nowadays, the application of both natural compounds and vitamins is essential for treatment of cancer cells instead of chemical compounds. In this study, we aimed to detect possible expression changes of piRNAs in order to compare 4- Hydroxycoumarin to the active form of vitamin D (1.25-Dihydroxyvitamin D) in hepatocellular carcinoma.

Methods: According to our previous study, HePG2 cells were treated with 4- Hydroxycoumarin, 1.25-Dihydroxyvitamin D and drug form of vitamin D at the optimal time and concentration. After treatment, the total RNA was isolated and expressions of piR-Hep-1 and piR-651 were determined by using Real Time Polymerase Chain Reactions.
Results: According to our obtained data, statistically significant upregulation of piR-651 expression was observed in 4-Hydroxycoumarin-treated HePG2 cells compared to control ($p<0.001$). However, the expression of piR-Hep-1 statistically was not affected from 4- Hydroxycoumarin treatment ($p>0.05$). In contrast, 1.25-dihydroxyvitamin treatment downregulated the expression of piR-Hep-1 statistically significant in HePG2 cells ($p<0.001$). piR-Hep-1 was not statistically significant effected from drug form of vitamin D treatment ($p>0.05$).
Conclusion: Our results indicated that some of the piRNAs might have special expression patterns in hepatocellular carcinoma and these expression patterns can be regulated by treated natural compounds. We suggest that substances that are observed to be effective in hepatocellular carcinoma individually may result in different piRNA expression changes contrary to the expectations.

Keywords: 1.25-Dihydroxyvitamin D, 4-Hydroxycoumarin, Hepatocellular Carcinoma, piR- 651, piR-Hep-1

ÖZ

Amaç: PIWI interacting RNA'lar (piRNA'lar) herhangi bir protein üretemeyen ancak hücrelerin transkripsiyon ve transkripsiyon sonrası mekanizmalarında etkili olan küçük kodlayıcı olmayan RNA'ların yeni üyeleridir. Günümüzde, kanser hücrelerinin tedavisinde kimyasal bileşikler yerine, hem doğal bileşikler hem de vitaminler uygulanabilirliği araştırılmaktadır. Bu çalışmadaki amacımız, 4-Hidroksikoumarinin ve aktif D vitamini formunun (1.25-dihidroksivitamin D) hepatoselüler karsinomada piRNA'ların olası ekspresyonları üzerindeki değişiklikleri belirlemektir.
Yöntemler: Önceki çalışmamızdan elde edilen verilere göre, optimal zaman ve konsantrasyonu belirlenen 4-Hidroksikoumarin, 1.25-dihidroksivitamin D ve D vitamininin ilaç formu HePG2 hücrelerine uygulandı. Uygulamadan sonra total RNA izole edildi. piR-Hep-1 ve piR-651'in ekspresyonları Gerçek Zamanlı Polimeraz Zincir Reaksiyonları kullanılarak belirlendi.
Bulgular: Elde edilen verilere göre, 4-Hidroksikoumarin uygulanan HePG2 hücrelerinde kontrole göre piR-651 ekspresyonunda istatistiksel olarak anlamlı bir artış gözlenmiştir ($p< 0.001$). Bununla birlikte, 4-Hidroksikoumarin uygulamasından sonra piR-Hep-1 ekspresyonundaki değişim istatistiksel olarak anlamlı değildir ($p> 0.05$). Buna karşılık, 1,25- dihidroksivitamin uygulaması HePG2 hücrelerinde piR-Hep-1 ekspresyonunu istatistiksel olarak anlamlı şekilde azaltmıştır ($p <0.001$). D vitamininin ilaç formunun uygulamasından sonra piR-Hep-1 ekspresyonundaki azalma istatistiksel olarak anlamlı değildir ($p> 0.05$).
Sonuç: Tüm bu veriler, piRNA'ların bazılarının hepatoselüler karsinomda özel ekspresyon paternlerine sahip olabileceğini ve bu ekspresyon paternlerinin, uygulanan doğal bileşikler tarafından düzenlenebileceğini göstermektedir. Hepatoselüler karsinomda tek tek etkili olduğu gözlenen maddelerin, beklentilerin aksine farklı piRNA ekspresyon değişikliklerine neden olabileceğini savunmaktayız.

Anahtar Kelimeler: 1.25-Dihidroksivitamin D, 4-Hidroksikoumarin, Hepatoselüler Karsinom, piR-651, piR-Hep-1

Received: 20.06.2020 Accepted: 25.09.2020 Published (Online):29.10.2020

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To cited: Öner Ç, Altiner N, Çolak E. How do the effective therapeutics for hepatocellular carcinoma treatment change PIWI Interacting RNA expressions? Acta Med. Alanya 2020;4(3):242-246. doi:10.30565/medalanya.755050

INTRODUCTION

PIWI Interacting RNAs (piRNAs) are the novel member of a small non-coding RNA family. piRNAs are short (26-31 nucleotide in length) and single stranded RNA sequences that work with Argonate proteins, which are called PIWI proteins. piRNAs are functional on transposon silencing, gene and protein regulation, genome rearrangement, spermatogenesis and survival of germ cells. piRNAs can be tumor suppressing or oncogenic according to the characteristics of the target regions they affect, such as miRNAs. Studies shows that these features may differ according to the cancer types [1-3]. piR-Hep-1 expression has been investigated especially in hepatocellular carcinoma and liver cancers. Furthermore, high piR- Hep-1 expression was detected in hepatic cancerous tumors and cell lines [4]. piR-651 is another piRNA which especially has oncogenic characteristics in gastric, colon, lung and breast cancer [3, 5]. Furthermore, there are some clinical evidence indicating that piR-651 and PIWI proteins might have an essential role on the development of pancreas, gastric and esophagus cancer [6, 7]. In mesothelioma and hepatocellular carcinoma, high piR-651 expressions were observed in tumors compared to healthy tissues. It is thought that piRNAs and PIWIs have a potential role in tumor formation [8].

Hepatocellular carcinoma (HCC) is the fourth most common cancer worldwide. The main causes of this type of cancer are Type II diabetes, obesity and alcohol [9]. Since HepG2 cells have properties similar to normal hepatocytes, they are used more in the study of liver toxicity and the metabolism of xenobiotics. Moreover, HepG2 cells can hydroxylate compounds to the active form of vitamin D, which is also known as 1.25-dihydroxyvitamin [1.25(OH)2D3] [10].

Vitamin D is a type of fat-soluble vitamin that is metabolized in the liver. It is used as a treatment or a preventive measure in various liver diseases. Excessive vitamin D deficiency is observed in liver diseases such as cirrhosis and hepatic hemangioma [11]. Vitamin D, obtained both from nutrients and directly from sunlight, is transformed into an active form in the liver, 1.25-Dihydroxyvitamin D, and then it is used throughout the body. The hormonal form of vitamin D (1.25-Dihydroxyvitamin D) is a transcription factor, which is the stimulating molecule of the vitamin D receptor (VDR), and which binds to Vitamin D responsive elements (VDRE) in DNA. 1.25-Dihydroxyvitamin D prevents cancer from occurring; it might also suppress tumor development [12].

The other therapeutic, which is used to detect the piR-Hep-1 and piR-651, is 4- Hydroxycoumarin. 4-Hydroxycoumaarin is a phenolic natural compound which is extracted from vanilla and cinnamon [13]. Coumarins are heterocyclic substances and are used in treatment of various diseases, especially cardiovascular diseases. Moreover, long ter

Coumarin usage causes an increase of coroner arterial calcification via inhibition of carboxylation Gla Protein through vitamin K. Coumarins (1,2-benzopyrone) and their hydroxylated forms (Hydroxycoumarins) triggers the formation of free radicals which leads cells to have oxidative stress [14]. Some clinical trials indicate the effect of Coumarins derivatives on the treatment of prostate cancer, malign melanoma and metastatic kidney carcinoma [15, 16].

Each therapeutic was used to treat HePG2 hepatocellular carcinoma cells individually and it was observed that each of them previously had a negative impact on the survival and the proliferation of HePG2 cells. In this study, we aimed to observe the effect of different types of therapeutics (4-Hydroxycoumarin and 1.25-Dihydroxyvitamin D), which are individually useful to treat hepatocellular carcinoma, on piRNA expressions.

MATERIALS AND METHODS

Cell Culture, 4-Hydroxycoumarin, 1.25-Dihydroxyvitamin D and Drug Form of Vitamin D Treatment: Hepatocellular carcinoma cell line HePG2 (ATCC, Washington D.C., USA) was maintained in a humidified atmosphere with 5% CO2 at 37°C. Dulbecco's Modified Eagle's Medium (DMEM; Wisent, Canada) with 10% fetal bovine serum (FBS; Capricorn, Germany) and 1% Penicillin/Streptomycin (FBS; Capricorn, Germany) were used to culture HePG2 cells.

Before treatment of 4-Hydroxycoumarin (Sigma, USA),1.25-Dihydroxyvitamin D (Cayman, USA) and drug form of vitamin D (1.25-Dihydroxyvitamin D, Butylhydroxyanisole and Sunflower oil) to HePG2 cells, 5x105 cells were seeded to each well of 6-well plate (Greiner, Germany). According to our previous study, the optimal concentration of 4-Hydroxycoumarin is 5 µM at the 48th hour, so 5 µM 4-hydroxycoumarin was treated to HePG2 cells for 48 hours. The optimal concentration of 1.25-Dihydroxyvitamin D is 250 nM at the 48th hour and

250 nM 1.25-Dihydroxyvitamin D was treated to HePG2 cells for 48 hours. To observe the drug form of vitamin D, 250 nM was treated to HePG2 cells for 96 hours [17].

Total RNA Isolation and Real Time Polymerase Chain Reaction (RT-PCR): Total RNA was isolated using a Nucleospin RNA Kit (Macherey-Nagel, Germany) in accordance with the manufacturer protocols. The total RNAs were converted to cDNA through a reverse transcription (Genaxxon, Germany). SYBR Green based primer sets for the amplification of piR-Hep-1, piR–651 and Glyseraldehide-3-phosphate dehydrogenase (GAPDH) were designed and supplied by Oligomer (Ankara, Turkey). The primer sequences are shown in Table 1. RT-PCR was carried out inside a Roche Lightcycler96 (Vedbaek, Denmark).

Table 1. The primer sequences used in RT-PCR

Primer Name	Forward Sequence	Reverse Sequence
piR-Hep-1	5'-TCCCTGGTGGT CTAGTGGTTAGAGAA-3'	3'-CCAGTCTCAGGG TCCGAGGTATTC-'5
piR-651	5' -AGAGAGGG GCCCGTGCCTTG-'3	3'-CCAGTCTCAGG GTCCGAGGTATTC-'5
GAPDH	5'-CGAGGGGG GAGCCAAAAGGG-'3	3'-GAAACTGC GACCCCGACCGT-'5

GAPDH was used as an internal control, and the expression of related genes was normalized in line with the expression of GAPDH. The SYBR Green RT-PCR was conducted at following conditions: pre-denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 10 s, and annealing/extension at 60°C for 30 s. Gene expression changes were quantified using the delta-delta CT (ΔΔCT) method. *Table 1

Statistical Analysis: The normal distribution of the continuous variables was enabled using the Kolmogorov-Smirnov suitability test. Multiple comparisons of gene expressions were compared using the Student t test. All analyses were carried out using the IBM SPSS Statistics 21.0 software package. The obtained data were indicated as mean ± standard deviation (sd). In the figures, only mean values have been shown.

RESULTS

According to our obtained data, treatment of 4-Hydroxycoumarin caused an increase in piR-Hep-1 expression in treated group (-5.72±0.379) compared to the control (-5.99±0.071). But we cannot detect statistically significant changes between groups (p>0.05). The piR-651 expression of 4-Hydroxycoumarin treated group (3.66±0.282) was upregulated compared to control (-5.87±0.071) and was statistically significant (p<0.001; Fig. 1).

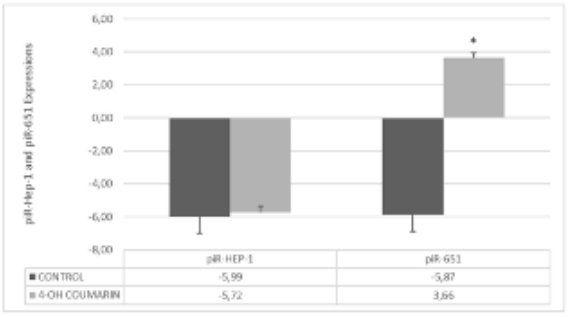

Fig 1. The effect of 4-Hydroxycoumarin treatment on piR-Hep-1 (p>0.05) and piR-651 expressions (p<0.001).

In treatment of Vitamin D active form (5.379±0.035), the piR-Hep-1 expression was downregulated compared to control group (12.223±0.072) and was statistically significant (p<0.001). Furthermore, we wanted to observe the drug form of vitamin D on piR-Hep-1 expression. The drug form of the vitamin D treated group (3.193±0.103) was also downregulated compared to control group (3.233±0.104). However, statistically significant differences cannot be detected between the group treated with the drug form of vitamin D and the control group (p>0.05; Fig. 2).

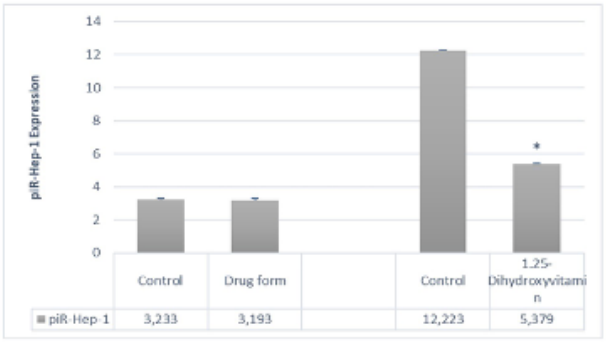

Fig 2. The effect of drug form of vitamin D ($p>0.05$) and 1.25-Dihydroxyvitamin D ($p<0.001$) treatment on piR-Hep-1 expression.

DISCUSSION

PIWI Interacting RNAs are the new perspective of small non-coding RNAs which are important transposon silencing. As a result of this epigenetic regulation, some gene regions become active while some of them become inactive [3, 18]. piR-Hep1 expression is high in HCC tumors and promotes viability and invasive characteristics. Law et al. determined that upregulation of piR-Hep1 by 46.6% in HCC tumors compared to healthy liver tissues causes an increase in viability, motility, and invasiveness depending on the amount of AKT phosphorylation in HCC [4]. In gastric, colon, lung and breast cancer tumors, piR-651 expression is aberrantly high [5]. Furthermore, inhibition of piR-651 in gastric cancer promotes cellular development. piR-651 expression, which were detected in the peripheral blood mononuclear cells from gastric cancer patients, downregulated significantly [2]. piRNA are also complicated and same piRNA might be tumor suppressing or have oncogenic characteristics in various cancer types. Our findings showed that 4-hydroxycoumarin treatment caused an increase in piR-651 expression. It is the first report that indicates that piR-651 might be tumor suppressive piRNA in HCC.

One of the main objectives of our research is to scientifically reveal that natural components applied in cancer treatment have a genetically significant effect in some regions while reducing the survival of cancer cells; while in some regions this natural compound treatment is ineffective. We wanted to show this situation by determining the expression changes of piR-Hep-1. piR-Hep-1 is an important piRNA region for hepatocellular carcinoma. piRNA studies in hepatocellular carcinoma especially showed that piR-Hep-1 is upregulated. Furthermore, high piR-Hep-1 expression was also detected in hepatocellular tumors [4]. Natural compounds extracted from plants have been regarded as a source of potential therapeutic agents, and are also are well known to play essential roles in a variety of cancer treatments. 4-Hydroxycoumarin is a polyphenolic natural compound. Recent studies about polyphenolic compounds and hepatocellular carcinoma indicate that these compounds represent a wide range of pharmacological properties like antioxidant and anti-carcinogenic activities [19, 20]. In cancerous cells, hydroxycoumarins enhances formation of free radicals and oxidative stress occurs. As a result of this mechanism, oxidative stress is effective in decreasing proliferation of cancer cells. In renal carcinoma, 7-Hydroxycoumarin was used as cytotoxic therapeutic in vitro [21]. Furthermore, hydroxylated Coumarins have anti- proliferative and cytotoxic activity in breast, sarcoma and skin cancer cell lines [22, 23]. We determined that piR-Hep-1 expression cannot be effected while piR-651 expression is upregulated. piR-651 expression is detected to indicate that 4-hydroxycoumarin can be related to another piRNA sequence. These results are the first results showing the link between 4-Hydroxycoumarin, piR-Hep-1 and piR-651 in hepatocellular carcinoma.

Vitamin D is not a natural compound but it is important in the treatment of hepatocellular carcinoma. Active form of vitamin D, 1.25-Dihydroxyvitamin D, and its analogs prevent cancer development or delay the recurrence and metastasis of previously developing cancer. Pourgholami et al., found that 1.25-Dihydroxyvitamin D inhibited the growth of HepG2 and Hep3B hepatocellular carcinoma cell lines [24]. Histone deacetylase 2 (HDAC2) is one of the target genes of piR-Hep-1 [18]. Decreased the proliferation of 1.25-Dihydroxyvitamin D treated HDAC2 inhibited HePG2 cells pointed to the possible link between vitamin D and piR-Hep-1 [25]. According to our data, although the drug form of vitamin D was not effective to downregulate piR-Hep-1 expression, 1.25-Dihydroxyvitamin D treatment caused a decrease in piR-Hep-1 expression in HePG2 cells. In the treatment of different forms of vitamin D, it is important to

emphasize however that the basis of molecule is the same, and that small changes can change the piRNA expressions. From this point of view, the drug form of vitamin D treatment did not cause a statistically significant change on the expression of piR-Hep-1, while active form of vitamin D (1.25-Dihydroxyvitamin D) had a statistically significant change on the piR-Hep-1. This is also the first report which identifies the relationship between vitamin D and piR-Hep-1.

As with the majority of studies, the design of the current study is subject to limitations. For example, the number of piRNAs is an important limitation, especially for evaluation. The results should be examined in large-scale studies. Furthermore, the studies of piRNAs increase day by day, however there is more studies needed to identify the cellular mechanisms of piRNA.

Conclusion

In scientific literature, piRNAs and their roles in cancer cells is identified day by day. However, there is a lack of piRNA cellular mechanisms. We have demonstrated in this study that natural compounds cannot always be effective in all genes and/or epigenetic mechanisms of cancer cells. We even showed that two different forms of the same substance may show different expression changes on the same piRNA region. More cellular and molecular mechanisms of piRNAs should be identified by future studies.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):247-253

DOI:10.30565/medalanya.736684

Coincidence of obstructive sleep apnea syndrome and systemic diseases in geriatric patients

Geriatrik Hastalarda Obstruktif Uyku Apne Sendromu Ve Sistemik Hastalık Birlikteliği

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ABSTRACT

Aim: To evaluate the coincidence of obstructive sleep apnea syndrome (OSAS) and systemic diseases in elderly patients presenting to our sleep disorders center.
Materials and Methods: Ninety-seven patients with ages older than 65 years, who had at least one of snoring, witnessed apnea, and excessive daytime sleepiness symptoms, and whose systemic diseases were under evaluation, were included in the study. The patients were divided into 4 groups according to their apnea-hypopnea indexes (AHI) and body mass index (BMI) values. The association of BMI and the polysomnography parameters such as AHI, arousal index, lowest oxygen saturation and mean oxygen desaturation in patients with and without systemic illnesses ,were evaluated.
Results: Seventy-one (73.2%) of the patients comprised the group with systemic illnesses, and 26 (26.80%) patients comprised the group without systemic illnesses. AHI value was found to be higher in patients with systemic diseases compared to patients without systemic illnesses, and this was statistically significant ($p<0.05$). A statistically significant association was found between the presence of systemic diseases and OSAS and increased BMI (BMI>25) ($p<0.05$). A statistically significant association was not detected between the arousal index, lowest oxygen saturation, mean oxygen saturation, and presence of systemic illness. No difference in the rates of presence of systemic illnesses was found among male and female patients.
Conclusion: Patients in the geriatric age group with increased BMI and OSAS should be evaluated for the presence of systemic illnesses.

Keywords: Obstructive sleep apnea syndrome, systemic diseases, geriatrics

ÖZ

Amaç: Uyku bozuklukları merkezimize başvuran yaşlı hastalardaki obstruktif uyku apne sendromu (OSAS) ile sistemik hastalık birlikteliğini ve bunlarla ilişkili parametreleri araştırmaktır.
Gereç ve Yöntem: Horlama, tanıklı apne, gündüz aşırı uyku hali semptomlarından en az birinden şikayeti olan ve sistemik hastalıkları araştırılan 65 yaş üstü 97 hasta çalışmaya alındı. Hastalar apne hipopne indeksi (AHİ) ve vücut kitle indeksi (VKİ) değerlerine göre 4'er gruba ayrıldı. Sistemik hastalığı olan ve olmayan hastalarda VKİ ile polisomnografi parametrelerinden AHİ, arousal indeksi, en düşük oksijen saturasyonu ve ortalama oksijen desaturasyonu arasındaki ilişki incelenmiştir.
Bulgular: Hastaların 71'i (%73.2) sistemik hastalığı olanlar, 26'sı (%26.80) sistemik hastalığı olmayanlar grubunu oluşturmuştur. Sistemik hastalığı olanlarda, sistemik hastalığı olmayanlara göre AHİ değeri daha yüksek saptanmış olup istatiksel olarak anlamlı saptanmıştır($p<0.05$). Sistemik hastalık varlığı ile OSAS ve artmış VKİ (VKİ>25) arasında istatiksel olarak anlamlı birliktelik saptanmıştır($p<0.05$).Arousal indeksi, en düşük oksijen saturasyonu ,ortalama oksijen desaturasyonu ile sistemik hastalık varlığı arasında istatiksel anlamlı bir birliktelik saptanmamıştır. Kadın ve erkek hastalar arasında sistemik hastalık görülmesi açısından fark saptanmamıştır.
Sonuç: Artmış VKİ ve OSAS'ı olan geriatrik yaş gurubundaki hastalar sistemik hastalık varlığı açısından değerlendirilmelidir.

Anahtar Kelimeler: Obstrüktif uyku apne sendromu, sistemik hastalıklar, geriatri

Received: 10.07.2020 Accepted: 29.09.2020 Published (Online):29.10.2020:

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To cited: Akdag M., Coincidence of obstructive sleep apnea syndrome and systemic diseases in geriatric patients. Acta Med. Alanya 2020;4(3):2417-251. doi:10.30565/medalanya.736684

INTRODUCTION

Along with aging, many changes occur in sleep structure. It is known that there is a deterioration in sleep quality and an increase in the prevalence of sleep disorders in the elderly [1,2]. The incidence of sleep disorders also increases in old age: it is reported that approximately 35% of people over 60 years old and about 50% of people over 65 years old, experience sleep disorders [3]. In old age, night sleep time decreases, sleep efficiency decreases to around 70-80%, and the frequency and duration of nighttime awakenings increase. Among the causes of sleep disorders seen in old age are changes in sleep duration and rhythm, changes in the metabolism, cardiovascular diseases, neurological diseases, psychiatric problems and multiple drug use. It is a problem to be able to distinguish normal or physiological sleep changes associated with age, from abnormal or pathological sleep, because there is a close relationship between comorbid conditions with high prevalence in elderly and sleep disorders. In addition to the changes occurring in sleep due to age in the elderly, the rate of sleep respiratory disorders increases [4]. It is difficult to obtain useful data with sleep-breathing disorders in the elderly, as it is more difficult to persuade the elderly to perform polysomnography (PSG) and to bring them to the sleep laboratory with the restrictive effect of the accompanying diseases [5].

Obstructive sleep apnea syndrome (OSAS) constitutes more than 90% of the most common sleep-related respiratory disorders. OSAS is a disease coursing with recurrent apnea and hypopnea episodes, that develop due to obstructions in one or more areas of the upper respiratory tract during sleep [6]. It has been reported that disruption between the neural and muscular factors responsible for keeping the airway open during sleep causes obstructions [7]. This can range from partial obstruction of the airway that causes simple snoring, to full obstruction of the airway that causes apnea formation. The reason for the closure of the upper airway during sleep is the disruption of the balance of the forces that keep the airway open, which causes it to collapse [8]. Although its prevalence varies with age, gender and genetics, it is 2-4% on average. The most common symptoms are respiratory standstill in sleep, snoring, and excessive daytime sleepiness. The gold standard in diagnosis is PSG [9] and continuous positive air pressure (CPAP) is the most effective method of treating the disease and preventing complications [10]. With increasing age, upper respiratory tract muscle tone decreases, snoring prevalence increases and increased comorbidities are thought to increase the tendency to upper respiratory obstructions.

In OSAS, besides respiratory disorders in sleep, serious complications occur in other systems, especially in the cardiovascular system, and these complications constitute the most important cause of mortality and morbidity in OSAS. With the prolongation of lifetime, the frequency of chronic diseases increases: 90% of people aged 65 and over have at least one chronic disease, while 35% of them have 2, 23% of them have 3, 15% of them have 4 or more diseases simultaneously [7].

The aim of this study is to investigate the coincidence of OSAS and systemic diseases and the related parameters, in elderly patients admitted to Akdeniz University Faculty of Medicine Otorhinolaryngology Sleep Disorders Center.

MATERIALS AND METHODS

A total of 118 patients over the age of 65 who complained of at least one of the symptoms of snoring, witnessed apnea and excessive sleep during the day admitting to Akdeniz University Faculty of Medicine Otorhinolaryngology clinic, were included in the study. The patient data related to the study were retrospectively taken from their files. Since 21 patients did not have a record of systemic disease in the file, they were not included in the study, so that 97 patients were included in the study. As the inclusion criteria, in addition to the complaints of respiratory disorders in the sleep of the patients, questioning about systemic disease and having related records, patients who were tested for PSG (Compumedics E-Series, Profusion) in the sleep laboratory, were included in the study. Those who were under the age of 65, who did not have records about their systemic diseases, those who had previously been diagnosed with OSAS, and those who were treated, were excluded from the study. The PSG records were scored manually by an

experienced otolaryngologist for sleep disorders according to the American Academy of Sleep Medicine (AASM) 2007 criteria [11]. In the scoring of respiratory events, the duration of respiratory effort (obstructive), lack of respiratory effort (central), or lack of initial respiratory effort, and then the initiation of respiratory effort (mixed) and the cessation of air flow for at least 10 seconds, were defined as apnea. Hypopnea was defined as the decrease of at least 3% in the oxygen saturation, or accompanied by arousal, and at least 10 minutes of the event with a decrease of minimum 50% compared to the initial value of the airflow. The patients were divided into four groups according to the severity of their disease, apnea-hypopnea index (AHI), and the results of the PSG [11]:

- AHI<5: Simple snoring

- AHI 5-15: Mild OSAS

- AHI 16-30: Moderate OSAS

- AHI> 30: Severe OSAS

BMI values of the patients were calculated. They were grouped as underweight (<18.5), normal (18.5-24.9), overweight (25-29.9), and obese (> 30) according to BMI values [12].

Statistical Analysis: The data of the study were analyzed by using the SPSS Software (version 16.0-SPSS Inc., Chicago, USA). The descriptive data were given as Mean ± Standard Deviation (SD). The results of the categorized data were given as percentages (%). The independent groups were compared with the Chi-Square Test, and the group ratios were compared with Student's t-test. The P<0.05 level was considered to be significant.

RESULTS

A total of 97 patients, 66 males (68%) and 31 females (32%), were included in our study. The ages of the patients were between 65-78 and the mean age was 70.56 (±4.52). The BMI values of the patients were between 21.4 and 46.2 and the mean was 29.83 (±4.94). After the PSG test, AHI values were found to be between 0 and 80.6, with an average of 28.96 (±22.46).

According to AHI values, 15 (15.46%) patients with simple snoring, 19 (19.59%) with mild OSAS, 19 (19.59%) with moderate OSAS, and 44 (45.36%) with severe OSAS were identified. (Figure 1).

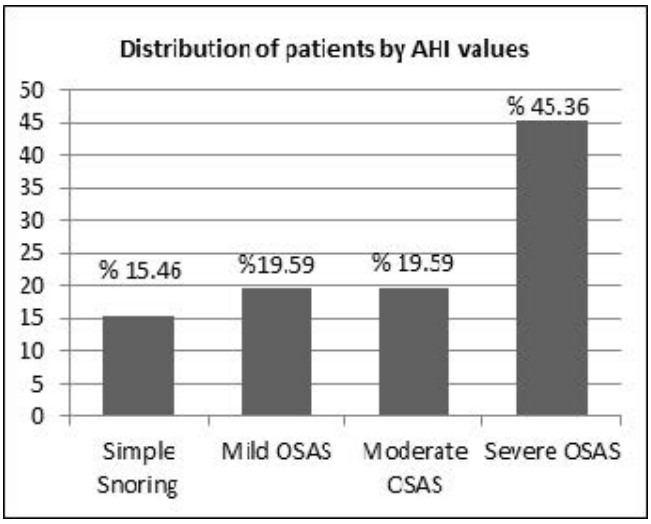

Figure 1. Distribution percentages of patients by AHI values

According to BMI values, 0 (0%) underweight, 20 (20.62%) normal, 33 (34.02%) overweight and 44 (45.36%) obese patients were found (Figure 2).

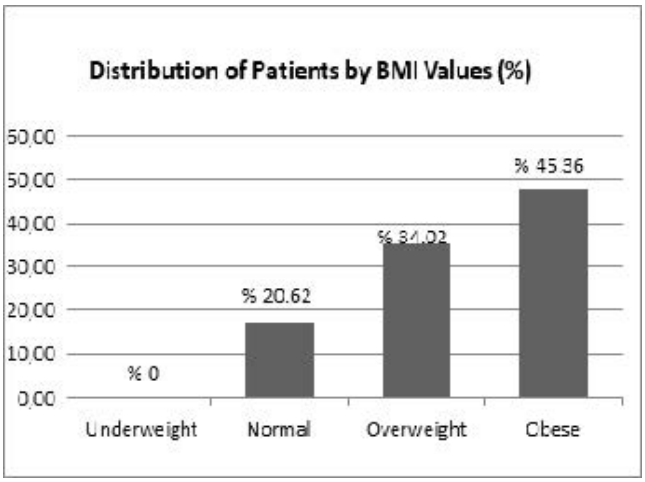

Figure 2. Distribution of Patients by BMI Values

The patients were divided into two groups as those with and without systemic disease: 71 (73.2%) of the patients made up the group with systemic diseases, which were detected in 49 (69.0%) of male patients and 22 (31.0%) of female patients. 26 of the patients (26.80%) made up the group without systemic diseases. No systemic disease was detected in 17 (65.4%) of male patients and 9 (34.6%) of female patients. When those with systemic diseases were evaluated, hypertension

(HT), diabetes mellitus (DM), hyperlipidemia (HPL), chronic obstructive pulmonary disease (COPD), heart failure, cardiac pathology such as coronary artery disease (CAD) and history of myocardial infarct, and depression were encountered alone or with other systemic diseases in 64 (90,1%), 27 (38%), 18 (25,4%), 12 (16,9%), 12 (16,9%), 8 (11,3%), and 4 (5,6%) patients, respectively.

While 21 (29.58%) of patients with systemic diseases had a single isolated systemic disease, 50 (70.42%) of them had two or more systemic disease comorbidities. The ages of the patients with systemic diseases were between 65-77 and the mean age was 70.31 (±4.52). AHI values range between 0-80.6 and the mean AHI was determined as 31.92 (±22.43). The BMI values range between 21.4-42.6 and the mean BMI was determined as 30.07 (±4.75). The ages of the patients without systemic diseases were between 65-78 and the mean age was 71.19 (±5.46). The AHI values range between 0-75.3 and the mean AHI was determined as 20.88 (±20.86). The BMI values range between 21.5-46.2 and the mean BMI was determined as 29.17 (±5.45) (Table 1).

Table 1. Distribution of patients with and without systemic diseases by age, AHI and BMI values

		Min.	Max.	Mean	SD
Those with Systemic Diseases (n:71)	Age	65.00	77.00	70.31	4.52
	AHI	0.00	80.60	31.92	22.43
	BMI	21.40	42.60	30.07	4.75
Those without Systemic Diseases (n:26)	Age	65.00	78.00	71.19	5.46
	AHI	0.00	75.30	20.88	20.86
	BMI	21.50	46.20	29.17	5.45

AHI=Apnea Hypopnea Index, BMI=Body Mass Index, Age (years)

Sixty-two (% 87.3) of the patients with a systemic disease had OSAS (AHI>5), and 34 (47.9%) had obesity (BMI>30). 20 (%77) of the patients without a systemic disease had OSAS (AHI>5), and 10 (38.5%) had obesity (BMI>30). The number and distribution (%) of the classified AHI and BMI values of the patients with and without systemic diseases are shown in Table 2.

AHI and BMI values were found to be higher in patients with systemic disease. A statistically significant relationship was found between the presence of systemic disease and OSAS (AHI>5) and increased BMI (BMI>30) ($p<0.05$).

Table 2. Number and distribution (%) of patients with and without systemic diseases by classified AHI and BMI values

		Those with Sys-temic Diseases (n:71)	Those without Systemic Diseas-es (n:26)	Total (n:97)
AHI	Simple snoring	9 (%12.7)	6 (%23.0)	15 (%15.5)
	Mild OSAS	11 (%15.5)	8 (%30.8)	19 (%19.6)
	Moderate OSAS	15 (%21.1)	4 (%15.4)	19 (%19.6)
	Severe OSAS	36 (%50.7)	8 (%30.8)	44 (%45.3)
BMI	Thin	0 (%0.0)	0 (%0.0)	0 (%0.0)
	Normal	12 (%16.9)	8 (%30.8)	20 (%20.6)
	Overweight	25 (%35.2)	8 (%30.8)	33 (% 34)
	Obese	34 (%47.9)	10 (%38.5)	44 (%45.4)

AHI=Apnea Hypopnea Index, BMI=Body Mass Index

The AHI values of 71 patients with systemic disease in the PSG test were detected as a minimum of 0.00, a maximum of 80.60, and an average of 31.92 (±22.43). Arousal Index values were detected as a minimum of 3.30, a maximum of 73.80, and an average of 30.09 (±19.39). The lowest oxygen saturation was determined as a minimum of 43.00, a maximum of 96.00, and an average of 81.35 (±9.74). The mean oxygen desaturation value was determined as a minimum of 0.00, a maximum of 20.00 and an average of 6.24 (±3.91).

The AHI values of 26 patients without a systemic disease in the PSG test were determined as a minimum of 0.00, a maximum of 75.30, and an average of 20.88 (±20.86). The arousal index values were determined as a minimum of 3.10, a maximum of 62.00, and an average of 22.71 (±17.16). The lowest oxygen saturation was determined as a minimum of 68.00, a maximum of 93.00 and an average of 82.81 (±6.81). The mean oxygen desaturation value was determined as a minimum of 0.00, a maximum of 11.00 and an average of 5.19 (±2.50). The AHI, arousal index, lowest oxygen saturation, mean oxygen desaturation values of the patients with and without systemic diseases are presented in Table 3.

The AHI value was found to be higher in patients with systemic diseases compared to patients

Table 3. AHI, Arousal Index, Lowest O2 Saturation, Average O2 Desaturation values of patients with and without systemic diseases

		Minimum	Maximum	Mean	SD
Those with Systemic Diseases (n:71)	AHI	0.00	80.60	31.92	22.43
	Arousal Index	3.30	73.80	30.09	19.39
	Lowest O2 Saturation (%)	43.00	96.00	81.35	9.74
	Average O2 Desaturation (%)	0.00	20.00	6.24	3.91
Those without Systemic Diseases (n:26)	AHI	0.00	75.30	20.88	20.86
	Arousal Index	3.10	62.00	22.71	17.16
	Lowest O2 Saturation (%)	68.00	93.00	82.81	6.81
	Average O2 Desaturation (%)	0.00	11.00	5.19	2.50

AHI=Apnea Hypopnea Index, O2=Oxygen

without systemic illnesses, which was statistically significant ($p<0.05$). In terms of the relationship between the presence of systemic disease and the arousal index, the arousal index was found to be higher in those with systemic disease, but no statistical relationship was found ($p>0.05$). The lowest oxygen saturation was found to be lower in those with systemic disease, but this was not statistically significant ($p>0.05$).

When we look at the association between systemic diseases and gender, the number of systemic diseases was found to be more in male patients, but no statistically significant difference was found between men and women in terms of the presence of systemic diseases ($p> 0.05$). While 21 (29.58%) of the patients with systemic disease had a single isolated systemic disease, 50 (70.42%) of them had two or more systemic diseases. Although no statistical significance was found, the frequency of systemic diseases increases with increasing AHI values ($p>0.05$). A statistically significant relationship was found between the presence of systemic diseases and OSAS (AHI> 5) and increased BMI (BMI>30) ($p<0.05$).

DISCUSSION

Studies show that the OSAS prevalence varies between 1% and 5%. According to the study of Kokturk et al., the OSAS prevalence in our society is estimated to be 0.9-1.9% [13]. In terms of the frequency of OSAS in the elderly, different results have been revealed in studies. Martin J. et al. found that 62% of the randomly selected population aged between 65-95 years had AHI>10 and 24% had AHI>5. Compiled epidemiological studies and reported that the incidence of SBD ranged between 28-62% in males and 19.5-60% in females. This figure was between 5.6-45% when gender differences were disregarded [14]. In the study of Ancoli-Israel et al., 427 cases over 65 years of age were followed for 5 years and it was shown that AHI increased with age. In the same study, the rate of those with AHI>20 between the ages of 40 and 60 was 10.9% in men and 5.3% in women [15].

Patients aged 65 and over were included in our study and among those, 82 (84.5%) were found to have AHI>5. The OSAS rate was found to be quite high among our elderly patients, since all of the patients admitted had at least one of the three major symptoms of OSAS and the patients were not randomly selected from the population.

OSAS has consequences regarding many systems, especially on the cardiovascular system. The main consequences regarding the cardiovascular system are hypertension, cardiac arrhythmias, ischemic heart diseases and myocardial infarction. Although the mechanisms of the consequences of OSAS regarding the cardiovascular system are still under investigation, blood gas changes and sympathetic nervous system activation due to respiratory events, are primarily held responsible [14,16]. Hypoxemia, which develops following apnea and hypopneas during sleep, stimulates the sympathetic nervous system and as a result, sudden increases in heart rate and blood pressure occur [17]. 30-50% of the patients with OSAS have systemic HT. In the prevalence studies of HT in the elderly, the rate of HT was found to be 41% in those between the ages of 60-69 and 53% in those between the ages of 70-79 [18]. The rate of HT in geriatric patients in our study was determined to be

90.1%. Many clinical and epidemiological studies have shown that dyslipidemia is an important cardiovascular risk factor in the elderly and this risk can be reduced with appropriate treatment. In our study, the rate of HPL in elderly patients was found to be 25.4%. As with the OSAS rate, the HT rate was found to be quite high among our elderly patients since all of the patients admitted had at least one of the three major symptoms of OSAS and patients were not randomly selected from the population.

Diabetes mellitus (DM) is a metabolic disease that negatively affects the quality and life of the elderly. In addition to the deterioration in carbohydrate metabolism that causes hyperglycemia, protein and lipid metabolism are also affected. With age, the incidence and prevalence increase gradually. Diabetic patients over the age of 65 make up almost 40% of all diabetics. Again, 20% of the geriatric age group have impaired glucose tolerance. In addition, 10% of the elderly population has undiagnosed diabetes [19]. In our study, the DM rate was found to be 38% in elderly patients.

Chronic obstructive pulmonary disease (COPD) is a disease that is not fully reversible and is characterized by airflow restriction, which is progressive in these patients, and an abnormal inflammatory response develops against harmful particles and gases (cigarettes). The prevalence of COPD in people over 65 years old is about 10% [20], in our study, COPD was found to be 16.9% in elderly patients.

A psychiatric disorder was found in 66% of those with sleep disorders. The frequency of sleep disorders in the elderly was found as 37%, and the frequency of depression as 31%. It is reported that sleep disorders in the elderly are a predictor of depression that may develop in the future [21] but that effective treatment of sleep disorders also delays or prevents the onset of major depression [22]. The rate of depression in the elderly varies according to where they live: the rates determined in the community are between 1-5%, and in our study, depression was found to be 5.6% in elderly patients.

Obesity has an important place in OSAS physiopathology. There is a poorly understood complex relationship between OSAS and obesity, insulin resistance, and daytime sleepiness. In addition to obesity, interruptions in sleep increased sympathetic activity and hypoxia have negative effects on insulin resistance and metabolic disorders[23]. Central obesity increases the OSAS tendency by affecting the upper respiratory tract patency and respiratory pattern, through fat accumulation around the upper respiratory tract and in the abdominal region [24]. In our study, 20.6% of all cases were normal, 34% were overweight and 45.4% were obese. When we look at the groups of BMI according to the AHI values, we determined that 46.7% of the patients with simple snoring were normal, 20% were overweight and 33.3% were obese, 31.6% of patients with mild OSAS were normal, 42.1% were overweight and 26.3% were obese, and 26.3% of the patients with moderate OSAS were normal, 26.3% were overweight and 47.4% were obese. It was found that 4.6% of the patients with severe OSAS were normal, 38.6% were overweight and 56.8% were obese. Our study showed that BMI values also increased in patients with increased AHI, and obesity was observed mostly in patients with severe OSAS. In the past, OSAS was known as a disease of obese people, however studies have reported that around 40% of the people with OSAS are in fact, not obese. The data obtained from our study shows that OSAS can also be seen in people over 65 years old who are not obese (BMI<30)

CONCLUSION

Patients in the geriatric age group with increased BMI and OSAS should be evaluated for the presence of systemic illnesses. We think that OSAS can cause cardiovascular, neurological, pulmonary, endocrine and psychiatric complications, and the complications that may occur due as a result may increase the morbidity and mortality of OSAS. This relationship will be understood more accurately with prospective studies.

Funding sources: There is no source of funding or financial interest in this study.

Conflict of Interest: The author has no conflict of interest related to this article.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):254-259

DOI:10.30565/medalanya.752398

The clinical outcomes of endovenous radiofrequency ablation of varicose veins: two year follow-up results

Kronik venöz yetmezlik olgularında Endovenöz Radyofrekans Ablasyonun Klinik Sonuçları: İki Yıllık Takip Sonuçları

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ABSTRACT

Aim: Our aim was to evaluate the two-year results of radiofrequency ablation (RFA) in the treatment of great saphenous vein (GSV) insufficiency.
Methods: A total number of 217 patients who underwent RFA (52.5 % male, mean age 42.7±11.4) were included in the study. RFA was performed in patients with great saphenous vein valvular incompetence and saphenofemoral junction incompetence. Occlusion status was recorded by ultrasonography. Venous clinical severity score (VCSS) was calculated pre- and post-intervention.
Results: The mean follow-up period of the patients was 22.2 ± 5.1 months. Cumulative survival rate (Kaplan–Meier) of 24-month follow-up was 84.3%. Complete occlusion rate for GSV was 100%, 98.6% ,97.6%, 97.6% and 97.6% for 1, 6, 12, 18 and 24 months follow up, respectively. VCSS was significantly different before RFA and 4 weeks after RFA ($p < 0.001$). No major complications were observed in the study.
Conclusion: In the treatment of GSV insufficiency, RFA is a safely applied method with high occlusion rates and obvious VCSS score decrease.

Key words: Great saphenous vein insufficiency, radiofrequency ablation

ÖZ

Amaç: Çalışmamızda büyük safen ven (GSV) yetmezliğinin tedavisinde radyofrekans ablasyonun (RFA) iki yıllık sonuçlarını değerlendirmeyi amaçladık.
Yöntemler: Çalışmaya RFA uygulanan toplam 217 hasta (%52,5 erkek, ortalama yaş 42.7 ± 11.4) dahil edildi. RFA işlemi büyük safen vende kapak yetersizliği ve safenofemoral bileşkede venöz reflü olan hastalara uygulandı. Oklüzyon takibi doppler ultrasonografi ile yapıldı. Müdahale öncesi ve sonrası Venous clinical severity score (VCSS) kayıt altına alındı.
Bulgular: Hastalar ortalama 22.2 ± 5,1 ay takip edildi. 24 aylık takipte kümülatif sağkalım oranı (Kaplan-Meier) %84,3 idi. GSV için tam oklüzyon oranı 1, 6, 12, 18 ve 24 aylık
takiplerde sırasıyla % 100,% 98.6,% 97.6,% 97.6 ve% 97.6 idi. RFA işleminden önce ve RFA uygulandıktan 4 hafta sonra hesaplanan VCSS değerleri anlamlı ölçüde farklıydı ($p <0.001$). Çalışma sırasında önemli bir komplikasyon gözlenmedi.
Sonuç: GSV yetmezliğinin tedavisinde RFA, yüksek oklüzyon oranları ve belirgin VCSS skoru düşüşü ile güvenli bir şekilde uygulanan bir yöntemdir.

Anahtar kelimeler: Büyük safenöz ven yetmezliği, Radyofrekans ablasyon

Received: 16.06.2020 Accepted: 06.09.2020 Published (Online):29.10.2020:

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To cited: Sanrı US, Özsin KK, Atlı FH, Toktaş F, Yavuz Ş. The clinical outcomes of endovenous radiofrequency ablation of varicose veins: two year follow-up results. Acta Med. Alanya 2020;4(3):254-259. doi:10.30565/medalanya.752398

INTRODUCTION

Varicose veins are common in many populations, with prevalence ranging from 10.4 to 23.0% for men and 29.5 to 39.0% for women [1]. Due to its high incidence it has an important role in health expenditures [2]. Many treatment options are currently available for varicose veins, including compression stockings, high ligation of the saphenofemoral junction (SFJ) accompanied by stripping of the great saphenous vein (GSV) and minimally invasive procedures, such as radiofrequency ablation (RFA), endovenous laser ablation (EVLA), sclerotherapy and cyanoacrylate embolization. Minimally invasive procedures are associated with lower morbidity rates and faster recovery than conventional surgery [3]. In our study, we retrospectively analyzed 217 patients which have great saphenous vein insufficiency and were treated with RFA.

PATIENTS AND METHODS

Patients who underwent endovenous RFA to GSV due to venous insufficiency between 2017 and 2018, were evaluated retrospectively. Venous insufficiency was diagnosed by duplex ultrasound (USG) in standing position. A diameter below 2 cm for SFJ, a diameter above 5.5 mm for proximal GSV and reflux lasting longer than 2 seconds was assigned as the main criterion for endovenous RFA. Although they meet the main criteria, treatment of RFA was not performed in patients with chronic renal failure, known cardiac disease, uncontrolled hypertension, deep vein thrombosis (DVT), coagulation disorder, malignancy, history of another invasive venous treatment method, allergy to the tumescent anaesthesia solution and local or systemic infection. Treated lower limbs were classified according to the Clinical-Etiology-Anatomy-Pathophysiology (CEAP) system. Before the procedure, patients' age, gender, body mass index (BMI), CEAP classification, GSV diameters and Venous clinical severity score (VCSS) scores were recorded. The study was approved by the local institutional Ethical Committee of Health Sciences University Bursa Higher Specialization Training and Research Hospital (Ethical Committee number: 2011-KAEK-25 2020/06-16).

Technique of Radiofrequency Ablation: We performed RFA under spinal anaesthesia in all cases. Knee level was preferred as the location of intervention to the GSV with insufficiency (Figure 1). The whole procedure was performed with the guidance of duplex USG. All treated GSVs had terminal valve incompetence. All interventions were performed by the same surgical team. The ClosureFAST (Covidien, Mansfield, Mass) catheter was used in all patients for the RFA procedure(Figure 2).

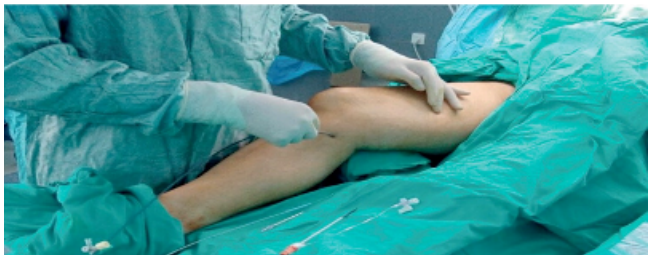

Figure 1. The location of intervention to the GSV.

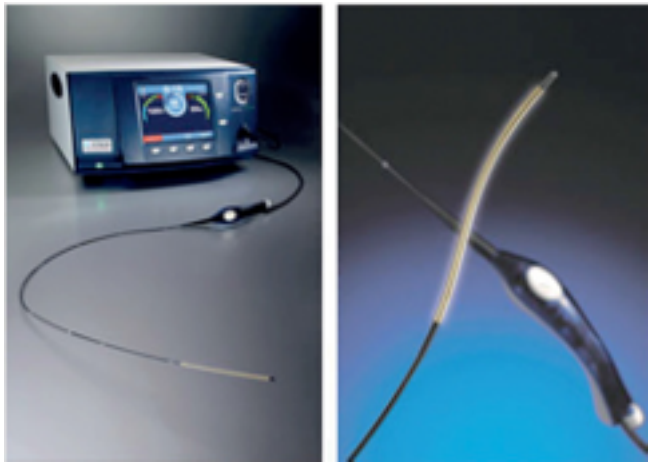

Figure 2. Covidien ClosureFast™ Endovenous Radiofrequency Ablation (RFA) Catheter.

After evaluating the GSV with duplex USG, intervention is performed with a 16 G - 70 mm needle. 7f sheath is applied to create the way to deliver the catheter. Through the sheath, the RFA catheter is delivered to the point where ablation will be initiated, again with USG guidance. The most appropriate point where the tip of the catheter is to be placed is 2 cm distal of the SFJ. To avoid heat damage, a classical tumescent anaesthetic mixture [4] consisting of 50 ml 1% lidocaine, 0.5 mg adrenaline and 10 ml 8.4% sodium bicarbonate and 450 ml isotonic NaCl was used (Figure 3). The average quantity was 350 to 450 mL. According to the manufacturer's recommendations in automatic mode, two cycles were performed for all segments. Each cycle consists of 20 seconds, that the 7 cm

active part of the catheter is kept at a constant temperature of 120 ° C.

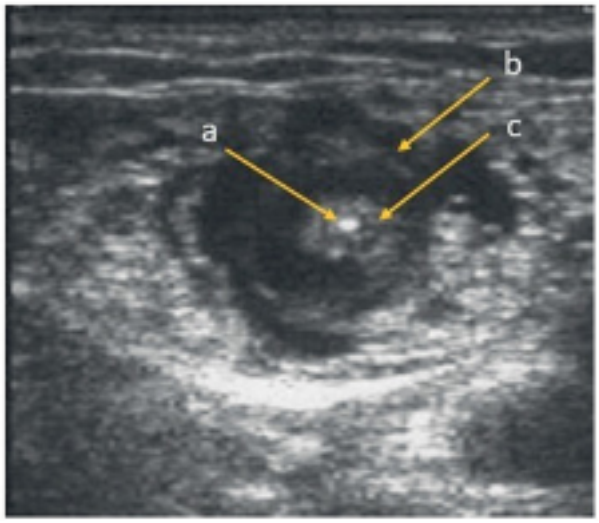

Figure 3. Tumescent anaesthetic application to avoid heat damage.

Following the procedure, an elastic bandage was applied around the extremity.

Compression therapy was performed for a period of two days.

Follow up protocol: All patients were followed at the outpatient clinic by the same surgeons. All phlebitis, edema, ecchymosis and any other complications were recorded. Duplex USG evaluation was performed in the standing position using a 9 MHz linear transducer (SonoSite Titan, SonoSite Ltd, Hitchin, UK). Duplex USG assessment results were classified as occluded vein (incompressible vein and no flow) and patent vein (partially incompressible vein and minimal flow pattern; compressible vein and presence of reflux for more than 2 s). All patients were evaluated using duplex USG at 4 weeks and 6, 12, 18 and 24 months after the procedure. In addition, clinical outcome measures were calculated using VCSS before and 4 weeks after the procedure.

Statistical analysis: Statistical analysis was performed with the Statistical Package for the Social Sciences (IBM SPSS Statistic Inc. Version 21.0, Chicago, IL, USA). Continuous and ordinal variables were expressed as mean ± standard deviation, and nominal variables were expressed as frequency and percentage. The Kolmogorov–Smirnov test of normality was used to identify the distribution of variables and the Wilcoxon signed ranks test was used to compare VCSS results before and 4 weeks after last endovenous RFA. Cumulative survival and complete occlusion rates were analyzed using the Kaplan–Meier method. For all tests, p value of <0.05 was considered statistically significant.

RESULTS

A total number of 217 patients who underwent RFA (52.5 % male, mean age 42.7±11.4) were recorded in the study. The demographic and clinical properties of the subjects are summarized in Table 1. The majority of patients (76%) were in the C3 and C4 groups according to the CEAP classification. The average size of the GSV was 7.9 ± 1.7 mm and the largest GSV diameter that was RFA applied was 14 mm (Table 1).

Table 1. Demographic features of the patients

	Patients n=217
Age (years)	42.7±11.4 (17-66)
Gender	
Male, n, %)	114 (52.5)
Female, n, %	103 (47.5)
BMI, kg/m2	27.4±4.2
Diamater of GSV(mm)	7.9±1.7 (6-14)
CEAP Class	
C2, n, (%)	48 (22.1)
C3, n, (%)	86 (39.6)
C4, n, (%)	79 (36.4)
C5, n, (%)	3 (1.4)
C6, n, (%)	1(0.5)
Baseline VCSS	6.4±1.2(4-10)
Follow-up time (Months)	22.2±5.1(1-24)

GSV: Great Saphenous Vein, CEAP: Clinical Etiologic Anatomic Pathophysiologic, VCSS: Venous Clinical Severity Score, BMI: Body Mass Index

The mean follow-up period of the patients was 22.2 ± 5.1 months. Due to the change of phone number and address, 34 patients could not be followed up clinically. Cumulative survival curve of RFA during follow up (Kaplan–Meier) was shown in figure 4 and the cumulative survival rate of 24-month follow-up was 84.3%. Complete occlusion rate for GSV was 100%, 98.6% ,97.6%, 97.6% and 97.6% for 1, 6, 12, 18 and 24 months follow up, respectively (Figure 5). GSV patency was observed in 5 patients among the patients that could be followed. While 3 of these patients

were detected at the 6th month of control, 2 were detected at the 12th month of the control. These patients underwent high SFJ ligation as an additional operation.

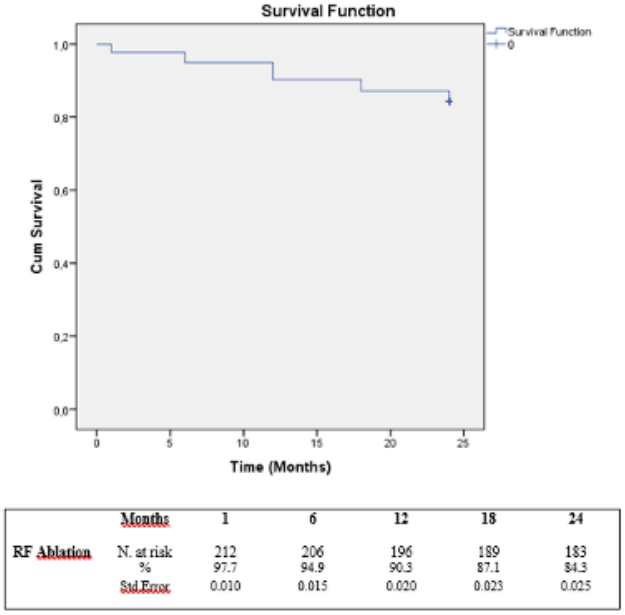

Figure 4. Kaplan–Meier survival curves of RF ablation during follow up. RF: Radiofrequency, N: Number of patients

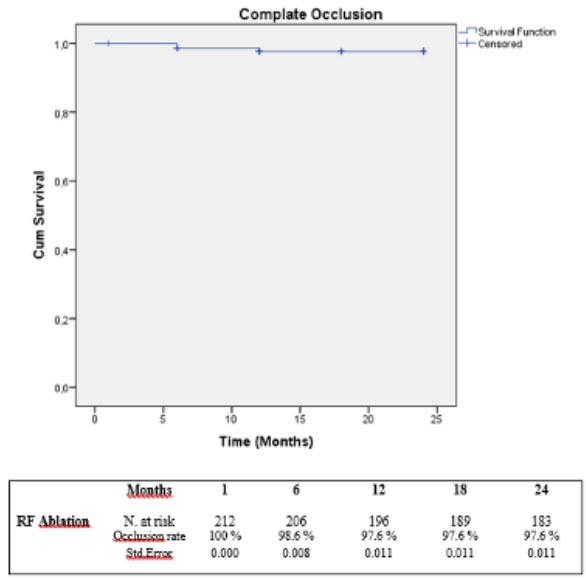

Figure 5. Kaplan–Meier cumulative complete occlusion rates of RF ablation. RF: Radiofrequency, N: Number of patients

We found that VCSS were 6.22±1.1 in preintervention and 1.3±0.5 in fourth weeks after RFA and found that VCSS was significantly different before and 4 weeks after RFA ($p < 0.001$, Wilcoxon Signed Ranks Test) (Table 2).

Table 2. Venous Clinical Severity Score results before and four weeks after RFA

	Before RFA	Four weeks after RFA	P value*
VCSS	6.22±1.1	1.3±0.5	< 0.001

RF: Radiofrequency ablation VCSS: Venous Clinical Severity Score, *Wilcoxon Signed Ranks Test

The most common postoperative finding was leg edema (26.7%), whereas local phlebitis occurred in 8 (3.7%) patients, and 42 (19.4%) patients complained of non-diffuse leg ecchymosis. Edema and ecchymosis findings improved after 2 weeks of medical treatment. In 14 (6.5 %) patients, palpable cordlike stiffness developed in the GSV region. Cordlike stiffness regressed within 2 months, except in one patient, whose cordlike stiffness was causing pain in limb movements and whose cordlike mass was removed surgically. In one patient (0.5 %), swelling was detected, after 1 month, in the region where the 7F sheath was inserted into the GSV with the Seldinger technique. The swelling was seen to be fluid in the USG and seroma was detected in the needle aspiration. With USG-guided needle aspiration, the seroma was emptied and a tight elastic bandage was applied. Although seroma was emptied twice, it continued to accumulate. Surgical exploration followed by vacuum treatment for 2 weeks was applied to the seroma region. When the accumulated amount was reduced to a minimum, capitonnage was performed surgically and seroma accumulation was not observed in the follow-up after surgery. DVT was not observed in any patient. Complications observed in patients are summarized in Table 3.

Table 3. Complications

	n=217
Edema, n,%	58 (26.7)
Ecchymosis, n,%	42 (19.4)
Palpable cordlike stiffness, n, %	14 (6.5)
Phlebitis, n,%	8 (3.7)
Seroma, n,%	1 (0.5)
Deep vein thrombosis	0

DISCUSSION

In present study, we investigated mid-term results of RFA in the treatment of varicose veins. We found that complete occlusion rates of GSV at 24 months of follow-up was %97.6. VCSS significantly

decreased after RFA at the first month follow-up. In addition, no life- threatening complications were observed.

The RFA method primarily effects to collagen matrix through heat-induced denaturation. Vein wall collagen contraction follows this effect. Shortly after, due to injury and inflammation of the vein wall, fibrotic sealing of the vessel lumen occurs [5]. Secondarily, endothelial denudation and swelling of the vein wall components occur due to heat-induced inflammatory processes. These mechanisms promise high rates of saphenous vein closure, therefore it has been claimed that it is an alternative treatment option to venous stripping, which leads to painful and prolonged post-operative recovery with high risk for hematoma formation, nerve damage and incidence of infection [6]. Consequently, due to improvement of the VCSS and CEAP levels after RFA procedure, the surgical treatment of symptomatic saphenous vein failure has evolved into less invasive endovenous treatments than GSV striping [7].

In a meta-analysis compiling randomized controlled studies presenting long-term results of endovenous procedures applied to the lower limb varices, no significant difference was found between RFA, EVLA or GSV striping in terms of recurrence rate, but RFA was reported to be superior to foam sclerotherapy with ultrasound [8]. Eroğlu et al. did not reveal a difference in their randomized controlled study comparing cyanoacrylate, RFA and EVLA procedures in respect to 2-year occlusion rates (occlusion rates were 92.6%, 90.9%, 91.5% respectively) (3). Bozoğlan et al. reported that the occlusion rates in their study that compare EVLA and RFA procedures, were respectively 100% and 94% after 6 months of follow-up [9]. In the study of 155 patients treated with RFA, Shepherd et al. reported 94.1% occlusion rate after an average follow-up of 12.2 months [10]. In another study evaluating only RFA results, they found occlusion rates as 94.6% -96% in the 2-year follow-up of the clinical results of RFA applied in 5 different centers in the Korean population [11]. In our study, similar to results of meta- analysis in the literature, comparative studies and studies in which the clinical results of RFA were published, we found the occlusion rate of 217 ClosureFastTM procedure to be GSV at 97.6% in a 24-month follow-up.

In a review evaluating frequently used quality of life and clinical scoring measurement techniques for venous diseases, it was found that VCSS was revised in 2010 and was the most common scoring method [12]. It is mentioned that VCSS is a scoring system that allows the measurement of minor changes in disease severity and enables evaluation of results at many levels such as technical success, patient reported success and clinical success. It is also mentioned that it has completed the CEAP classification. Our patients are routinely classified according to the CEAP classification and in our study as well, we evaluated the RFA procedure using VCSS scoring. In a study in which 12-month VCSS scores of RFA patients were evaluated, they stated that VCSS scores improved even in patients with recanalization after RFA [13]. Studies evaluating VCSS scores after endovenous RFA intervention have represented that the VCSS score is significantly reduced after the procedure [3,9-11]. In parallel with the literature, in our study, we found a significant decrease in the VCSS score after 4 weeks.

Similar to the literature, only minor complications were seen in present study (Table 3), and their quality of life improved one week after RFA and more improvement was observed in 12 months [3,9-11,14]. Since we recommend routine NSAIDs medication during the postoperative three-day follow-up, not many complaints of post-procedural pain were encountered, and postoperative pain was only observed in patients with phlebitis and cordlike stiffness. In the literature, among the complications that occur following endovenous procedures, we see the definition of "cord-like stiffness" in very few publications [9,11]. We consider that this situation is discussed under the title of thrombophlebitis. We think that vascular diameter, insufficient compression and insufficient leg elevation are effective in the development of cord-like stiffness. We found no evidence of classical phlebitis in our patients who developed cord-like stiffness in the GSV region: we encountered this complication in our study at a rate of 6.5%, and only one patient underwent surgical excision due to pain, but we think more studies investigating this clinical situation are needed. When we search

the literature, we were unable to find any seroma case in the region where the sheath was placed in the GSV, for the RFA procedure. Therefore, we think that our case may be the first, which we were able to treat with a series of procedures including puncture, compression, vacuum and surgical applications. We found that the treatment of seroma is challenging and therefore, although rare, it should be considered that it can develop at the puncture site. In early period of our RFA practice, we recommended enoxaparin for a week in the postoperative period and aspirin for a month, therefore the rate of ecchymosis was high in our series. However, our rate of ecchymosis was no higher than previous published studies, and a cure was achieved within two weeks [3,9,11] and we no longer administer this medication. Although DVT prevalence after RFA is believed to be between 0.2% and 1% [15], we did not observe any acute thrombosis or DVT in present study.

In a study comparing RFA with ligation and stripping, it was reported that most of the patients returned to their normal activities after the procedure, within 3-7 days [16]. Similarly, in our study, since spinal anaesthesia was performed in all our patients, they were all mobilized at the 6th postoperative hour, and all were discharged on the postoperative 1st day. The patients were advised to return to their normal activities within 3 days and to reprise work within a maximum of 5 days. We considered that this was a recommendation in accordance with the current literature [17].

Limitations: We have some limitations in present study, namely that that this was a retrospective effort. Additionally, the number of patients included in our study was small and it was not comparative.

Conclusion: RFA has improved patients' quality of life and our results are consistent with the results of studies in the literature. Consequently, we claim that RFA is a safe intervention that is successfully applied with high occlusion rates and significant VCSS score improvement in GSV failure.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):260-263

DOI:10.30565/medalanya.791230

Actinomyces in Tonsillectomy Materials

Tonsillektomi Materyallerinde Aktinomiçes

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ABSTRACT

Aim: Tonsillectomy is one of the most common surgeries in the pediatric age group. Actinomyces species are Gram-positive, non-spore-forming facultative anaerobic bacteria. Actinomycosis is a disease characterized by abscess formation, ranging from subacute to chronic infection.
Materials and methods: We studied with the tonsillectomy materials of 185 patients who underwent tonsillectomy in our clinic between January 2016 and January 2019.
Results: A total of 185 patients, 98 (53%) males and 87 (47%) females, who underwent tonsillectomy were included in the study. The mean age was 12.6 (range, 3-56) years. A total of 64 patients showed the presence of Actinomyces.
Conclusion: Actinomyces species are bacteria found in the tonsil tissue flora that cause opportunistic infection. Tonsillar Actinomyces colonization is more common in adults and in older children.

Key words: Tonsillectomy, Histopathology, Actinomyces

ÖZ

Amaç: Tonsillektomi çocuk yaş grubunda en sık yapılan ameliyatlardan birisidir. Actinomiçes türleri Gram-pozitif, spor oluşturmayan fakültatif anaerobik bakterilerdir. Aktinomikoz, subakuttan kronik enfeksiyona kadar apse oluşumu ile karakterize bir hastalıktır.
Yöntem: Ocak 2016 - Ocak 2019 tarihleri arasında Alanya Alaaddin Keykubat Üniversitesi Eğitim ve Araştırma Hastanesinde tonsillektomi uygulanan 185 hastanın tonsillektomi materyalleri ile çalıştık.
Bulgular: Tonsillektomi yapılan 98 (% 53) erkek ve 87 (% 47) kadın 185 hasta çalışmaya dahil edildi. Ortalama yaş 12.6 (dağılım 3-56) idi. Toplam 64 hastada Actinomiçes varlığı gösterildi.
Sonuç: Actinomyces türleri tonsil dokusu florasında fırsatçı enfeksiyona neden olan bakterilerdir. Tonsiller Aktinomikoz kolonizasyonu yetişkinlerde ve büyük çocuklarda daha yaygındır.

Anahtar kelimeler: Tonsillektomi, Histopatoloji, Aktinomiçes.

Received: 31.01 2019 Accepted: 06.04.2019 Published (Online):29.10.2020

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To cited: Gunizi OC, Gunizi H. Actinomyces in Tonsillectomy Materials. Acta Med. Alanya 2020;4(3): 260-263. doi:10.30565/medalanya.791230

INTRODUCTION

Tonsillectomy is one of the most common surgeries in the pediatric age group. Tonsillectomy involves the surgical excision of palatine tonsils. Recurrent tonsillitis and obstructive sleep apnea caused by tonsillary hypertrophy are the most common indications for tonsillectomy. [1,2]

Actinomyces species are Gram-positive, non-spore-forming facultative anaerobic bacteria that cause infection in the cervicofacial, abdominopelvic, and pulmonothoracic regions. [3] Actinomycosis is a disease characterized by abscess formation, ranging from subacute to chronic infection. The term Actinomyces colonization defines colonization in tissues and does not constitute active disease. Actinomyces israelii and Actinomyces naeslundii are the most commonly isolated Actinomyces species, and all species except Actinomyces bovis are members of the natural flora of the human oral cavity.[4-6] These bacteria are found in gingival clefts and tonsillar crypts in the normal structure of oral flora, especially in periodontal pockets, dental plaques, decayed teeth, and the upper respiratory tract. They are known to cause infection via dental caries in the head and neck area, interventional dental treatments, maxillofacial traumas, and mucosal trauma in tonsils. Proteolytic enzymes produced by the bacterium cause infection to progress to deeper tissues.[7] Actinomycosis can be diagnosed by showing reproduction in culture or by observing sulfur granules in biopsy samples. [8,9]

In this study, the results of routine histopathologic examinations of patients who underwent tonsillectomy in our clinic were investigated for the presence of Actinomyces and the results were evaluated in view of the literature.

MATERIALS AND METHODS

In our study, the tonsillectomy materials of 185 patients who underwent tonsillectomy in our clinic between January 2016 and January 2019 due to recurrent tonsillitis and obstructive sleep apnea caused by tonsillary hypertrophy were retrospectively examined.

Patients' age, sex, vital signs, indications for tonsillectomy and preoperative data were recorded. The patients underwent tonsillectomy under general anesthesia. The surgically removed tonsil tissues were fixed with 10% formalin, embedded in paraffin wax and stained with hematoxylin-eosin. Preparations were evaluated under light microscopy, and histopathologic findings were recorded.

Our retrospective study was approved by Alaaddin Keykubat University İnstitutional Review Boards and Ethics Comittee (Ethics no:20-11).

Means and standard deviations of groups were calculated. Fisher's exact test was used to determine significant differences between non-parametric data from the groups. The data obtained in the study were evaluated statistically and the results with a p value of <0.05 were evaluated significantly.

RESULTS

A total of 185 patients, 98 (53%) males and 87 (47%) females, who underwent tonsillectomy were included in the study. The mean age was 12.6 (range 3-56) years. Of the patients, 39 were aged under 6 years, 100 were aged between 6 and 18 years and 46 were older than 16 years. Of the patients, 104 (56.2%) underwent tonsillectomy due to recurrent tonsillitis and 81 (43.8%) due to chronic tonsillar hypertrophy and this issue is detailed in figure. (Figure 1). A total of 64 (34.6%) patients showed the presence of Actinomyces. The presence of Actinomyces by gender is shown in the table. (Table 1). Allergic rhinitis was present in 16.8% (n=31) patients. We found a significantly increased incidence of Actinomyces in patients receiving treatment for allergic rhinitis (67.7%).

Table 1. Actinomyces colonization and gender

	Gender		Total
	male	female	
Actinomyces -	66	55	121
Actinomyces +	32	32	64
Total	98	87	185

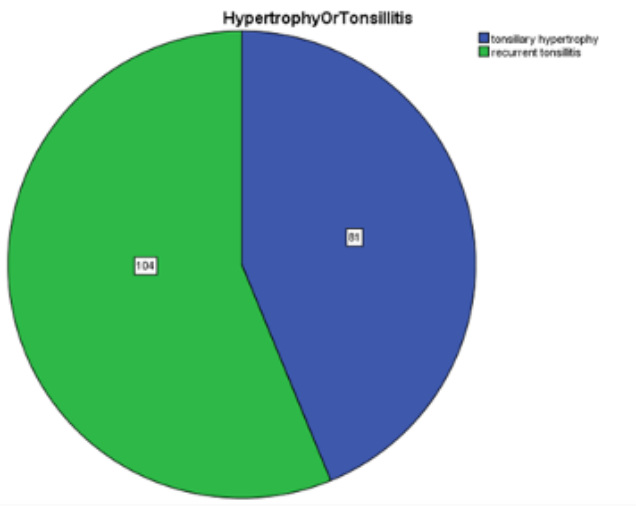

Figure 1: Number of Tonsillectomy due to recurrent tonsillitis or due to chronic tonsillary hypertrophy

DISCUSSION

The palatine tonsil is a large lymphoid tissue settled in a fossa formed by palatopharyngeal and palatoglossal muscles on both sides of the oropharynx. It reaches the largest volume at the ages of 5-6 years due to hyperplasia and atrophies at older ages. Absolute indications for tonsillectomy include tonsillar hyperplasia with obstructive sleep apnea, chronic tonsillitis, peritonsillar abscess, suspicion of malignant disease and hemorrhagic tonsillitis. Tonsillectomy is one of the frequent operations performed in the pediatric age group.

Actinomyces species were originally evaluated as fungi due to their branching fibrous structure, but were later shown to be Gram-positive bacteria.[10] Actinomyces israelii and Actinomyces naeslundii are the most commonly isolated Actinomyces species from humans, and these bacteria, which are normal flora elements, cannot overcome the robust mucosal barrier.[4-6] In the event that the mucosa is penetrated, it can cause infection by crossing the mucosal barrier.[11] Clinically, it may cause different pathologies such as fistula, abscess or pseudotumor. Typical microscopic findings include necrosis with yellowish sulfur granules and filamentous Gram-positive fungal-like pathogens. Actinomyces develops a chronic granulomatous infection characterized by the formation of tiny clumps, called sulfur granules because of their yellow color. These formations of 0.1–1 mm in diameter, composed of an internal tangle of mycelial fragments and a rosette of peripheral clubs, are stabilized by a protein–polysaccharide complex,.It is a homogeneous, eosinophilic saprophyte with structures extending towards the periphery in a radiative style in light microscopy. Besides clinical findings, microbiologic and histopathologic findings are important in diagnosis and the gold standard test for diagnosis is polymerase chain reaction. It is a penicillin-sensitive bacterium and penicillin is the first choice in treatment.[12]

Actinomyces species are detected in 1.8-37.0% of tonsillectomy materials.[11-13] In our study, we found colonization in 64 (34.6%) patients. Erkilic et al. found colonization in 8.2% of 1220 patients, and studies reporting a higher proportion are also available in the literature.[13] Toh et al. found colonization in 35.6% of 834 patients. [14] Antibiotic use before surgery is reported to affect the presence of Actinomyces in tonsillectomy materials. This major difference between the studies may be related to the different age groups in studies, different laboratories in which tests were performed and potentially the amount of antibiotics used before surgery.

Tonsillar Actinomyces are more frequent in adults, older children and men. Aydin reported that more Actinomyces were detected in adults in a study on 1820 patients.[15,16] Van Lierop and Melgarejo reported that this rate was higher in children aged over 5 years.[6,16] In our study, we found the presence of Actinomyces in 39.1% of the adult age group, which was more than in the pediatric age group, in line with the literature. There are also studies that report that there are more male patients with Actinomyces, that there are more female patients with Actinomyces, or that female and male patients with Actinomyces are equal.[4-6,,13,17] In our study, we found a higher rate of colonization in the female sex (36.8%), but this result was not statistically significant.

Systemic diseases such as sickle cell anemia and thalassemia were noted to increase the presence of Actinomyces.[18] In our study, we found a significantly increased incidence of Actinomyces in patients receiving treatment for allergic rhinitis (67.7%). However, we found no studies on this

subject in the literature. The classic symptoms of the disorder are nasal congestion, nasal itch, rhinorrhea and sneezing. A thorough history, physical examination and allergen skin testing are important for establishing the diagnosis of allergic rhinitis. Second-generation oral antihistamines and intranasal corticosteroids are the mainstay of treatment. Intense postnasal influx in allergic rhinitis may aid colonization of this bacterium.

More Actinomyces species are found in patients with tonsillar hypertrophy than in patients with recurrent tonsillitis and it is thought that Actinomyces infection causes tonsil hypertrophy and increases apnea. It was explained that the presence of bacteria increases lymphoid hyperplasia and causes apnea due to tonsillar hypertrophy. Toh et al. found more Actinomyces in patients who underwent tonsillectomy due to sleep apnea than in patients who underwent tonsillectomy due to recurrent tonsillitis in their study.[14] The researchers thought that Actinomyces could play a role in the etiologic mechanism of tonsillar hypertrophy and reported that antibiotic treatment would reduce the symptoms of apnea and tonsil size in these patients.[18,19] In our study, the number of patients who underwent surgery for recurrent tonsillitis (56.2%) was higher than for patients who underwent surgery for tonsillitis causing sleep apnea (43.8%). We found more (37.5%) Actinomyces in patients who underwent surgery for recurrent tonsillitis. However, we detected no significant link between Actinomyces colonization and tonsil hypertrophy and recurrent tonsillitis.

Conclusion: As a result, Actinomyces species are bacteria found in the tonsil tissue flora that cause opportunistic infection. Tonsillar Actinomyces colonization is more common in adults and in older children and is thought to contribute to obstructive apnea.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):264-269

DOI:10.30565/medalanya.738966

The relations of traumatic life events with depression, loneliness, anxiety, posttraumatic growth and pain in refugee university students

Mülteci Üniversitesi Öğrencilerinde Travmatik Yaşantilarin Depresyon, Yalnizlik, Anksiyete, Travma Sonrasi Gelişim ve Ağri ile İlişkisi

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ABSTRACT

Aim: The purpose of this study was to investigate the rate of traumatic experiences and the relationship between traumas and anxiety, loneliness, depression, posttraumatic growth and pain, in refugee university students.

Materials and Methods: This study was conducted at the Gaziantep University between September 2017 and September 2018. UCLA (University of California, Los Angeles) Loneliness Scale, Posttraumatic Growth Inventory (PTGI), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Traumatic Events Checklist (T.E.C) were used for assessment purposes.

Results: A total of 71 students were included in our study. There was a positive correlation between UCLA and BDI, T.E.C and a negative correlation between UCLA and PTGE. There was a positive correlation between BDI and BAI, T.E.C. A negative correlation was determined between PTGE and BDI and also T.E.C. A significant and positive correlation was observed between duration of pain felt during a day and T.E.C, BDI, and a negative correlation between duration of pain and PTGE.

Conclusion: Excessive traumatic experiences were positively related with depression and negatively related with traumatic growth. The duration of pain felt during a day was positively related with the excess of depressive symptoms and traumatic events, however there was no relationship between the presence of pain/ localization of pain and assessment scales.

Key words: Refugees, trauma, anxiety, depression, pain, posttraumatic growth

ÖZ

Amaç: Bu çalışmanın amacı, mülteci üniversite öğrencilerinde travmatik deneyimlerin oranını ve travmalar ile anksiyete, yalnızlık, depresyon, travma sonrası gelişim ve ağrı arasındaki ilişkiyi araştırmaktır. Gereç ve

Yöntemler: Bu çalışma Eylül 2017 ve Eylül 2018 tarihleri arasında XXX Üniversitesinde gerçekleştirilmiştir. Değerlendirme için UCLA (California Üniversitesi, Los Angeles) Yalnızlık Ölçeği, Travma Sonrası Büyüme Envanteri (TSBE), Beck Depresyon Envanteri (BDE), Beck Anksiyete Envanteri (BAE), Travmatik Yaşantilar Kontrol Listesi (TYKL) kullanılmıştır.

Bulgular: Çalışmaya toplam 71 öğrenci dahil edildi. UCLA ve BDE, TYKL arasında pozitif, UCLA ve TSBE arasında negatif korelasyon vardı. BDI, BAE ve TYKL arasında pozitif korelasyon vardı. TSBE ve BDE ile TYKL arasında negatif bir korelasyon saptandı. Bir gün içinde hissedilen ortalama ağrı süresi ile TYKL, BDE arasında anlamlı ve pozitif bir korelasyon ve ağrı süresi ile TSBE arasında negatif korelasyon gözlendi.

Sonuç: Mülteci öğrencilerde travmatik yaşantıların fazla olması depresyon ile pozitif, travmatik büyüme ile negatif ilişkiliydi. Gün içinde hissedilen ortalama ağrı süresi, depresif belirtilerin fazlalığı ve travmatik olayların fazlalılığı ile pozitif ilişkilidir, ancak ağrının varlığı / ağrının lokalizasyonu ve değerlendirme ölçekleri arasında bir ilişki saptanmamıştır..

Anahtar kelimeler: Travma, Depresyon, Yalnizlik, Anksiyete, Travma Sonrasi Gelişim, Ağrı

Received: 17.05.2020 Accepted: 27.07.2020 Published (Online):2910.2020

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To cited: Şahin ŞK, Altındağ Ö, Elboğa G, Elçiçek S, Akaltun MS, Altındağ A, Gür A, Doğan İ. The relations of traumatic life events with depression, loneliness, anxiety, posttraumatic growth, and pain in refugee university students. Acta Med. Alanya 2020;4(3):264-269. doi:10.30565/medalanya.738966

INTRODUCTION

Traumatic events are described in the 5th Edition of Diagnostic and Statistical of Mental Disorders as exposure to actual or threatened death, serious injury or sexual violation (DSM-5) [1]. Refugees comprise a high-risk population with regard to trauma exposure [2] and have higher risks of mental disorders in the long term, such as anxiety, depression, posttraumatic stress disorder and somatic complaints, such as pain. These mental disorders emerge in refugees after their exposure to war, violence or exile [3] and moreover, chronic pain and anxiety/depression are largely comorbid [4].

Turkey continues to be the world's largest home for refugees, with an approximate population of 4 million refugees according to statistics data by the United Nations High Commissioner for Refugees (UNHCR) from August 2018 [5]. Syrian refugees started to attend Turkish Universities as early as 2013 [6] and Gaziantep University, where the present study was carried out, has been the university with the highest number of refugee students in Turkey during the 2016-2017 academic year [7].

Posttraumatic growth (PTG) is an interesting concept that articulates that people can benefit from traumas, or even grow to a more optimal functioning [8]. Social support and coping styles facilitate posttraumatic growth and in Turkey, where social support for immigrants continues on an on-going basis, this relationship has not yet been studied.

Loneliness is a subjective concept based on the individual's perceptions. In other words, loneliness is the discrepancy between the relationships that an individual wants and the relationship that the person actually has. Loneliness and trauma symptoms were found to predict levels of PTG, and loneliness was found to moderated the relationship between PTG and trauma [9].

The most frequent traumatic events reported in Syrian refugees were being forced to flee their home country, witnessing drumfire and being confined to home [10]. Posttraumatic stress disorder and anxiety disorders have been investigated in refugees in previous studies: although the relationship between anxiety, depression symptoms and also chronic pain symptoms in refugees have been documented [11], it is not yet clear which trauma is associated with which variable, nor how traumatic growth in immigrants staying in our country for at least 5 years is related to these variables. Our aim in this study was to analyze the traumas that refugee university students suffer from the most, as well as to investigate the relationship between loneliness, anxiety, depression symptoms, psychosomatic pain, posttraumatic growth and traumatic experiences.

METHODS

Refugee university students were included in this descriptive study carried out between the dates of September 2017 and October 2018 in Gaziantep, Turkey. The approval for the study was obtained from the Ethics Committee of Gaziantep University (Ethical code: 2019/482), and written and verbal informed consent was recorded from the voluntary participants. A total of 71 students were selected to participate and the minimum duration of their status as a refugee was five years for all participants. Exclusion criteria were neurological diseases, acute infections, diabetes mellitus and other endocrinopathologies, a medical disease that might change pain thresholds, using medication for pain or anxiety, as well as being a patient with malignant diseases. Sociodemographic variables such as age and marital status were recorded and the participants were asked about the following traumas: the death of a family member, witnessing violence, witnessing drumfire, witnessing death, being homeless and being a subject of torture. Also, participants were asked if they have pain, to localize such pain in addition to the duration of pain during a day (no/not every day/0-30 min/30-60 min/more than 60 min). A visual analog scale was used to assess pain severity.

The participants were asked to complete all the scales. However, since some participants did not respond to certain questions or disregarded the scale, each test was evaluated according to the participants who completed those tests.

ASSESSMENT TOOLS

UCLA (UNIVERSITY OF CALIFORNIA, LOS

ANGELES) LONELINESS SCALE

The UCLA consists of 20-items and a 4-point Likert-type self-report questionnaire. It assesses the general subjective feelings of loneliness and social isolation for individuals. The scale was developed in 1978 and revised in 1980. The UCLA was investigated according to psychometric qualities for the Turkish version [12, 13]. Cronbach's alpha was 0.72

POSTTRAUMATIC GROWTH INVENTORY (PTGI)

The PTGI consists of 21-items and a 6-point Likert-type questionnaire. The test assesses favorable posttraumatic changes: higher scores mean favorable psychological changes owing to the hostile life events [14]. The PTGI was investigated according to psychometric qualities for Turkish version [15]. Internal consistency was found at 0.88 for self-perception subscale, 0.78 for philosophy of life subscale, 0.77 for relationship subscale and 0.92 for overall items.

BECK DEPRESSION INVENTORY (BDI):

The BDI consists of 21 items and a 4-point Likert-type questionnaire which evaluates depression severity over the previous week. The total score is an indication of the severity of depression. Cronbach's alpha was 0.91. The reliability and validity studies were carried out for this scale in Turkish [16].

BECK ANXIETY INVENTORY (BAI):

The scale was developed in 1988 and consists of 21 items and a 4-point Likert-type questionnaire for evaluating anxiety severity. The reliability and validity studies were conducted for this scale in Turkish [17, 18]. Cronbach's alpha was 0.93.

TRAUMATIC EVENTS CHECKLIST (T.E.C)

The T.E.C consists of 29-items for potentially traumatizing events and a Likert-type self-report questionnaire. The total score is an indication of potentially traumatizing experiences (range 0–29). The reliability and validity studies were carried out for this scale in Turkish [18].

The SPSS 22.0 program (IBM Corporation, Armonk, NY) was used for analyzing the variables. Descriptive statistics were used for the demographic characteristics of the 71 students. Data were described as mean±standard deviation. The normal distribution of numerical data was tested via the Shapiro-Wilk test. Comparisons for the variables which are compatible with normal distribution were performed by the t test, otherwise using the Mann-Whitney u test. $P<0.05$ was considered as significant.

RESULTS

A total of 71 cases were included in our study. The mean age was 22±4, 97.1% of the students were single, 39.1% were smokers. A total of 41 participants were Syrian, whereas 23 participants didn't indicate their nationality. Of the total number of students, 14 reported the death of a family member, while 34 stated that they witnessed violence, 28 witnessed drumfire, 43 witnessed death, 20 experienced being homeless and 19 of them were subject to torture. Sixty-two participants complained of pain (Table 1).

Table 1: Traumatic events

		n	%
Witnessing drum-fire event	Yes	28	%40
	No	42	%60
Death of a family member during process	Yes	14	%20
	No	56	%80
Witnessing violence	Yes	34	%48.6
	No	36	%51.4
Witnessing a death except that of a family member	Yes	43	%61.4
	No	27	%38.6
Being homeless	Yes	20	%28.6
	No	50	%71.4
Being subject to torture	Yes	19	%27.1
	No	51	%72.9
Pain	Yes	62	%87.3
	No	9	%12.7

The mean assessment scales scores were as follows: UCLA: 45.2±10.4, PTGE: 56.5±12.3, BDI: 19.4±12.5, BAI: 17.4±15.5; T.E.C:15.7±18.8. A positive correlation was observed between UCLA and BDI, T.E.C and a negative correlation with PTGE. There was a negative correlation between PTGE and BDI and also T.E.C, whereas a positive correlation was found between BDI and BAI,

T.E.C. (Table 2).

Table 2: Correlations of assessment tools

Spearman's rho		UCLA	PTGE	BDI	BAI	T.E.C	Duration of pain
UCLA	R	1,000	-,376**	,391**	,133	,316**	,433**
	P		,001	,001	,274	,008	,000
	N		70	70	70	70	70
PTGE	R		1,000	-0.341	-,032	-,292*	-,245*
	P			,004	,793	,014	,041
	N			70	70	70	70
BDI	R		-0.341	1,000	,341**	,500**	,409**
	P				,004	,000	,000
	N				70	70	70
BAI	R				1,000	,206	,180
	P					,088	,135
	N					70	70
T.E.C	R					1,000	,466**
	P						,000
							70

There were no significant differences between death of a family member/witnessing drumfire and five assessment tools. There were significant differences witnessing violence and BDI, BAI, T.E.C. There was significant differences between death of a family member and BAI, T.E.C. There was significant differences being homeless and BAI, BDI, and there were significant differences being a subject of torture and UCLA, BAI (Table 3).

Of the participants with pain, 49 (79%) described widespread pain, 5 (7.1%) complained of waist and back pains, 6 (8.6%) of headaches and 2 (2.9%) of leg-knee-foot pain. There were no significant differences between pain/pain localization and trauma exposure types and assessment tools. A statistically significant correlation could not be determined between presence of pain and assessment tools. However, there was a significant positive correlation between duration of pain and T.E.C, BDI and a negative correlation between duration of pain and PTGE (Table 2). Moreover, a significant and positive correlation was determined between duration of pain and witnessing violence (p=0.001, r: 0.471), being homeless (p=0.002, r: 0.356) and being a subject of torture (p=0.003, r:0.354).

A significant and positive correlation was also determined between the duration of pain and witnessing violence (p=0.001, r: 0.471), being homeless (p=0.002, r: 0.356) and being a subject of torture (p=0.003, r: 0.354).

Table 3: The significant differences between trauma types and five assessment scores

	Witnessing violence	N	Mean	Std. Deviation	P
BDI	No	36	16,56	13,40	0,022
	Yes	34	22,56	10,99	
BAI	No	36	12,47	14,77	0,002
	Yes	34	22,79	14,77	
T.E.C	No	36	11,81	20,74	0,001
	Yes	34	19,91	15,89	
	Witnessing a death except that of a family mem-ber	N	Mean	Std. Deviation	P
BAI	No	27	12,04	16,22	0,002
	Yes	43	20,91	14,27	
T.E.C	No	27	15,22	23,80	0,047
	Yes	43	16,07	15,28	
	Being homeless	N	Mean	Std. Deviation	P
BDI	No	50	17,66	12,63	0,050
	Yes	20	24,00	11,50	
BAI	No	50	14,10	14,61	0,001
	Yes	20	25,95	14,94	
	Being subject to torture	N	Mean	Std. Deviation	P
UCLA	No	51	43,16	10,26	0,003
	Yes	19	50,95	9,13	
BAI	No	51	15,63	15,60	0,031
		19	22,47	14,69	

DISCUSSION

Our country is home to refugees and ensures the continuity of the university education of refugee students [5-7]; it is believed their attendance in school facilitates their adaptation to the host country [19]. This study investigated traumas and related mental symptoms, including psychosomatic pain and post traumatic improvement, in refugee university students who are expected to adapt to their host country.

Witnessing a death (61.4 %) was observed to be the maximum traumatic events in our study. A relationship was found between PTSD and depression and also PTSD and anxiety in a meta-analysis [11]. Our study confirms that there was

a positive correlation between depression and anxiety symptoms/traumatic events; witnessing violence was also observed to be related with higher depressive symptoms, according to trauma type.

The UCLA is an indication of subjective feelings on loneliness but does not provide information on causes [12]. Positive correlations between UCLA and depressive symptoms and negative correlations with PTGE, suggest that the feeling of loneliness increases with increasing depressive symptoms and that those feelings of loneliness decrease as refugees recover from posttraumatic symptoms. Being subject to torture was also observed to be related to higher loneliness symptoms, according to trauma type. A strong relationship has been observed in previous studies between depression and loneliness [20] and these results indicate the importance of coping with post traumatic depressive symptoms.

Traumatic life events were found to be a major risk factor for depression. Migrants were also more likely to report feelings of loneliness and greater overall depression, in comparison with non-migrants [21]. Similarly, our study showed that there is a strong relationship between traumatic events and depression. A negative correlation was also observed between T.E.C. and PTGE, and these results suggest that "the greater number of traumas means fewer posttraumatic recoveries". Moreover, the minimum period of time the participants spent as a refugee was five years, which reveals that the majority of the participants had traumatic experiences before the age of 18. Therefore some of these traumatic experiences can be evaluated as childhood traumas, which in turn are associated with mixed anxiety-mood disorders [22]. Being homeless, being subject to torture, witnessing violence, the death of a family member, were all observed to have significantly higher BAI scores, whereas witnessing violence and being homeless were also observed to have significantly higher BDI scores. These results suggest that some childhood traumas may highly contribute to anxiety and depression symptom levels in adulthood.

There is strong evidence purporting to the relationship between chronic pain and anxiety/depression [4]. There is also consistent evidence on the relation between chronic pain and PTSD: indeed patients with PTSD report greater chronic pain severity [23]. Since the majority of the students did not fill out the VAS - the scale related to pain - we were unable to reach a conclusion on pain severity. We did not find any relationship between the presence of pain/localization of pain and assessment scales. However, a significant and positive correlation was observed between duration of pain and T.E.C, UCLA, BDI. There was also a significant and positive correlation between duration of pain and witnessing violence, being homeless and being subject to torture. These results suggest that more traumatic events and depression severity increased the duration of the pain in our refugee students. In addition, trauma type is also related to chronic pain: there was a particularly strong relation between chronic pain and witnessing violence. The mechanisms for the relationship between pain and trauma are not fully known, but one hypothesis is that pain is a maladaptive coping strategy against arousal, that triggers traumas leading to PTSD. Another hypothesis is that high levels of anxiety sensitivity lead to a traumatic stressor or pain [24].

The limitations of our study were the low number of participants, the self-report evaluation, the fact that participants did not answer all questions in the questionnaires, and that the precise duration of time spent as a refugee is unknown – though a five year minimum duration of time spent as a refugee was selected as a criteria for inclusion in the study of some 71 individuals. In addition, most of the participants preferred not to indicate their gender, therefore we were unable to determine if there was a difference between the sexes. Furthermore, though studies aim to eliminate the mental health challenges and improve preventive strategies for mental health in these populations, refugees may be suspicious of research, may feel some degree of shame or may fear a loss of confidentiality, thereby making them less likely to participate in research. We think that participants may have been particularly overly concerned about revealing their identity.

Trauma rates are high among refugee university students and the most common trauma type reported by students in our study was witnessing

a death (61.4 %). Feelings of loneliness were positively correlated with depressive symptoms and negatively correlated with post traumatic growth in refugees. Growth after trauma is difficult if traumatic experiences are high; our results indicate the significance of coping with post traumatic mental symptoms. Exercise was negatively associated with pain, but they were not associated with anxiety depression. The duration of pain is positively proportional with excessive depressive symptoms and traumatic events, while no relationship could be determined between the presence of pain/localization of pain and assessment scales. Further large scale studies are needed to better evaluate the association between traumas and anxiety/depression/pain in refugees.

Funding sources: The authors declared that this study has received no financial support.

Conflict of Interest: No conflict of interest was declared by the authors.

Acknowledgements: Authors would like to thank Enago (www.enago.com) and jy.blanchard@alanya.edu.tr for the English editing and language review

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Acta Medica Alanya

REESRCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):270-277

DOI:10.30565/medalanya.792279

Thoracic outlet syndrome: efficiency of surgery

Torasik Outlet Sendromunda Cerrahi Tedavinin Etkinliği

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ABSTRACT

Aim: In this study, we aimed to evaluate the effectiveness of surgical treatment in patients diagnosed with thoracic outlet syndrome.

Patients and Methods: This study was conducted by analyzing the age, sex, occupational distribution, anamnesis and physical examination findings, symptoms, preoperative examinations, operation findings, postoperative complications, postoperative hospital stay and operation results of thirty seven patients who were diagnosed with Thoracic Outlet Syndrome, and were operated on between 1991 and 2004.

Results: Most of the patients were in the 15-35 age group (72.97%) and female (91.9%). Most of these cases were housewives (51.4%). The most common symptoms were pain (94.6%) and numbness (78.4%). The most commonly used test in physical examination was the Adson test, which provided an 86% positive rate. The most common pathology encountered in radiological tests was cervical rib anomaly (37.8%). It was found that EMG of upper extremity supported TOS with a rate of 48.6% and arterial doppler of upper extremity supported TOS with a rate of 13,5%. We applied first rib and cervical rib resection scalenectomy and cutting of fibromuscular bands as a surgery by transaxillary approach. A 78.4% complete recovery was observed in the early period after surgery and there was no mortality in any of the cases.

Conclusion: In this study, the results were satisfactory in TOS patients on suitable cases and with good surgical technique. Surgical success rate can reach up to 97%.

Key Words: thoracic outlet syndrome, cervical costa, transaxillary approach.

ÖZ

Amaç: Bu çalışmada, Torasik outlet sendromu tanısı konulan hastalarda cerrahi tedavinin etkinliğini değerlendirmeyi amaçladık.

Hastalar ve Yöntem: 1991 ile 2004 yılları arasında Torasik Outlet Sendromu tanısı almış ve tedavi yöntemi olarak cerrahi uygulanmış otuz yedi olgunun yaş, cins, meslek dağılımı, anamnez ve fizik muayene bulguları, semptomları, preoperatif tetkikleri, operasyon bulguları, postoperatif komplikasyonları, postoperatif hastanede kalış süreleri ve operasyon sonuçlan incelenerek yapıldı.

Bulgular: Olguların çoğu 15-35 yaş grubunda (% 72.97) ve kadınlardan (% 91,9) oluşmaktaydı. Bu olguların çoğu ev hanımıydı (% 51.4). En sık rastlanılan semptomlar ağrı (%94,6) ve uyuşma (% 78.4) idi. Fizik muayenede en sık kullanılan test Adson testi idi. Adson testi %86 oranında pozitif bulundu. Radyolojik testlerde en sık karşılaşılan patoloji servikal kosta anomalisiydi (% 37.8). üst ekstremite EMG sinin %48.6, üst ekstremite arteriel doplerinde% 13.5 oranında TOS u desteklediği bulundu. Cerrahide transaksiller girişim ile birinci kosta ve servikal kosta rezeksiyonu. skalenektomi fibromüsküler bantların kesilmesi işlemlerini uyguladık. Cerrahiden sonra erken dönemde %78.4 oranında tam düzelme görüldü. Olguların hiçbirinde mortalite olmadı.

Sonuç: Bu çalışmada TOS hastalarda, uygun olgularda ve iyi cerrahi teknik ile sonuçlar tatmin edici bulundu. Cerrahi başarı oranı %97 lere kadar çıkabilmektedir.

Anahtar kelimeler: Torasik Outlet Sendromu, Servikal Kosta, Trnsaksiller yaklaşım.

Received: 10.09.2020 Accepted: 28.09.2020 Published (Online):29.10.2020

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To cited: Aslaner O. Thoracic outlet syndrome: efficiency of surgery. Acta Med. Alanya 2020;4(3):270-277. doi:10.30565/medalanya.792279

INTRODUCTION

Thoracic outlet syndrome is a group of symptoms occurring due to the vascular and neurological structures at the upper thorax outlet being compressed by other upper thorax outlet structures, for congenital or acquired causes. While only one of the vascular or neurological symptoms can be seen in one person, symptoms shared by both can also be observed in the same person. The severity of the symptoms of thoracic outlet syndrome does not correlate clinically with the severity of anatomic pressure. For this reason, thoracic outlet syndrome does not have any physical examination results or laboratory tests that will lead to a definite diagnosis. While there was no evidence of severe pressure in the physical examinations, EMG findings or operations of some patients with severe symptoms, severe pressure findings may be observed in the physical examinations, EMG findings or operations of patients with low level symptoms. Therefore, it is not possible to talk about a complete correlation between anatomic pressure and symptoms, which leads to difficulties in the diagnosis. Taken together, the clinical experience of the physician in said cases is very important [1,2]

In diagnosed patients, physical therapy methods accompanied by medical treatment are suggested as a first line treatment[3]. Medical treatment combined with physical therapy can lead to serious clinical relief, although it can never completely improve the current mechanical pressure[6]. In patients where the desired level of clinical relief could not be maintained despite physical therapy and medical treatment, surgical treatment can be recommended [4,5]. The purpose of surgical treatment is to completely remove the components that cause pressure[6], and they include the first rib and if present, cervical ribs and scalene muscles [7-11]. Patient's complaints almost completely disappear shortly after the surgical treatment performed without damaging the plexus brachialis, subclavian artery and subclavian vein, and as long as there are no leaking bleeding spots left in the operation area to cause future cohesions, the likelihood of recurrence of thoracic outlet syndrome in later periods is very low [6,12,13].

In this study, we retrospectively examined our cases where we applied surgical treatment and tried to demonstrate the effectiveness of surgical treatment in thoracic outlet syndrome .

PATIENTS AND METHODS

Our study included 37 patients diagnosed with thoracic outlet syndrome in the Department of Thoracic Surgery of the Akdeniz University Medical Faculty between 1991-2004, who were expected to benefit from surgical treatment in the light of preoperative evaluations and examinations, and were operated on. The cases were examined with retrospective evaluation by observing patient anamnesis forms, surgical reports, preoperative examinations (EMG, upper extremity arterial doppler, cervical MR, etc.) and postoperative clinical monitoring from patient records. Preoperative symptoms, physical examination findings and preoperative examination results were evaluated by comparison and the consistency was examined. Also, postoperative healing was compared with the length of stay in the hospital to detect how much the patients had benefited from the surgery.

Our exact diagnosis criteria in our clinical approach; The positivity of the Adson test was that the nerve compression was compatible with TOS in the EMG in neurogenic TOS, and the Adson test positivity in the vascular TOS was the compression findings compatible with TOS in the vascular doppler Pressure signs in vascular doppler Venous thrombosis in the thoracic outlet and distal, arterial thrombosis, and deceleration in arterial flow velocities distal to the thoracic outlet. It was accepted as definite TOS in cases who had complaints consistent with TOS and had no other pathological condition that could cause these complaints and also had a positive Adson test. In cases with complaints compatible with TOS, no other pathological condition that could lead to these complaints, and EMG and vascular doppler results that do not support TOS, cases with suspicious positive Adson test were accepted as possible TOS.

Patients who were suspected of having definite or possible TOS and whose other diseases were excluded in the differential diagnosis were firstly treated medically with NSAI, whereas the cases that could not be obtained were directed to the

physical therapy and rehabilitation clinic, and received physical therapy.

The surgical technique we clinically adopted and applied in TOS was the transaxillary approach. We operated all our cases with this surgical method and we removed first rib in all patients (Image 1 and Image 2).

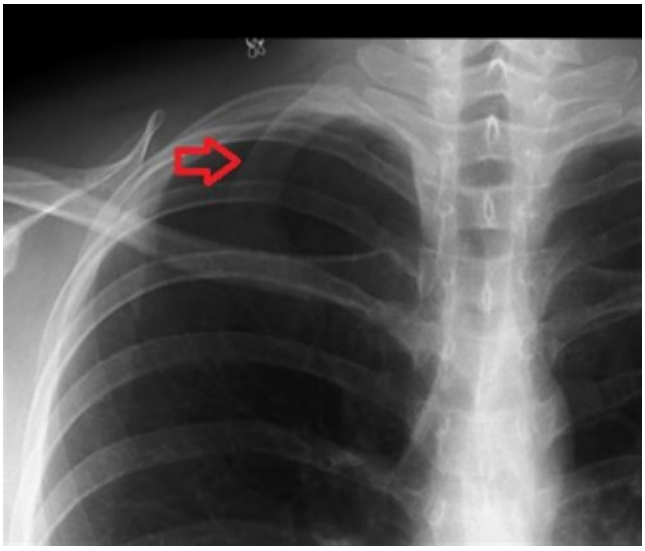

Image 1. Preop, presence of 1st rib

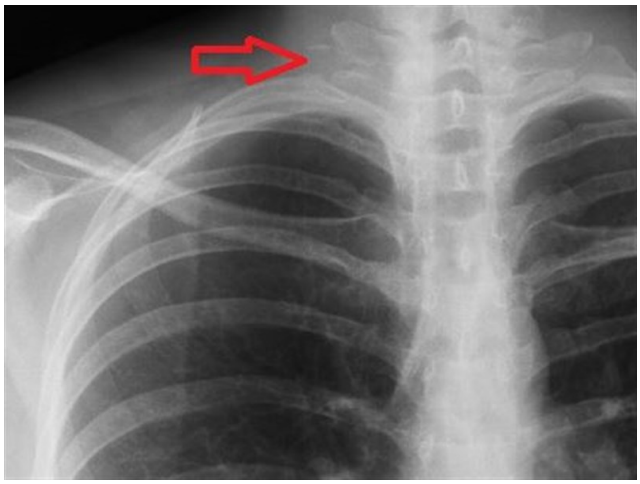

Image 2. Postop, after 1st rib resection.

Wound dressing was applied daily and antibiotic prophylaxis was applied to all patients. Tube thoracostomy cases were followed up with daily chest x-ray, and daily chest tube maintenance was performed. The aim was to remove the risk of infection and tube pain by pulling the chest tubes as soon as possible.

In the first week, the patients were advised not to get the wound areas wet, to make movements to strengthen the upper extremity and to prevent restriction, not to put excessive load on the extremity and not to force it excessively. All patients were called for control at regular intervals within the first year. Clinical examination and direct radiological imaging were performed routinely during the controls. During certain periods of the controls, EMG in neurogenic TOS and doppler tests in vascular TOS were requested and compared with preoperative findings.

Our criteria for improvement were the statements of the patient about the improvement level of their complaints, the negative Adson test on physical examination, as well as the improvement in the EMG and Doppler findings. Statements of the patients about recovery were categorized as complete recovery, partial improvement and no change: We determined our recovery results using these criteria and we ensured the study was carried out according to ethical rules.

Statistical methods: The data obtained was compared with the chi-squared analysis test using the SPSS 10 statistics program in a Windows XP processor. Firstly, demographic data of the patients were recorded and descriptive statistics were made, comparing the presence of the patients' clink healing preop and postop symptoms. The investigation of cases with positive Adson test and EMG positive cases was done with the chi-square. The relationship between the presence of cervical ribs and the severity of symptoms was investigated by the Pearson correlation.

RESULTS

The ages of the patients differed between 18 and 60 years and the average was 33.2432. Thirty-four of the patients (91.9%) were female and 3 were male (8.1%). Thoracic outlet syndrome was detected unilaterally in 35 cases (94.59%) and 2 bilateral cases (5.41%) were identified. Housewives accounted for the majority of the patients (51.4%), whereas students and workers were in the second and third place at an equal 18.9% (Table 1).

In our study, we separated the symptoms into the two main groups of vascular and neurological. In 33 of the cases (89.2%), there were symptoms of neurological origin and 2 of them (5.4%) had vascular symptoms. Two cases (5.4%) had both

neurological and vascular symptoms; the Adson tests of all the cases were examined. Upper extremity arterial doppler was performed in 22 (59.5%) of the patients and 5 (13.5%) of the findings were compatible with pressure, while arterial doppler results of 17 (45.9%) came in normal. Arterial doppler was not performed on 15 of the patients (40.5%), while on radiological examination, cervical graphs and cervical MR images of the cases were examined. All of the cases had cervical graphs, cervical MR scans were performed on 11 (29.72%) cases and positive findings were encountered in 5 (45.45%) of these. In cervical graphs, a cervical rib was encountered in 14 cases (37.8%) and not encountered in 23 cases (62.2%). Another test that we used for electrophysiological diagnosis was the EMG, which was performed on all cases except for 3 (%8.81). It turned out positive in 18 cases (48.6%) and negative in 16 (43.2%) (Table 2).

Table 1. Demographic Data

Age (Mean±SD)	39±10
Gender (Female/Male)	34 (%91.9)/ 3 (%8,1)
Side (Right/Left)	21/18
Occupation (House-wife/Worker/ Student/Other)	19/7/7/4

Table 2. Clinical Signs

	Preop	Postop	P value
Adson test (+)	39	3	0,03**
	-		
Emg *(+)	18	3	0,001**
	-		
Cervical Costa	14	0	0,001**
Vascular symp-tom***	4	0	0,001**
Neurological***	35	5	0,001**

*3 were not performed, ** Chi Square test(Monte Carlo Method's),
*** both symptom was positive: neurological and vascular

Among the 37 cases in our study, the most common complication was pneumothorax, at 14 (37.8%). In contrast to the other series where the Horner syndrome was not observed at all, hemothorax occurred in 2 cases (5.4%) and infection occurred in 1 case (2.7%). When the early results of the cases were evaluated, 29 patients (78.4%) were fully recovered and 5 patients (13.5%) were partially recovered, whereas in 3 cases (8,1%), there was no change in symptoms (Table 3).

Table 3. Early Postop Recovery Results

Results	Number	%
Total Recovery	29	78,4
Partial Recovery	5	13,5
No Changes	2	8,1
TOTAL	37	100

DISCUSSION

The main result of this study is that there was 78.4% complete recovery in the early period after surgery. The debate over whether the method used for TOS treatment should be a conservative approach or a surgical approach, has not yet been clarified. Despite the fact that there are many studies advocating both treatment methods in the literature, the view that is prevailing in recent years is that the cases should be treated with conservative treatments, including physical therapy and rehabilitation, until the last moment, and that surgical treatment should only step in if the complaints continue or are not reduced to a satisfactory level. Krusen et al. reported in his study published in 1968 that physical therapy and rehabilitation is the first treatment to be done in TOS [3]. According to them, it is possible to obtain results in the middle age group through neck-region massages, active neck exercises, exercises performed by stretching and loosening the upper trapezius muscle and scalene muscles, as well as hot applications. All cases included in our study had firstly applied various conservative treatments. When early results of the cases were evaluated, 29 cases (78.4%) were fully recovered and 5 cases (13.5%) were partially recovered, whereas in 3 cases (8,1%), there was no change in symptoms. With all cases in which partial recovery was achieved as a result of surgical treatment, complete recovery was achieved after physical therapy and rehabilitation. This rate is higher than many series reported in the literature. The full recovery rate of 78.4% in our study was also in accordance with the success rates of the studies done in our country, but one of the best success rates in the literature belongs to Sanders, who reported a 70% success rate [1,4]. The highest success rate ever reported was by Urschel in his series, at 97%.

In the surgical treatment of thoracic outlet syndrome, the most commonly used intervention

since the 1960s is transaxillary intervention. It can easily reach the first rib, cervical rib and neurovascular structures, and protection of neurovascular structures can be achieved during the removal of the first rib [4,9,15]. We too have performed transaxillary intervention on all of the cases in our operations and we performed first rib resection and scalenectomy in all cases. In cases with cervical rib and fibromuscular bands, we had to excise these structures.

In a long-term study, success rates were reported as 92% for transaxillary intervention, 83% for supraclavicular intervention, and 86% for posterior intervention. Postoperative complication rates do not exceed 4% in the literature [1,2,9,10,16], whereas in our study, the most common complication in 37 cases was pneumothorax, at 14 (37.8%). In contrast to the other series, there was no occurrence of the Horner syndrome, while hemothorax occurred in 2 cases (5.4%) and infection in 1 case (2.7%). None of the cases developed phrenic nerve palsy, but Acinetobacter Baumannii occured in the case with infection: the patient remained in the hospital for a total of 10 days, during which time the infection was controlled without further progress and the patient was discharged with a complete recovery. Our complication rate was high, however, in comparison to the literature, such as with the series from Roos [7] and Urschel [17] which were around 1%. We attributed the fact that pneumothorax made up the highest portion of our complications to the fact that sufficient care was not shown to protect the pleura during operation and in addition, our complication rate would be down to 8% if we excluded pneumothorax. In some publications for thoracic outlet syndrome, the recurrence rate is reported to be 25% within the first two years [18,19] and Zatocil reported a recurrence rate of 45% in a series of 112 cases published in 1997 [20]. Many of the recurrent cases later benefited from physical therapy and rehabilitation methods and they were able to carry on asymptomatic or with symptoms that do not affect their lives [4,9,21,22]. Two of our cases (5.4%) applied with recurrence complaints about one year later, and both benefited from physical therapy and rehabilitation methods. Long-term routine follow-ups of the patients were not performed, but we did not have similar complaints again except with these 2 cases.

On the other hand, we found that the demographic findings of the cases included in our study were partially compatible with the literature and on occasion, quite different. For instance, in series with large numbers of cases, the age group of 20-40 years is reported as the most frequent age group of thoracic outlet syndrome [1,2,4,9]. Nelson et al. reported an average age of 35 years [9], while Jamieson et al. reported 36 years in a group of 409 patients [23]. In our study, which included 37 cases, the average age was found to be 33.25 and this average was in accordance with the literature. Women account for 60-80% of thoracic outlet syndrome cases in all published series [1,2,4,9] and Jamieson reported this rate at 86% in his publication in 1996 [23]. In our study, 91.9% of the cases were females, however the regional situation of the clinic where the study was conducted and the gender distribution of the patients included may have had some effect on these results.

One of the most important points to note when examining the etiology history of thoracic outlet syndrome is the occupation of the person, as professional predisposition must definitely be known in TOS. The likelihood of thoracic outlet syndrome is an increasingly reported disease in occupations such as computer operators, secretaries, and bodybuilders who use the upper extremities for a long period of time [1,2,24-27]. In our study, we have found that 19 patients who constituted 51.4% of thoracic outlet syndrome cases were housewives and did not actively work in any particular occupation. However, the most frequent occupation in the literature is reported to secretaries in thoracic outlet syndrome female cases. We hypothesized that the majority of females in countries where thoracic outlet syndrome related studies were conducted participated in active work lives, and that the rates of housewives included in the cases of our study were critically low.

In the meta-analysis of 17 multicenter studies by Sunders, published in various years, they reported trauma at 54%, headache at 36% and neck pain at 68% between 1964 and 1971. In 1972-1979, these rates reached 89%, 74% and

92% respectively, and between 1980 and 1985, they reached 91%, 83% and 85% respectively. What can be understood from these ratios is that trauma is becoming an increasing etiologic cause in thoracic outlet syndrome. In this, the role of traffic accidents takes up a lot of space, whereas none of the cases in our study had trauma stories. Despite the increasing number of traffic accidents in our country in recent years, it was an interesting finding that there was no trauma as the etiologic cause in any of our cases. We related this due to the fact that thoracic outlet syndrome was neglected because of not being suspected by physicians and not being examined in this respect, or perhaps because of more serious complications rising in cases resulting from trauma.

Anamnesis and physical examination have the most important place in diagnosis of thoracic outlet syndrome, as in the diagnosis of many other diseases, however some radiological diagnostic methods must be used for determinative diagnosis. Upper extremity EMG and upper extremity doppler are now routinely performed in cases where thoracic outlet syndrome is suspected in many centers. Many centers also routinely recommending cervical MR imaging in these cases. In our study, we observed that 34 of the cases (91.9%) had the EMC test, 22 patients (59.5%) underwent upper extremity arterial doppler and 11 patients (29.7%) underwent cervical MR examination. The upper trunk consisting of the C5-C7 roots of the brachial plexus is the most anatomic formation under pressure, affected by the hypertrophy of the scalene muscles: the pressure of the upper trunk leads to symptoms such as pain, numbness, weakness. The most common symptoms in TOS in the literature are reported as numbness, pain, weakness and the occurrence of these goes up to 95% in the literature [1,2,4,6,9,13]. In many publications, neurological symptoms are more common than vascular symptoms[1,2,9], therefore in our study we distinguished the symptoms into two main groups of vascular and neurological: of all the cases, 33 (89.2%) had symptoms of neurological origin and 2 (5.4%) had symptoms of vascular origin, whereas 2 cases (5.4%) had both neurological and vascular symptoms. In most of the cases, more than one symptom was present together, the most common one being pain, at 94.6%. As the second most common symptom, numbness was seen in 78.4% of the cases and the next common complaints were chills at 13.5%, weakness at 27%, atrophy at 10.8% and cyanosis at 10.8%; these findings were also consistent with the literature.

The most commonly used test for physical examination in thoracic outlet syndrome is the Adson test, the aim of which is to cause a contraction of the anterior and medial scalene muscles and consequently, to pressurize the subclavian artery and brachial plexus. While the susceptibility of the Adson test remained at 27% in some series, Murphy reported a 100% positive response to the it [28]; in our study, this rate was 86.5%. False positivity is much lower in the Adson test than in the costoclavicular test and hyperabduction test [28,29]: Rayan reported a false positive rate of 13.5% for the Adson test and 47% for the costoclavicular test in a study in 200 extremities of 100 volunteers [29]. Pleva reported a false positive rate of 62% for the hyperabduction test and 11% for the Adson test in a study of 53 medical students with no symptoms [30]. All these suggest that the Adson test is not a definite diagnostic criterion for thoracic outlet syndrome, but is a supporting finding for thoracic outlet syndrome . Despite the fact that Adson test was applied to all cases in our study, a healthy statistical evaluation could not be performed since no regular records were found about the hyperabduction test in retrospective examination.

In patients suspected to have thoracic outlet syndrome , the first radiological examination to be requested is a two- or four-way direct cervical graph. The detection of cervical ribs on direct cervical graphs implies a highly possible thoracic outlet syndrome diagnosis if the clinical data support it. The diagnosis should be supported by EMG, and if necessary, with arterial doppler [4].

Though cervical graphs were present for all the cases, cervical MR examinations were performed in only 11 (29.72%) of the cases and 5 (45.45%) had positive findings. In cervical graphs, 14 cases (37.8%) had cervical ribs, whereas 23 cases (62.2%) did not. In thoracic outlet syndrome, the rate of normal radiological examinations in literature varies between 1% and 10% [8,13,17]. In our study, this rate is 62.2%, which is in fact

incompatible with the literature.

EMG is the most reliable test showing pressure on brachial plexus in thoracic outlet syndrome [3,4]. In our study, EMG was performed on all cases except for 3 (8.1%): it was positive in 18 cases (48.6%) and negative in 16 (43.2%).

While the main cause of neurogenic symptoms in thoracic outlet syndrome is scalene muscle hypertrophy [2,13,18], the main cause of vascular symptoms is bone anomalies with type 7, type 8 and type 9 congenital fibromuscular bands. In our study there were no bone anomalies in any case. Scalene muscle hypertrophy and congenital fibromuscular bands were present in many cases and this explains why the symptoms in our cases are mostly neurological. However, since these data were not recorded regularly in operation notes, healthy statistics could not be made regarding scalene muscle hypertrophy and congenital fibromuscular bands. Arterial doppler is the most reliable and the most practical method of showing vascular pressure in TOS [1,2,9,20,25]: the examination is widely used because it is both non-invasive, relatively inexpensive and applicable to all patients. Upper extremity arterial doppler was performed in 22 (59.5%) of our cases and of these, 5 (13.5%) were found to have findings compatible with compression, whereas 17 (45.9%) had normal arterial doppler results. Arterial doppler was not performed on 15 of the patients (40.5%). There was a correlation between low rates of arterial pressure symptoms and low arterial doppler positivity in our study.

Limitations: The most important limitation of our study is that it is retrospective. Other limitations were the fact that the EMG tests of the patients were not performed by the same person and that the patient statements - one of the recovery criteria - were subjective.

CONCLUSION

Our results showed that a higher rate of complete recovery was achieved in the early postoperative period. We think that physical therapy and rehabilitation methods should be tried first in TOS, and favorable results can be obtained with appropriate and effective surgery in suitable cases. Moreover, these results indicated that higher surgical success rates can be obtained by using this strategy.

Funding sources: The authors declared that this study has received no financial support

Conflict of Interest: No conflict of interest was declared by the authors.

Acknowledgements

The author wishes to thank Professor Doctor Abid DEMİRCAN, who supported in the study as thesis advisor.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):278-284

DOI:10.30565/medalanya.797406

Gastroprotective effect of tarantula cubensis extract in the indomethacin-induced peptic ulcer model in rats

Sıçanlarda indometazin ile indüklenmiş peptik ülser modelinde tarantula cubensis ekstraktının gastroprotektif etkisi

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ABSTRACT

Background: Gastric ulcers are the most common gastrointestinal disease, due to several factors in the industrialized world. They may also occur after many pharmacological agents, combined with gastroprotective agents such as proton pump inhibitors (PPI) or anti-acids, are used as a preferred approach to maintaining gastrointestinal health. Unexpected adverse effects in these combinations make natural products an important alternative option.
Methods: Therefore, the main aim of this study is to investigate one natural compound, the Tarantula cubensis extract (TCE), in the experimental peptic ulcer model which was created by a single administration of indomethacin (40 mg/kg, body weight) to fed state Wistar-albino rats. The animals were pre-treated with two-doses of the TCE (0.2 ml/kg) before the indomethacin administration. After six hours, they were euthanized and the stomach tissue was isolated for biochemical and immunohistochemical analysis. Total antioxidant status/total oxidant status (TAS/TOS), prostaglandin E2 (PGE2), and nuclear factor kappa-B (NF-κB) levels were determined with ELISA in tissue homogenates. Caspase-3, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and tumor necrosis factor-alpha (TNF-α), were visualized with immunohistochemistry in intact tissues.
Results: Pre-treatment with TCE increased PGE2 levels and decreased total oxidative status (TAS/TOS). Additionally, TCE alleviated the increase of (NF-κB) levels due to the indomethacin administration. Histopathological and immunostaining results showed that TCE mitigated elevated immunoreactivity of the caspase-3, COX-2, iNOS, and TNF-α which were the results of the indomethacin administration.
Conclusion: Our study demonstrated that pre-TCE treatment ameliorated indomethacin-induced peptic ulcers via antioxidant and anti-inflammatory actions.

Keywords: Tarantula cubensis extract, indomethacin, peptic ulcer, oxidative stress, inflammation

ÖZ

Amaç: Gastrik ülser, endüstrileşmiş dünyada çeşitli faktörler nedeniyle en sık görülen gastrointestinal hastalıktır. Bununla birlikte mide ülseri, gastrointestinal sağlığın korunması için genellikle tercih edilen proton pompa inhibitörleri (PPI) veya anti-asitler gibi birçok gastroprotektif farmakolojik ajanın kombine kullanılmasından sonra da nüks edebilmektedir. Bu kombinasyonlardaki beklenmedik olumsuz etkiler doğal ürünleri alternatif bir seçenek haline getirir. Bu nedenle, bu çalışmanın temel amacı, tek bir indometasin (40 mg/kg) ile oluşturulan deneysel peptik ülser modelinde doğal bileşiklerden biri olan Tarantula cubensis (TCE) ekstraktının etkisinin araştırılmasıdır.
Yöntemler: İndometazin uygulanmasından önce iki doz TCE (0.2 ml/kg) s.k yoldan uygulanmıştır. Tek doz Indometazin (40 mg/kg) uygulamasından altı saat sonra anestezi altında mide dokusu biyokimyasal ve immünohistokimyasal analiz için çıkarılmıştır. Total antioksidan durum/total oksidan durum (TAS/TOS), prostaglandin E2 (PGE2) ve nükleer faktör kappa-B (NF-κB) düzeyleri doku homojenatlarında ELISA ile belirlenmiştir. Kaspaz-3, siklooksijenaz-2 (COX-2), indüklenebilir nitrik oksit sentaz (iNOS) ve tümör nekroz faktörü-alfa (TNF-α) immünohistokimya ile görüntülenmiştir.
Bulgular: TCE ile ön tedavi PGE2 düzeylerini artırdı ve toplam oksidatif durumu (TAS / TOS) azaltmıştır. Ek olarak, TCE indometazin uygulamasına bağlı olarak NF-κB düzeylerindeki artışı hafifletmiştir. Histopatolojik ve immünboyama sonuçları, TCE'nin, indometazin uygulamasının sonuçları olan kaspaz-3, COX-2, iNOS ve TNF-α'nın yüksek immünoreaktivitesini azalttığını göstermiştir.
Sonuç: Çalışmamız TCE tedavisinin, antioksidan ve antiinflamatuvar etkiler yoluyla indometazine bağlı peptik ülserleri iyileştirdiğini göstermiştir.

Anahtar kelimeler: Tarantula Cubensis Ekstraktı, indometazin, peptik ülser, oksidatif stres, inflamasyon

Received: 04.05.2020 Accepted: 05.07.2020 Published (Online):29.10.2020

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To cited: Cankara FN, Özmen Ö, Savaş HB, Şahin Y, Günaydın C. Gastroprotective effect of tarantula cubensis extract in the indomethacin-induced peptic ulcer model in rats. Acta Med. Alanya 2020;4(3):278-284. doi:10.30565/medalanya.797406

INTRODUCTION

Gastric ulcer is the most prevalent gastrointestinal system disease and nearly 15 patients in every 15,000, results in death in the world [1]. This chronic and multifactorial disease frequently relapses 10% over of the lifetime and many factors, such as Helicobacter pylori, smoking, stress, alcohol consumption and non-steroidal anti-inflammatory drug (NSAIDs) use, increase risk and incidence of the disease throughout the lifetime [2].

Sustained production of the free radicals and antioxidant mechanisms against these radicals are in the balance of the normal homeostasis and dysregulation of this balance is related to more than one hundred disease pathologies [3]. However, this bidirectional balance between oxidative stressors and antioxidant mechanisms is crucially maintained in the human body, as reactive products are continuously formed by exogenous and endogenous sources [4]. Owing to the complex pathophysiology of gastric ulcers, antioxidant and anti-inflammatory compounds that can affect gastric prostaglandin synthesis have major importance in the ways to seek gastric ulcer treatment [5]. Although proton pump inhibitors are frequently prescribed drugs against NSAID-induced gastrointestinal damage, long-term gastric ulcer treatment with these drugs were reported to be a risk factor for bone fractures and unwanted cardiovascular events [4]. The main reason for gastric ulcers seen in NSAID users is that activated 5-Lipooxygenase (5-LOX) pathways becomes more dominated due to the suppressed arachidonic acid/cyclo-oxygenase cascade [4]. Various studies have demonstrated the gastroprotective effects of natural products in experimental gastric ulcer models [6].

Tarantula cubensis extract (TCE) is commonly used in the veterinary field [7],[8]. Although, major mechanism behind the therapeutic effects of TCE is not clearly identified, it is commonly believed that stimulation of cellular defense mechanisms and boosting of the anti-inflammatory mechanism occurs in favor of the healing of the inflammatory lesions, such several ulcers and abscess [9],[10]. Therefore, it is rational to think that the suggested anti-inflammatory and wound-healing properties of TCE might be have beneficial effects in the gastric ulcer treatment.

The aim of this study was the investigation of the effects of TCE administration on an experimental gastric ulcer model in rats. Because the inflammation and dysregulated oxidative balance are key mediators in disease pathophysiology, we tried to investigate the effects of TCE on the oxidative status and well-known inflammatory markers on stomach tissue, and reveal the possible effects and mechanisms in the peptic-ulcer disease.

MATERIAL AND METHODS

Animals

Experiments were performed with 8-10 weeks old male Wistar-albino rats, which were kept under 12-hour light/dark cycles with constant room temperature (22±2□C) and humidity (%55-60), in separate cages. The study was conducted in accordance with the guideline for the care and use of laboratory animals approved by Experimental Animals Research Council (2018-416) and all efforts were made throughout the experiment in reducing animal suffering.

Chemicals

Indomethacin was purchased from Sigma Aldrich (St. Louis, MO, USA) and Theranekron® alcoholic extract (1:100) of Tarantula cubensis in alcoholic solution 1 mg/ml, was purchased from Richter-Pharma AG, Wels, Austria. Indomethacin was dissolved in the saline and the TCE dose was selected based on the previous studies [9].

Experimental groups and drug treatments

Animals were divided into 4 equal groups: Control (n=8), IDM (n=8), TCE (n=8), TCE+IDM (n=8). Throughout a 24-hour fasting period in all the groups, animals were provided with unrestricted access to water. Experimental groups and the administration schedules were designed as follows:

1-Control group: 0.2 ml saline (1st and 4th day) + 24 hour fasting (5th day) + 1 ml saline (i.p, 6th day) + 6 hours after last administration, sacrification.

2- IDM group: 0.2 ml saline (1st and 4th day) +

24 hour fasting (5th day) + IDM 40 mg/kg [11] intraperitoneal (i.p, 6th day) + 6 hours after last administration, sacrification.

3- TCE group: 0.2 ml/kg TCE solution (14) subcutaneous (s.c) (1st and 4th day) + 24 hour fasting (5th day) + 1 ml saline (i.p, 6th day) + 6 hours after last administration, sacrification.

4- TCE+IDM group: 0.2 ml/kg TCE solution (s.c, 1st and 4th day) + 24 hour fasting (5th day) + IDM 40 mg/kg (i.p, 6th day) + 6 hours after last administration, sacrification.

Biochemical analysis

After all animals were euthanized, abdomens were dissected and opened along the greater curvature for observation of the number and locations of gastric lesions. A piece of stomach was dissected and homogenized in an ice-cold phosphate buffer (pH 7.4) and stored in -80°C until analysis. The thawed samples of homogenized tissue were centrifuged at 10,000 g for 10 minutes at 4oC. Protein concentrations in the supernatant was measured by using the Lowry method [12]. TAS and TOS were determined from the same samples after the determination of protein levels. Total Antioxidant Capacity (TAS) and Total Oxidant Capacity (TOS) levels were measured spectrophotometrically by using commercially available ELISA kits [13]. Results were given in mmol Trolox Eq/L unit for TAS and μmol H2O2Eq/L unit for TOS. The TAS and TOS results for the tissues were calculated via division by the protein value and OSI (Oxidative Stress Index) ratio was expressed by using the TOS/TAS formula.

Histopathological analysis

Stomach tissues were harvested for microscopical evaluation in all rats, during the necropsy. Routine tissue processing method were performed by using an automatic tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany) for formalin-fixed tissues. Then the gastric tissue samples were embedded in paraffin, and five serial sections taken from the blocks at 5 μm thickness by a fully automatic microtome (Leica RM 2155, Leica Microsystem, Nussloch, Germany). One of the sections was stained routinelly with hematoxylin–eosin (H&E) and the remaining sections on poly-L-lysine slides were used for the immunohistochemical method. The sections were immunostained with active caspase-3 [Anti-caspase-3 antibody, (ab4051; Abcam-Cambridge, UK)], TNF-α [anti-TNF-α antibody (ab6671; Abcam -Cambridge, UK)], inducible nitric oxide synthase [Anti-iNOS antibody (ab15323; Abcam -Cambridge, UK)] and COX-2 (Cat. no: RM- 9121-S0, Thermo scientific, Fremont, USA) antibodies, according to the instructions of the manufacturer's streptavidin biotin peroxidase method. Primary antibodies were incubated for a period of 60 minute using a 1/100 dilution. For secondary antibody, a ready-to-use commercial kit [EXPOSE Mouse and Rabbit Specific HRP/ DAB Detection IHC kit (ab80436)] was used. As chromogen 3,3-diaminobenzidine (DAB) were applied for 5 minutes for all slides. For negative controls, the incubation with primary antiserum was omitted. All evaluations were performed by a pathologist from another university, in a blinded manners. To evaluate the percentage of immune-positive cells for each marker, 100 cells were counted in 10 different fields for every section, at a magnification of X40. Statistical analyses were subjected of the results obtained from the image analyzer and the morphometric analysis for microscopical evaluation were performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical analysis

All data obtained from experiments was expressed as mean±SD and analyzed with the one-way analysis of variance (ANOVA), followed by Bonferroni's correction which was used for multiple comparison. P values less than 0.05 were considered significant and all analysis carried out with the SPSS (v21.0, Chicago, Illinois).

RESULTS

Pre-TCE treatment decreased observable gastric hemorrhages due to the indomethacin administration

At the overall examination there was no lesions seen in the control group (shown in Fig. 1. a). IDM induced severe gastric damage, such as mucosal lesions characterized by brown-colored marked

hemorrhages (shown in Fig. 1. b). Additionally, there were no observable lesions in the TCE group as with the control group (shown in Fig. 1. c). Furthermore, TCE, before IDM administration, decreased observable gross hemorrhages at the gastric mucosa (shown in Fig. 1. d).

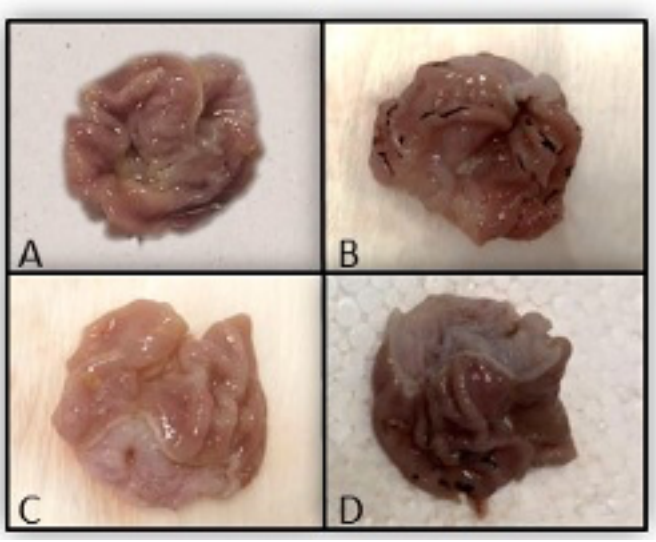

Fig. 1. Macroscopic images of stomach tissues are shown as control (a), IDM (b), TCE (c) and TCE+IDM (d) groups, respectively. Normal mucosal view in control and TCE groups (a, c) Hemorrhages and damage in stomach tissue clearly visible (b), Reduced hemorrhagic areas compared with IDM group (d).

Indomethacin-induced oxidative stress was alleviated with pre-TCE treatment

As expected, TCE alone administration did not cause any change on TAS/TOS levels and OSI ratio. IDM administration increased TAS (shown in Fig. 2. a) levels and decreased TOS (shown in Fig. 2. b) levels, resulting in a significantly increased OSI ratio (data not shown) ($p<0.05$), while TCE alleviated the alteration of the oxidative stress parameters as a result of IDM administration ($p>0.05$).

Pre-TCE attenuated the increase of PGE2 and NF-κB levels

Gastric PGE2 (shown in Fig. 2. c) levels were significantly decreased in the IDM group ($p<0.05$). Additionally, NF-κB (shown in Fig. 2. d) increased in the IDM group, as expected. Although, these parameters did not change in the TCE group, pre-TCE treatment significantly ameliorated the decrease in the PGE2 and increase in the NF-κB levels due to the IDM insult ($p<0.05$).

Indomethacin-induced increased inflammatory immunoreactivity and apoptosis inhibited by pre-TCE treatment

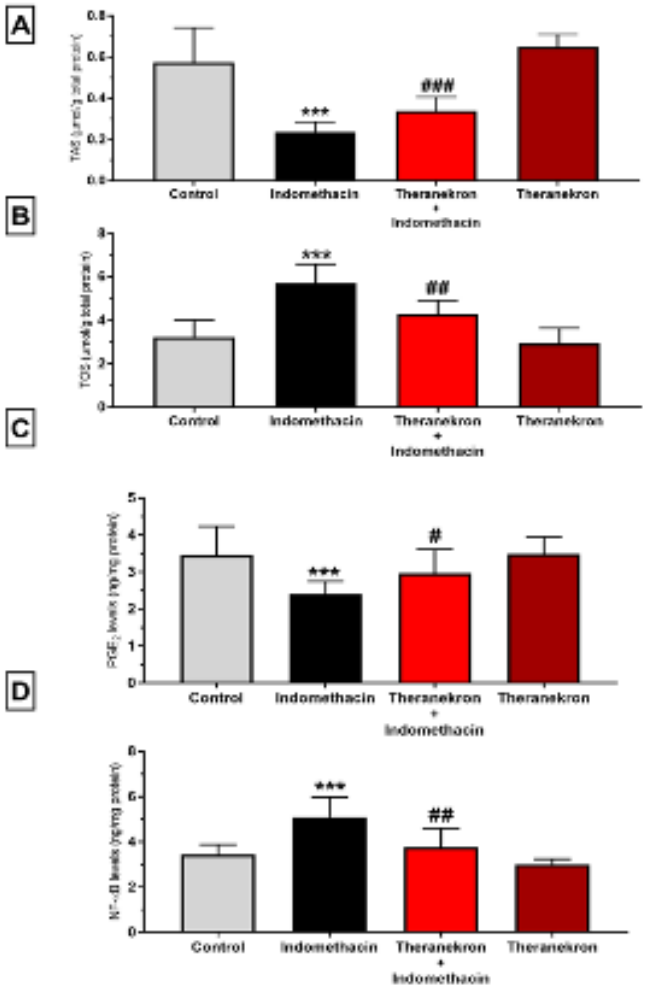

Fig. 2. TAS (a), TOS (b), PGE2 (c) and NF-kB (d) levels in the all experimental groups were expressed as mean±SD. *** $p<0.001$ versus control group, ### $p<0.001$, ## $p<0.01$, # $p<0.05$ versus IDM group.

Histopathological examination of the stomachs revealed large and deep ulcers in the IDM group. In these rat's stomachs, deep damage, mucosal hemorrhage, marked edema and numerous leucocytes infiltration of the submucosal layer were observed. The TCE treatment caused a decrease in both the diameter and depth of the ulcers. The gastric mucosa of the TCE and control groups demonstrated normal tissue and structure; the histopathological findings between the groups are shown in Figure 3. Marked Caspase-3 (shown in Fig. 3A), COX-2 (shown in Fig 3. b), iNOS (shown in Fig. 3. c) and TNF-α (shown in Fig. 3. d) expression was observed in stomach cells, in the lesioned areas of the indomethacin administered

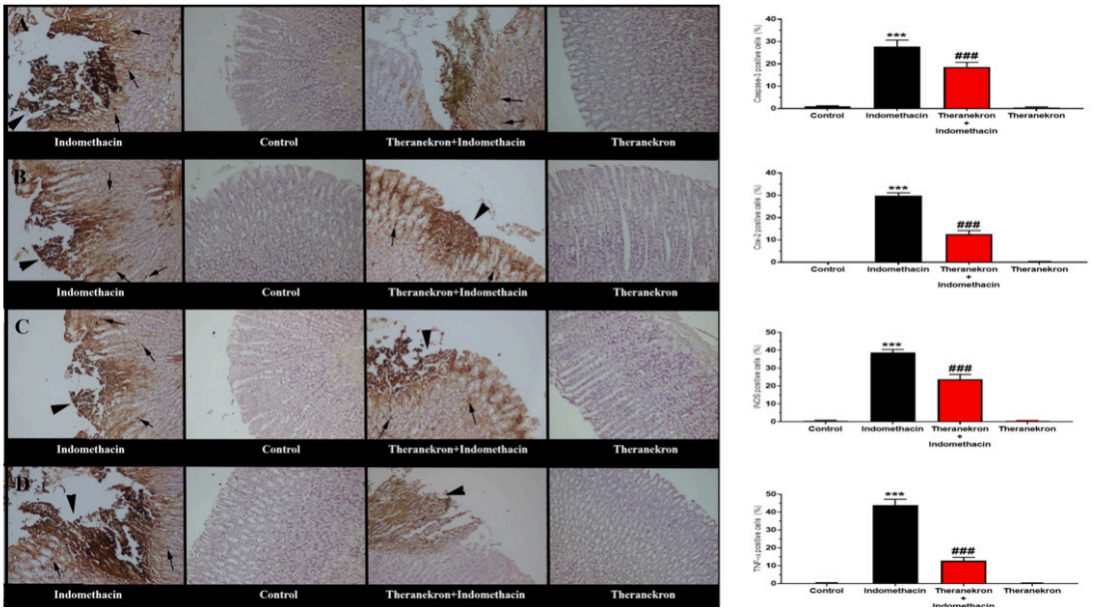

Fig. 3. Histopathological examination of all experimental groups. Hematoxylin and eosin staining of gastric tissues (A) with severe mucosal lesion (arrow head) and hemorrhage in the IDM group, normal gastric mucosae in control group, decreased lesion and hemorrhage (arrow head) in the TCE+ IDM group, normal tissue architecture in a rat's in stomach belonging TCE group, HE. Caspase-3 (a), COX-2 (b), iNOS (c) and TNF-α (d) immunoreaction between the groups. Severe expression in cells (arrows) in IDM group around the lesion, (arrowhead), no expression in control group, decreased expression (arrows) in IDM+TCE group, negative expression in TCE group. Streptavidin biotin peroxidase method, Bars=50μm. Additionally, caspase-3 (a), COX-2 (b), iNOS (c) and TNF-α (d) immunoreactivity in the all experimental groups were expressed as mean±SD. All data represented as mean±SD. *** p<0.001, ** p<0.01 versus control group, ### p<0.001 versus IDM group.

group. Both epithelial and mesenchymal cells expressed the markers. The cells near the lesions also expressed all markers. There was no or only slight reactions in the TCE and control groups. There was no positive reaction in the primary antibody omitted sections. Additionally, in a number of positive cells stained with Caspase-3, COX-2, iNOS, TNF-α significantly increased in IDM group and that increase reversed with TCE administration (shown in Fig. 3).

DISCUSSION

This study demonstrated that pre-TCE treatment ameliorated IDM-induced gastric damage. TCE increased the antioxidant response and decreased the inflammatory levels as well as immunoreactivity in the gastric tissue.

IDM, a commonly prescribed drug for its anti-inflammatory properties, is also used for experimental model of gastric ulcer in rats [14]. Free radical generation because of inhibited prostaglandin synthesis by IDM, has been accepted as crucial biochemical reaction in the physiopathology of gastric ulcer [15]. Utsumi et. Al. suggested that over-activation of the inflammatory response caused by IDM, could be dependent on the antioxidant/oxidant status of gastric cells [16]. Thus, compounds that can cause antioxidant actions could be targeted as a potential treatment option in NSAIDs-induced gastric lesions. In the abundance of oxidative markers, TAS and TOS plasma levels and OSI ratio are generally deemed a better indicator of the body's total anti-oxidant defense [17]. IDM-induced gastric ulcer via oxidative and inflammatory actions with increasing OSI parameters have been already studied [18] and compounds with antioxidant actions showed protective actions in the indomethacin-induced gastric ulcers by several groups [19]. In line with these studies, ours demonstrated that pre-treatment of TCE alleviated increased OSI ratio, which means that the antioxidant properties of TCE might have a role in the gastroprotective actions. However, caspase-3 is a non-selective apoptosis marker and it is well-known that increased ROS and oxidative stress, results in the cellular apoptosis via cytochrome C activated caspase-3. Inhibition of the elevation of the ROS levels in the gastric tissue, and the resulting decrease in the caspase-3 levels, are suggested as a protective mechanism against oxidative stress in the gastric tissue. Several groups demonstrated that decreasing ROS levels and increasing antioxidant enzymes in the gastric tissue, resulted in increased cellular survival and decreased caspase-3 apoptosis

immunoreactivity. Maintaining gastric mucosal integrity against oxidative stress and ROS are elegant targets as gastroprotective therapy options. In accordance with these studies, robust increases of caspase-3 immunoreactivity after indomethacin administration alleviated with the pre-TCE treatment, which indicates diminished caspase-3 cellular loss. Additionally, antioxidant effects of pre-TCE treatment supports this notion and demonstrates that decreased caspase-3 immunoreactivity might be the result of the antioxidant properties of the TCE.

Owing to the general mechanism of action of NSAIDs, decrease in the PGE2 levels and inhibition of COX isozymes in gastric mucosa exacerbates gastric ulcers in most animal species [20]. COX 2 in particular, has been known to modulate glandin-mediated mucosal defense in the gastric tissue [21]. Although it seems controversial, increased expressions of COX-2 mRNA after indomethacin administration have been considered as a gastric mucosal defense against increased inflammatory injury [22]. As a marker of the gastric inflammatory damage, inhibition of the increased COX-2 immunoreactivity is attenuated with pre-TCE treatment. In addition to its antioxidant action, that anti-inflammatory action of TCE possibly contributes effects on the PGE2 levels. Decreased PGE2 levels, which are a potent protector in the gastric tissue, is possibly the result of the selectivity of indomethacin on COX-1 more than COX-2. PGE2 naturally protect gastric mucosa against acidic damage through increasing mucus and bicarbonate secretion, and compounds that increase PGE2 act as a protector in gastric acid induced peptic damage [23]. Also, Abood et al. suggested that PGE2 could inhibit TNF-α production, which is another anti-inflammatory action in the context of the gastroprotection [24]. In line with these results, inhibition of the decrease in the PGE2 levels and increase in the TNF-α immunoreactivity in the gastric tissue by pre-TCE treatment seen in our study, suggest that gastroprotective action of TCE might be mediated with PGE2. Besides PGE2, NO is another important mediator in the NSAIDs-induced gastric ulcers [25] after discovering the role of NO in mucosal damage, several NO-containing NSAIDs were used as anti-inflammatory drugs, which deprive of the gastric damage potential. NO has been shown to regulate gastric pH through maintaining blood flow and vasodilation, and drugs that activate NO synthesis (iNOS) or increase free NO levels, could diminish gastric ulcer damage [19]. In parallel with that knowledge, increased iNOS levels after indomethacin administration is considered as a natural defense mechanism against mucosal injury, as seen in the COX-2. Therefore, the alleviated iNOS immunoreactivity increase seen in our study suggests that pre-TCE treatment protected against indomethacin-induced inflammatory gastric damage, in line with COX-2 immunoreactivity.

The empirical results reported herein should be considered in the light of some limitations. First, the study focused on the effects of venom that was extracted from a tarantula. Limited studies showed its beneficial effects in the treatment of cutaneous papillomatosis, mammary adenocarcinomas, as well as in traumatic tendon injuries in rats, in many inflammatory lesions and in particular in the healing processes of open wounds. According to studies about TCE, it was shown that TCE has antioxidant and anti-inflammatory properties, and can be used beneficially for its effects on open wound healing. Therefore, it is rational to think of possible protective effects of TCE on peptic ulcer pathology, which have similar pathophysiological regenerative mechanism [26].

In conclusion, to the best of our knowledge ours is the first study regarding the effects of pre-TCE treatment in the experimental gastric ulcer models. Our study demonstrated that pre-TCE treatment decreased oxidative stress and alleviated disruption of the mucosal integrity. Pre-administration of TCE also alleviated inflammatory response in the gastric tissue, via effecting COX-2, iNOS and PGE2 expression in the gastric tissue.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2020;4(3):285-290

DOI:10.30565/medalanya.804454

Evaluation of exposure and awareness of radiation in healthcare professionals exposed to ionizing radiation

İyonlaştırıcı Radyasyona Maruz Kalan Sağlık Çalışanlarında Radyasyon Maruziyeti ve Farkındalığının Değerlendirilmesi

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ABSTRACT

Aim: To evaluate radiation exposure and awareness of radiation in healthcare professionals exposed to ionizing radiation.

Material and Methods: The study was carried out at the Alanya Alaaddin Keykubat University Training and Research Hospital and the Süleyman Demirel University Hospital. Physicians, nurses, paramedics, anesthesia technicians, caregivers and cleaning staff exposed to radiation, participated in the study. An inquiry form was prepared to carry out the study, in which the demographic characteristics of the healthcare professionals, the characteristics related to the radiation they were exposed to, the effects that may occur due to this exposure were asked; the relationship between these was statistically analyzed according to the answers.

Results: One hundred twenty-nine healthcare professionals, 81 men and 48 women, participated in the study. Most of the healthcare professionals exposed to radiation have been working for more than 10 years and at a distance of less than 3 meters to the radiation source. Thirty-two of the participants (24.8%) did not use any personal protective equipment. On the day of exposure to radiation, 91 persons (70.54%) were experiencing symptoms such as weakness, fatigue, and headache, unlike other days. It was determined that personal protective equipment use did not correlate to education levels and radiation training background.

Conclusion: Most of the healthcare professionals exposed to radiation have not previously received any training on radiation and do not use personal protective equipment regularly. This situation causes these persons to complain of weakness, tiredness or headaches at the end of the day.

Keywords: Radiation, ionizing radiation, questionnaire, X-ray, computed tomography

ÖZ

Amaç: İyonize radyasyona maruz kalan sağlık çalışanlarında radyasyon maruziyeti ve farkındalığını değerlendirmek.

Gereç ve Yöntem: Çalışma Alanya Alaaddin Keykubat Üniversitesi Eğitim ve Araştırma Hastanesi ve Süleyman Demirel Üniversitesi Hastanesi'nde gerçekleştirildi. Çalışmaya radyasyona maruz kalan hekimler, hemşireler, sağlık memurları, anestezi teknisyenleri, hasta bakıcıları ve temizlik personelleri katıldı. Çalışmayı gerçekleştirmek için bir sorgulama formu hazırlandı. Bu formda çalışanların demografik özellikleri, maruz kaldığı radyasyon ile ilgili özellikler, bu maruziyete bağlı oluşabilecek etkiler soruldu ve cevaplara göre aralarında ilişki olup olmadığı istatistiksel olarak incelendi.

Bulgular: Çalışmaya 81 erkek, 48 kadın olmak üzere 129 sağlık çalışanı katıldı. Radyasyona maruz kalan sağlık çalışanlarının çoğu 10 yıldan daha uzun süredir ve radyasyon kaynağına 3 metreden daha yakın mesafede çalışmaktaymış. Çalışmaya katılanlardan 32 (%24,8) si hiçbir kişisel koruyucu donanım (KKD) kullanmıyordu. Radyasyona maruz kalındığı gün 91 kişi diğer günlerden farklı olarak halsizlik, yorgunluk, baş ağrısı gibi bir şikayet hissediyormuş. KKD kullanımının öğrenim düzeyi ve radyasyon eğitimi almakla ilişkisi olmadığı saptandı.

Sonuç: Radyasyona maruz kalan sağlık çalışanlarının çoğu radyasyon ile ilgili daha önce bir eğitim almamış ve KKD'leri düzenli kullanmamaktadır. Bu durum kişinin gün sonunda halsizlik, yorgunluk ya da baş ağrısı gibi şikayetleri olmasına neden olmaktadır.

Anahtar kelimeler: Radyasyon, iyonize radyasyon, sorgulama formu, X-ışını, bilgisayarlı tomografi

Received: 31.01 2019 Accepted: 06.04.2019 Published (Online):29.10.2020

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To cited: Uçar M, Akkoç A, Topçuoğlu M, Öztürk SA, Demir M. Evaluation of exposure and awareness of radiation in healthcare professionals exposed to ionizing radiation. Acta Med. Alanya 2020;4(3):285-290. doi:10.30565/medalanya.804454

INTRODUCTION

Radiological imaging has recently been used frequently in the diagnosis, treatment and follow-up of diseases [1]. Departments such as surgical branches, radiology, anesthesia, cardiology, neurology and operating theatre employees, are the main units exposed to radiation. The degree of exposure to ionizing radiation varies according to the radiological imaging applied and computed tomography, conventional radiography, C-arm fluoroscopy, angiography are the best-known ionizing radiation sources used in hospitals.

The most basic way to protect from ionizing radiation is to use the radiation as little as possible [2]. Surgeons, interventional radiologists, cardiology, and neurology departments that use fluoroscopy frequently in particular, will find that the less fluoroscopy they perform during the procedure, the less radiation they are exposed to. Another way to reduce radiation exposure is to stay away from the radiation source as much as possible during fluoroscopy or radiological imaging [3]. In order to reduce the effect of ionizing radiation, it is absolutely necessary to use personal protective equipment (PPE) and, if possible, to put a screen such as a glass partition between the radiation source and the operators [4].

Although the harmful effects of radiation are generally known, the degree of damage it causes in the chronic process is not fully understood. Health workers are exposed to the harmful effects of radiation at least as much as patients and when we consider chronic exposure, this damage can reach serious levels and lead to life-threatening diseases that can result in death [5]. In this study, we aimed to evaluate the awareness of the harmful effects of radiation in healthcare professionals exposed to ionizing radiation, at the Alanya Alaaddin Keykubat University Training and Research Hospital and the Süleyman Demirel University Hospital.

MATERIALS AND METHODS

Study Population

We conducted this study at the Alanya Alaaddin Keykubat University Training and Research Hospital and the Süleyman Demirel University Hospital, where physicians, nurses, paramedics, anesthesia technicians, caregivers as well as cleaning staff exposed to radiation, participated in the study. The units where the study participants worked in were urology, orthopedics, neurosurgery, radiology, interventional radiology, angiography, general surgery, otolaryngology, gynecology and obstetrics, plastic and reconstructive surgery, ophthalmology and the operating theatre. The total service time in the unit where they are exposed to radiation was stated in months, the daily exposure time in minutes and their distance to the radiation source in meters (m). X-ray, C-armscopy or fluoroscopy, computed tomography were sources of ionizing radiation to which they were exposed to.

Questionnaire

We prepared a questionnaire to carry out the study, in which we noted the demographic characteristics of the participants and the department they worked in. We asked how long they were exposed to radiation, how far they worked from the radiation source, the side effects they fear the most, how many days in a month and how much time they were exposed to radiation in a given day. In addition, they were asked whether they had radiation training, whether they were given time away from the radiation environment, whether they used a personal dosimeter (PD), whether they used PPE, which PPEs they used, whether they felt any symptoms such as weakness, fatigue, headaches different from other days on the day of radiation exposure, whether they had a disease due to radiation exposure. They were asked whether they used lead gowns, thyroid shields, gloves, glasses, glass partitions or screens as PPE. They were offered the following options as side effects of radiation that they may be aware of and feared the most: cancer, infertility, genetic disorder, shortening of life span, cataract, hair loss, skin disorders, growth retardation in children and other.

Analysis and Statistics

The personal and professional demographic characteristics of the participants were recorded and descriptive statistics were compiled with the data obtained. If the data showed normal

distribution, the results were given with mean ± standard deviation, otherwise median value + minimum-maximum values were given. Working periods in the department with radiation exposure were divided into 3 groups from 0-5 years, 5-10 years and more than 10 years. In terms of dosimetry use, the participants were divided into 3 groups: those who use it regularly, those who never use it and those who occasionally use it. The relationship between formal education levels and regular usage of PPE, the relationship between having radiation education and regular usage of PPE, and the relationship between the distance to the radiation source and the presence of radiation-related complaints on that day, were statistically analyzed. In comparison of qualitative data, Pearson's chi-square test was used for those whose expected value was less than 5 and the ratio of cells was less than 20%, and the Fisher exact test was used for those with an expected value greater than 20%. If the parametric test assumptions were not been provided, the Mann-Whitney U test was used to compare the quantitative data in the two groups. A threshold value of <0.05 for the level of significance was considered significant. Whether the data was normally distributed was determined by Shapiro-Wilk test and if the p value as a result was >0.05 , the data was considered to be normally distributed. Statistical measurements were made with the SPSS 22 package program (SPSS Inc., Chicago, IL).

Ethical considerations

All procedures in this study involving human participants were performed in accordance with the ethical standards of the Institutional Research Committee and with the 1964 Helsinki Declaration and its later amendments, or comparable ethical standards. This study was approved by Alanya Alaaddin Keykubat University Medical Ethics Committee, (10354421 - 2019/13-47) Turkey

RESULTS

One hundred twenty-nine healthcare professionals, 81 men and 48 women, participated in the study. The mean age of the participants in the study was 36.8 ± 8.61, 95 were married and 34 were single. There were 21 doctors, 88 nurses or health officers or technicians, 20 caregivers or staffs in the study. In terms of working experience, there were 27 persons between 0-5 years, 21 persons between 5-10 years and 81 persons who had been working more than 10 years. When we looked at the education levels, 72 persons had associate and lower degrees, 57 had undergraduate and higher degrees. There were 31 persons using extra holiday time due to radiation, whereas 98 persons were not. Fifty-seven of the participants had previously had formal radiation training and 72 had not. When we looked at the use of PD badge, 29 persons used it regularly and 93 persons never used it, whereas 7 persons sometimes did. Among those using dosimeters, 4 persons were using one for 12 months or less, 9 persons for 13-60 months and 23 persons for more than 60 months (Table 1).

Table 1. Demographic characteristics of the study participants

Parameter	Number (n)	Percentage (%)
Male	81	62.8
Female	48	37.2
Married	95	73.64
Single	34	26.35
Doctor	21	16.27
Nurse or health officer or technician	88	68.21
Other health personal	20	15.5
Education background		
Undergraduate or higher	57	44.18
Associate or lower	72	55.81
Working year		
0-5 years	27	20.93
5-10 years	21	16.27
>10 years	81	62.79
Education for Radiation		
Yes	57	44.18
No	72	55.81
Radiation Rest		
Yes	31	24.03
No	98	75.97
Dosimeter usage		
Always	29	22.48
Never	93	72.09
Sometimes	7	5.42

It was found that, on a monthly basis, 60 persons were exposed to radiation for 10 days or less, 52 persons were exposed to radiation for 11-20 days and 17 persons for 21-30 days. During a given radiation exposure day, 48 persons were exposed to an average of 0-60 minutes,

41 persons averaged 61-180 minutes and 40 persons averaged more than 180 minutes. As a radiation source, 97 persons were exposed to C-arm scope, 26 to X-ray, 18 to tomography and 22 of them to more than one source. During the process, 36 persons were closer than 1 meter to the radiation source, 65 persons were 1-3 meters away, whereas 28 persons were more than 3 meters away (Table 2).

In the questionnaire, to the question "whether protective equipment (PE) is enough" 16 persons answered yes, 44 persons no, 69 persons answered that it was partially sufficient. To the question of "do you use PPE", 64 persons answered that they used it, 29 did not and 36 persons used it sometimes. Twenty-two persons who did not use PPE were closer than 3 meters to the radiation source. As PE, 95 persons used lead vests, 73 persons used thyroid shields, 6 persons goggles, 3 of them used gloves and 47 persons used lead screen. Thirty-two participants (24.8%) answered that they do not use any PE during the procedure (Table 2).

Of the participants in the study, 5 persons did not know that radiation can cause cancer, 17 persons understood it can cause infertility and 39 persons that it can cause genetic disorders. On average, half of the participants did not know that radiation can shorten the life span, cause cataracts, hair loss, skin disorders or lead to growth retardation in children. To the question "What is the side effect you fear the most from radiation?" 122 persons answered cancer, 11 answered infertility and 8 persons answered cancer and infertility. On the day of exposure to radiation, 91 persons (70.54%) complained of symptoms such as weakness, fatigue and headache, unlike other days. Sixteen of the participants (12.4%) in the study had a disease that they thought was caused by radiation. Five of them were thyroid cancer, 4 were chronic headache or cranial mass, 2 were preterm birth or recurrent abortion (Table 2).

When the relationship between education level and regular usage of PPE were evaluated, the rate of regular PPE use for those with an undergraduate or higher level was 54.4%, while it was 45.8% for those with an associate or lower education level. The difference was not statistically significant according to Pearson's chi-square test (X2(1) = 0.931, p = 0,335). When the relationship between radiation education and regular use of PPE was evaluated, the rate of usage of PPE in for those who had received radiation training was 56.1%, while it was 44.4% in those who did not have such training. The difference was not statistically significant according to the Pearson's chi-square test (X2(1) = 1.741, p=0,187). It was therefore determined that PPE usage is unrelated to education levels or radiation training.

Table 2. Results about radiation source, protective equipment and disease related radiation

Parameter	Number (n)	Percentage (%)
Radiation source		
C armscopy	97	75.19
Conventional X-ray	26	20.15
Computed tomography	18	13.95
Distance to radiation source < 1 mt	36	27.9
1-3 mt	65	50.38
> 3 mt	28	21.7
Protective equipment Lead vest	95	73.64
Thyroid shield	73	56.58
Screen	47	36.43
Goggles	6	4.65
Gloves	3	2.32
Most feared side effect Cancer	122	94.57
İnfertility	11	8.52
Genetic	5	3.87
Possibly radiation-related illness	16	12.4
Thyroid cancer	5	3.87
Headache	4	3.1
Gynecological problem (abortus, prematurity)	2	1.55

The median value of the distance to the radiation source was found to be 2 m (0.1-8 m), while the median distance to the radiation source was found 2 m (0.1-5 m) in those who experienced a different complaint due to radiation exposure, and the median distance to the radiation source was found 3 m (0.3-8 m) in those who did not feel any complaint due to radiation exposure. The relationship between the presence of complaints such as weakness, fatigue and headache that the person felt, different from the other days and resulting from radiation and the distance to the radiation source, was examined with the Mann-Whitney U test: the results were found to be significant (U: 1324, p: 0.035). The closer

the person works to the radiation source, the more likely they are to feel complaints such as weakness, fatigue and headache that day due to radiation exposure.

DISCUSSION

Healthcare professionals are generally aware of the long-term damage that radiation exposure may cause in the body. However, their knowledge of the actual harmful effects of radiation exposure is very poor. In a study conducted in the UK on physicians' estimation of the radiation emitted by X-rays, 97% of the physicians estimated it was lower than the actual dose [5]. In a study involving 1184 persons in which European urology assistants participated, the knowledge of the participants in the study about the damages of ionizing radiation was found to be weak, and it was concluded that on average, half of the participants had no idea that ionizing radiation causes fatal cancer [6]. In our study, it was found that only 5 persons did not know that it caused cancer and 17 did not know that it caused infertility. The least known side effect of radiation was found to be cataracts and growth retardation in children. The reason for these low rates may be the recent increase in cancer and infertility rates, as well as the fact that radiation exposure is known by the majority of people in society to cause these diseases.

PPE must absolutely be used to eliminate or minimize the harmful effects of radiation [7,8]. In a study conducted by Bowman et al., they found that 33.8% of 518 persons did not use lead protection equipment [9]. Only 54.2% of the participants had their own lead vest and thyroid shields, while 12% had a fully equipped protection system consisting of vest, thyroid shield and goggles, which was a comparable rate to the one found in our study. The reason for the thyroid shield being used less frequently than the lead vest may be the discomfort it causes on the neck. Other PPE usage rates were found to be quite low in our study; the reason for the low usage of goggles and gloves, for instance, may be that the institution does not provide this equipment, that they have a high cost as well as the inconvenience they may cause during their usage.

Raising the awareness of healthcare professionals about the harmful effects of radiation and providing training on this subject, reduces exposure to radiation and increases the usage of PPE, resulting in less exposure to harmful effects [10]. In addition, formal training on radiation provides more accurate usage of radiation sources and similarly, provides less exposure to radiation [11]. In our study, only a small portion of the participants received radiation training. We observed that there was no relationship between the person's undergraduate education level and the rate of using PPE. In order to increase the use of PPE in healthcare professionals exposed to radiation, training should be given at regular intervals during the course of their professional life. Training about radiation should be focused on how to minimize exposure, which PPEs should be used and how much protection these PPEs actually provide, as well as the importance of PD in general.

It is necessary to use a PD badge to clearly understand the cumulative amount of radiation the person is exposed to. Dosimeter badges for instance, are controlled periodically to measure the amount of radiation exposure, though if there is more radiation exposure than expected in these, it may be because of their misuse, insufficient PPEs or exposure of the person to unusually intense radiation [12]. In our study, the usage of PD badges was found to be very low and in the literature, in one particular study, when asked "why don't you use PD badges", most of the participants answered that "I work in many different places and I cannot remember the dosimeter badge" [13]. In Turkey, dosimeter badges are usually provided by the institution and the reason for the low usage in our study may be that the specific institutions did not provide these or, as in the literature, participants simply forgot to wear them.

Although every organ can be affected in chronic radiation exposure, the hematopoietic system is particularly affected [14]. In our study most of participants had been working for more than 10 years and closer than 3 meters to the radiation source, and most of them did not use PPE regularly. Malignancy, infertility, skin lesions, retinopathy, cardiovascular diseases, chronic fatigue syndrome, thyroid diseases, fetal malformation and growth retardation, are the best known pathologies caused by ionizing radiation [15-19]. In our study, the rate of those who thought they

had radiation-related disease was 12.4%. Thyroid cancer was found to be the most common disease resulting from radiation exposure, whereas the prevalence of thyroid cancer in the community is very low [20]. In our study, the reason for the higher rate of thyroid cancer compared to the general population is most likely radiation exposure. In addition, participants, to a considerable extent, reported that on the days they were exposed to radiation, they experienced different symptoms from other days, such as weakness, fatigue and headaches. Park et al. demonstrated the effects of radiation on the brain's hippocampus in an animal study [21] and therefore, it is reasonable to think that these complaints may occur in the person as a result to damage caused by the radiation in that region of the brain.

Our study has some limitations, one of which was that the exact radiation dose exposed by the healthcare professionals could not be calculated. If the usage of PD badges were sufficient and the data obtained from them could have been examined, a more obvious relationship could have been established with the diseases. Another deficiency was that not all healthcare professionals exposed to radiation in the two institutions were included in the study. If this would have been possible, clearer information about these would have been obtained and it would be possible to correct errors regarding radiation exposure.

Conclusion: The number of departments and healthcare professionals exposed to radiation in the hospitals was quite high. Also, the knowledge required on the part of the healthcare professionals about radiation, in order to minimize exposure, was insufficient. In our study, it was found that healthcare workers exposed to ionizing radiation have low radiation exposure awareness. For this reason, it was concluded that the level of knowledge of healthcare professionals about radiation, the side effects that may occur as a result of exposure, as well as the importance of PPE usage, should all be increased.

Conflict of Interest: The authors have no relevant conflicts of interest in this study.

Funding Sources: The authors received no financial support for this study.

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Acta Medica Alanya

CASE REPORT

OLGU SUNUMU

Acta Medica Alanya

2020;4(3):291-295

DOI:10.30565/medalanya.714362

A rare cause of interstitial lung disease in rheumatology clinic: case report for sulfasalazine-induced acute pulmonary injury

Romatoloji Kliniğindeki Ender Bir İnterstisyel Akciğer Hastalığı Sebebi: Sulfasalazinin Tetiklediği Akut Akciğer Hasarı Olgusu

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ABSTRACT

Infection, primary lung pathology, rheumatic involvement, malignancy and drug-induced involvement can be suggested as differential diagnosis of a case with rheumatic disease ,who applied to an emergency service with pulmonary symptoms. The drugs cause 2.5-3% of all interstitial lung diseases. Sulfasalazine has been widely used in the treatment of inflammatory rheumatic conditions and it is an extremely rare reason for interstitial lung disase. Here, we aimed to present the rarely seen sulfasalazine-induced interstitial pulmonary disease and its treatment. Sulphasalazine-induced lung disease can mimic the symptoms of infectious and rheumatic lung involvement, and can cause serious diagnostic confusion.

Key Words: Sulphasalazine, Acute Pulmonary Injury, Interstitial Lung Disease, Ankylosing Spondylitis

ÖZ

Enfeksiyon , primer akciğer hastalıkları, romatolojik tutulum, malignite ve ilaca bağlı tutulumların hepsi interstisyel akciğer hastalığı bulgularıyla acil servise başvuran bir romatizma hastasında akla gelebilecek ayırıcı tanılardır. İlaçlara bağlı tutulumlar tüm interstisyel akciğer hastalığı olgularının %2.5 -3 arasındaki sebebini oluşturur. Sülfasalazin inflamatuar romatolojik hastalıkların tedavisinde geniş kullanım alanı olan bir ajan olup interstiyel akciğer hastalıklarına oldukça seyrek de olsa sebep olabilmektedir. Ayrıca sülfasalazine bağlı akciğer tutulumu enfeksiyonlar ve romatolojik tutulumları da taklit edip ciddi tanısal karışıklığa sebep olabilir. Bu yazımızda, nadiren karşılaşılan sülfasalazinin tetiklediği akciğer olgumuz ve bu durumun tedavisine değindik.

Anahtar Kelimeler: Sülfasalazin, İntestisyel Akciğer Hastalığı, Akut Akciğer Hasarı, Ankilozan Spondilit

Received: 04.04.2020 Accepted: 09.06.2020 Published (Online):29.10.2020

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To cited: Ketenci S, Salbaş E, Canbaz M, Özşahin F. A rare cause of interstitial lung disease in rheumatology clinic: case report for sulfasalazine-induced acute pulmonary injury. Acta Med. Alanya 2020;4(3):291-295. doi:10.30565/medalanya.714362

INTRODUCTION

In a case with a rheumatic disease who applied to an emergency service due to pulmonary derived symptoms, such as cough and shortness of breath: infection, primary lung pathology, rheumatic involvement, malignancy and drug-induced involvement should be considered in the differential diagnose list. More than 380 drugs can cause toxicity in the lung and the estimated ratio of drugs role for interstitial lung disease (ILD) is 2.5-3% [1, 2]. Differentiating drug-induced lung disease (DILD) from lung involvement of the disease in a rheumatic patient can be quite tricky because of indistinguishable common symptoms. The lack of specific clinical, laboratory, radiological or even histological findings, complicates the diagnosis and treatment process. For this reason, getting medical records as well as examining the onset of complaints and drug use history are necessary, and to be done with utmost care.

In this article we aimed to present the rarely seen sulfasalazine-induced interstitial pulmonary disease and its treatment.

CASE REPORT

A 43-year-old male patient with no known history of lung disease was reported to have cough, sputum, fever and wheezing for 15 days. In his first appliance to the emergency service, infiltration was detected a on chest X-ray and empirical antibiotherapy treatment was started. The patient, whose complaints persisted despite the therapy, referred to the emergency department of the university hospital. A pulmonologist consulted him with his chest X-ray at the emergency service and the patient was admitted to the chest diseases service with an acute pneumonia diagnosis. Ceftriaxone b.i.d. 1 gr and clarithromycin b.i.d. 500 mg (intravenous) were administered. On the 7th day of antibiotherapy his symptoms had not retreated. The patient had consulted by a rheumatologist while he was bedridden and on physical examination, blood pressure was found to be 120/80mmHg, and fever was 38.5°C, pulse was 75/min. There were no pathological findings other than bilateral crepitant rale in auscultation and other physical examination findings were normal. Laboratory findings provided the following results: leukocyte: 9400/μL, hemoglobin: 14g/dL, platelet: 325.000/μL, eosinophil: %1.5, neutrophil: %78, creatinine: 0.69mg/dL, urea: 27 mg/dL, aspartate aminotransferase (AST): 23U/L, alanine aminotransferase (ALT): 32U/L, C-reactive protein (CRP): 2,32 mg/L and erythrocyte sedimentation rate (ESR) 38 mm/h. Proteinuria and active sediment were not found in spot urine. Chest X-ray showed bilateral non-homogeneous reticular opacities in the upper zones. High-Resolution Computed Tomography (HRCT), requested by the chest diseases clinic, showed more common ground-glass opacities and minor fibrotic changes in the bilateral upper lobes (Figure 1). The pulmonary function test (PFT) of the patient was in a restrictive pattern. The carbon monoxide diffusion capacity (DLCO) test showed mild diffusion limitations. There was no cardiac failure found after being consulted with cardiology. HIV serology was negative. Immunological markers were requested, and the results were Rheumatoid factor (RF) (-) antinuclear antibody (ANA) (-), perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) (-), Cytoplasmic antineutrophil cytoplasmic antibodies (C-ANCA) (-). An expert performed bronchoscopy and a bronchoalveolar lavage (BAL) fluid was obtained from the patient. BAL fluid was slightly hemorrhagic but not as much to consider as a finding of diffuse alveolar hemorrhage. The polymerase chain reaction (PCR) test was negative for Pneumocystis Carinii.

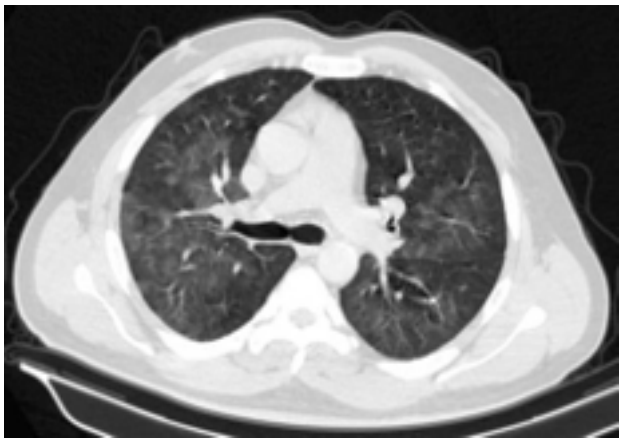

Figure 1: Ground-glass opacities, upper lobes

Evaluated with this information, the patient has no connective tissue diseases and vasculitis that may often cause ILD. Rheumatologic examination revealed chronic knee arthritis and significant inflammatory low back pain for more than five

years. His sibling has a diagnose of psoriasis and were in a follow-up by a dermatology clinic. After a more detailed examination, it was learned that the patient was still using sulfasalazine for three months, which has started by another rheumatology clinic. The patient did not reveal this information to the pulmonologist when giving his medical history. At this time, a sacroiliac X-ray was requested from the patient and bilateral stage 3 sacroiliitis was detected (Figure 2).

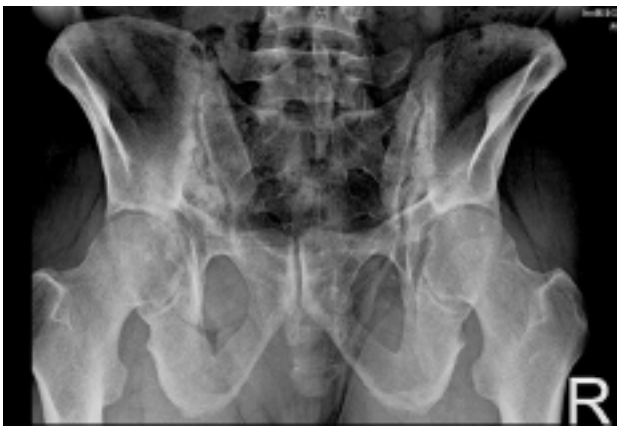

Figure 2: Bilateral grade-3 sacroiliitis

As clinical and radiological findings suggested, sulfasalazine-induced lung injury was considered in the foreground in the patient who was found to be followed up due to spondyloarthritis, and the diagnosis of pulmonary involvement due to primary rheumatic disease was excluded. Sulfasalazine and antibiotherapy were discontinued. Viral infection couldn't be ruled out, so steroid treatment was not started. After five days of sulfasalazine discontinuation, the patient's clinical symptoms wholly resolved. The rate of dyspnea decreased and no fever was observed. A significant regression was seen in the findings of involvement on the HRCT taken 15 days later (Figure 3).

Ethics: Informed consent was taken from the patient, and all procedures followed while writing case report were in accordance with the Helsinki Declaration of 1975 (in its most recently amended version).

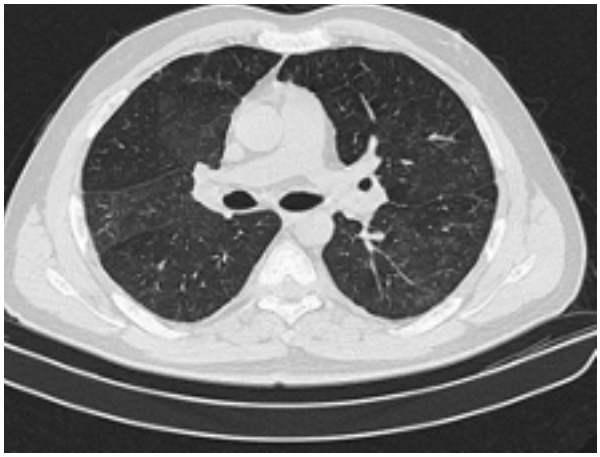

Figure 3: Regression of ground-glass opacities after drug cessation

DISCUSSION

Gas exchange takes place in the alveolocapillary membrane, which is part of the lung interstitium. ILD is the common name for a group of diseases that affects the alveolocapillary membrane in the foreground [3]. The most common cause was idiopathic pulmonary fibrosis, which was reported in most series ranging from 22-37% [4]. Genetic and environmental factors, occupational exposures, infections, malignancy, drug use and autoimmune diseases are other factors that can cause ILD [5].

It has been shown that lung involvement of rheumatic diseases can cause ILD up to 13% of all rheumatic diseases [2]. However, before deciding that ILD is a sign of rheumatic disease, infection, malignancies and pulmonary involvements caused by anti-rheumatic drugs, should be excluded. Radiological and even pathological data may not be helpful to determine the cause of the disease. Because of this, cooperation between rheumatologists and pulmonologist is required, and essential [6].

In cases without known lung disease, the development of new infection should first be excluded in the presence of acute-subacute clinical findings, concomitant fever and ground-glass appearance on HRCT. In our patient, there was no improvement with empirical antibiotic treatment and further investigations were requested. RF, ANCA and ANA tests were negative, microbiological tests could not find the cause and no additional findings were observed in BAL except hemorrhagia. The rheumatologic evaluation found

the spondyloarthritis (SpA) diagnosis of the patient. Acute interstitial pneumonia due to axial spondyloarthritis is an unexpected condition, but rather, bilateral apical fibrosis is more expected in advanced involvement of SpA [7]. Because of this unanticipated situation, the drugs which were being used by the patient were reconsidered as a cause of the drug-induced ILD.

Drug-induced ILD occurs due to the toxic and immunological effects of drugs [8]. They may be seen as cough, bronchospasm, diffuse lung disease, pulmonary edema, pleural diseases, pulmonary vascular diseases and mediastinal diseases [9]. Of the antirheumatic drugs, biological agents, methotrexate and sulfasalazine are often and particularly responsible for side effects in the lungs.

Sulfasalazine has highly miscellaneous side effects. Up to 20% of the patients receiving treatment may experience significant forms of these, such as nausea, vomiting, skin rash, arthralgia, and fever. It is responsible for initiating a number of immune reactions. Typical examples are sulfasalazine-induced ANA positivity, drug-induced lupus and drug-induced vasculitis DRESS syndrome [10], but pulmonary toxicity and blood dyscrasia can be seen in very few cases.

Acute interstitial pneumonitis, eosinophilic pneumonia, pleural effusion, sarcoidosis-like granulomatous lung disease, ANCA-related capillaritis and pulmonary fibrosis, have been identified in patients treated with sulfasalazine [11]. Eosinophilic pneumonia is the most common pulmonary complication: clinical presentation is characterized by fever, lung infiltration and/or skin rash and/or peripheral eosinophilia. Dyspnea, cough and fever trio can be observed in half of the cases [12]. Increased CRP and ESR values often appear, and as they are not specific to the disease, they require careful examination for discriminating from infections. Before the clinical presentation of ILD, usually, 2-6 months of drug use history is present. Radiological findings typically consist of bilateral, peripherally localized, consolidation areas. Upper lobe localization is predominant in these kinds of consolidations and in a few cases, lower lobe or diffuse involvements have been found. A high level of regression will be observed after the discontinuation of sulfasalazine treatment, the predominance of eosinophil and lymphocyte may be seen in BAL fluid; the use of steroids accelerates the treatment [13].

Of the other complications, acute interstitial pneumonia presents with flare-ups within the period following drug intake. Fever and dyspnea gradually increase, and in the pathological specimens, diffuse alveolar damage is detected. The drug should be rapidly discontinued, immunosuppressive agents should be added to the steroid treatments, and when necessary, plasmapheresis should be applied. Despite all, mortality occurs in approximately 50% of cases [14]. Another fatal complication is ANCA-related vasculitis due to sulfasalazine. In general, p-ANCA positivity is found in patients; the drug should be discontinued, and primary ANCA-related vasculitis treatment should be applied [14].

In our case, there was no peripheral eosinophilia and skin rash, but the diffuse involvement was predominant in the upper lobe. BAL had no eosinophilia, and hemorrhagic fluid was detected. Lung biopsy could be very valuable in the differential diagnosis but could not be performed. In our case, sulfasalazine induced acute interstitial pneumonia was considered in higher precedency due to radiological and clinical findings. Regression was observed with the discontinuation of the drug.

As a result, drug-induced lung disease is a rare condition. It can be reversible if diagnosed early and the drug is discontinued, however a delay in diagnosis or treatment causes fatal consequences. Acute phase responses, routine biochemical markers, radiological, or even pathological data often do not produce consistent findings for differential diagnosis. In the emergency service, if the acute pneumonia diagnosed and the patient has a concomitant rheumatic disease, rheumatic lung disease and drug-related lung disease should be kept in mind and obtaining detailed anamnesis (medical records) in this respect, is essential.

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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Acta Medica Alanya

REVIEW

DERLEME

Acta Medica Alanya

2020;4(3):296-308

DOI:10.30565/medalanya.788960

General approach to diabetic neuropathy

Diyabetik Nöropatiye Genel Yaklaşım

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ABSTRACT

Diabetic neuropathy is the most common complication of diabetes mellitus. It causes microvascular and macrovascular damage and diagnosis can easily be overlooked by most physicians. Generally, the diagnosis of DN can be omitted by physicians dealing with diabetes treatment since it starts with non-specific findings, shows slow progression and can be confused with complaints in many diseases. It is estimated that there will be 578 million people diagnosed with DM in the world in 2030. Chronic hyperglycemia, microvascular insufficiency, oxidative and nitrosative stress, impaired neurotropism and autoimmunity are some of the factors that cause nerve destruction. Paresthesias such as tingling, burning, electrical shock-like sensations, numbness, throbbing, compression, pinpricks to the skin, complaints are the most common symptoms. There is no treatment for pathogenetic mechanisms in diabetic neuropathy that eliminates neuronal damage. The purposes of treatment are slowing down the progression of the disease, controlling the pain, preventing complications, quick and adequate treatment of occurred complications, maintaining the functional capacity of the patient. In this review, we aimed to comprehensively address the general approach to diabetic neuropathy, diagnosis and treatment.

Keywords: diabetes mellitus, neuropathic pain, neuropathy, diagnosis, treatment

ÖZ

Diyabetik nöropati (DN), diabetes mellitusun en yaygın komplikasyonudur. Mikro ve makrovasküler hasara neden olur ve çoğu hekim tanıyı kolaylıkla gözden kaçırabilir. Genel olarak; DN tanısı, spesifik olmayan bulgularla başladığı, yavaş ilerleme gösterdiği ve birçok hastalığa ait şikayetlerle karışabileceği için diyabet tedavisi ile uğraşan hekimler tarafından ihmal edilebilir. 2030 yılında dünyada 578 milyon kişinin DM tanısı alacağı tahmin edilmektedir. Kronik hiperglisemi, mikrovasküler yetmezlik, oksidatif ve nitrozatif stres, bozulmuş nörotropizm ve otoimmünite sinir harabiyetine neden olan faktörlerden bazılarıdır. Karıncalanma, yanma, elektrik çarpması benzeri hisler, uyuşma, zonklama, bası, deriye iğne batması gibi parestezik yakınmalar en sık görülen semptomlardır. Diyabetik nöropatide nöronal hasarı tamamen ortadan kaldıran patogenetik mekanizmaların tedavisi yoktur. Tedavinin amacı hastalığın ilerlemesini yavaşlatmak, ağrıyı kontrol altına almak, komplikasyonları önlemek, oluşan komplikasyonların hızlı ve yeterli tedavisi ve hastanın fonksiyonel kapasitesini korumaktır. Bu derlemede, diyabetik nöropati, tanı ve tedaviye genel yaklaşımı kapsamlı bir şekilde ele almayı amaçladık.

Anahtar Kelimeler: Diabetes mellitus, tanı, tedavi, nöropati, nöropatik ağrı

Received: 01.09.2020 Accepted: 07.09.2020 Published (Online):

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To cited: Uzuner B, Ketenci S, Salbaş E. General Approach to Diabetic Neuropathy. Acta Med. Alanya 2020;4(3):296-308. doi:10.30565/medalanya.788960

Introduction

Diabetic neuropathy (DN) is the most common complication of diabetes mellitus (DM) [1]. In developed countries, it is the most common cause of neuropathy, leading to more hospitalizations than any other diabetes complication. It is also the major cause of non-traumatic amputations [2]. Generally, neuropathic pain is the first symptom that makes the patient consult a physician in type 2 diabetes, which is asymptomatic for many years. Diagnosis of DN can be omitted by physicians dealing with diabetes treatment since it starts with non-specific findings, shows slow progression and can be confused with complaints in many diseases [3]. While 50% of pathological findings were detected in simple diagnostic tests (vibration detection threshold (VDT) test, pinprick test), in 90% of the same patients, positive findings (for changes in autonomic function and peripheral sensitization), were found to support positive DN by complex tests [4]. The major cause of morbidity associated with diabetic neuropathy is foot ulcers induced by somatic neuropathy and extremity losses due to gangrene. Foot ulcer prevalence varies from 3% to 30% among patients with diabetes, and amputation risk increases 8 to 23-fold if there is a preexisting ulcer in the extremities [5]. In autonomic nervous system involvement due to diabetes, mortality is observed in 25-50% of the patients within 5-10 years.

Since DN was first identified, several classifications have been made. Commonly used simple classification defines patients as symmetric polyneuropathy (e.g., sensorimotor polyneuropathy), focal/multiple neuropathies (e.g., diabetic amyotrophy). Sensorimotor polyneuropathy is the most common form of the polyneuropathy of DM. Polyneuropathy is seen in approximately 25% of diabetic patients in the general population [6]. Diabetic polyneuropathy (DP) is generally one of the late complications of diabetes. It has been shown that long-term high blood glucose values associated with the duration of the disease contribute to metabolic and vascular changes and accelerate the risk and course of symmetrical sensorimotor polyneuropathy. At first, there is a decrease in the awareness of pain and heat due to the involvement of small nerve fibers and in the following process, the sensation of touch and vibration decreases with the involvement of large nerve fibers. Positive symptoms such as paresthesia and pain may be observed by the involvement of sensory nerve fibers. However, 50% of cases are asymptomatic. In DP, cardiovascular autonomic system involvement increases mortality risk with a 5-year mortality rate of 16%–50% [7]. In light of recent data, diabetic autonomic neuropathy or autonomic imbalance in the sympathetic-parasympathetic nervous system might be an important cardiovascular risk.

Epidemiology

It is estimated that there will be 578 million people diagnosed with DM in the world in 2030 [8]. It has been shown that the prevalence of diabetes in Turkey is 7.2% and the prevalence of impaired glucose tolerance is 6.7% [9]. The data on the frequency of DN varies according to the characteristics of the selected methods for diagnosis. Symptoms, examination findings, quantitative sensory tests and electrophysiological tests are the methods used for diagnosis. In epidemiological studies based on different diagnostic criteria, the prevalence of chronic sensorimotor neuropathy was found to be 4-64%. In Turkey, in the most recent study performed by Erbas T. et al., 14% of patients with DM had painful neuropathy, while 40% of patients had clinical diabetic peripheral polyneuropathy findings. Poor glycemic control, retinopathy, microalbuminuria, hyperlipidemia, diabetic foot and foot amputations, have been commonly observed in patients diagnosed with diabetic peripheral neuropathy [10].

Diabetic Neuropathy Classification

Although there are many neuropathy classifications prepared considering anatomical and clinical features, most of these classifications are similar to each other. The classification summarized in Table 1 is one of the most commonly used [11].

Etiopathogenesis

Chronic hyperglycemia, microvascular insufficiency, oxidative and nitrosative stress, impaired neurotropism and autoimmunity are some of the factors that cause nerve destruction. DN progress with a wide variety of clinical symptoms and consists of clinical pictures with

these symptoms having different pathological mechanisms. The elucidation of pathogenesis is important for the development of causative treatments (see Figure 1).

Table 1. Classification of Diabetic Neuropathy

1. Quickly reversible
a. Hyperglycemic neuropathy
2. Generalized symmetrical polyneuropathies
a. Sensorimotor (chronic)
b. Acute sensorial
c. Autonomous
3. Focal and multifocal neuropathies
a. Cranial neuropathies
b. Thoracolumbar radiculoneuropathies
c. Focal extremity neuropathies
d. Proximal motor neuropathies (Amyotrophy)
4. Chronic inflammatory demyelinating polyneuropathy

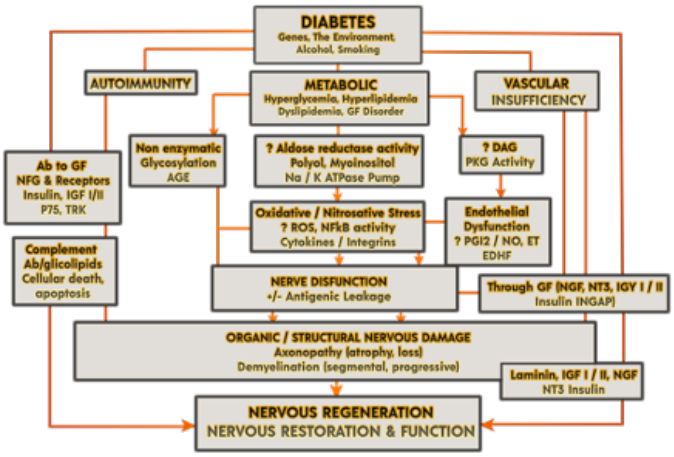

Figure 1. The pathogenesis of diabetic neuropathy. (Ab, antibody; AGE, advanced glycation end products; ATPase, adenosine triphosphatase; C, complement; DAG, diacylglycerol; EDHF, endothelial derived hyperpolarizing factor; ET, en-dothelin; GF, growth factor; IGF, insulin-like growth factor; NFkb, nuclear factor kappa b; NGF, nerve growth factor; NO, nitric oxide; NT3, neurotrophin3; PGI2, prostaglandin I2; PKC, protein kinase c; ROS, reactive oxygen species; TRK, tyrosine kinase (12).)

Symptoms and Clinical Features

DN clinic shows a rich variety. Diabetes has widespread involvement from the somatic peripheral nerves to the autonomic nervous system. There are very different clinical manifestations depending on the affected nerve segment: from cardiovascular symptoms to erectile dysfunction, neuropathic pain to foot ulcers, it may present with various clinical findings. Half of the DP patients present with neuropathy related symptoms and the other half are asymptomatic. A good neurological examination is very important for the detection of asymptomatic patients. Long axons are more sensitive to nerve damage and complaints usually begin distally to the lower limbs and more rarely from the distal upper limbs. Involvement of myelin-free C, thin myelin A delta, thick myelin Aα and A_β type neurons are typical. Although it little known, there is a great deal of evidence showing that thin fibers are involved earlier and neuropathic pain begins earlier than sensory loss and decreased nerve conduction velocity [12]. In the skin biopsies taken at the initial stage of DP, all findings showing thin fiber damage, especially decrease in the density of intraepidermal nerve fibers, were detected, however nerve conduction velocity, quantitative sensory tests and neuropathic disability scores indicating thick never fibers were found to be completely normal [13].

The most prominent feature of DN is that sensory symptoms begin much earlier than the symptoms of motor involvement. Sensory symptoms may be positive or negative: positive findings are symptoms of neural hyperactivity that are felt as stimuli without stimulation. Paresthesias such as tingling, burning, electrical shock-like sensations, numbness, throbbing, compression, pinpricks to the skin, complaints such as dysesthesia, hyperpathy, hyperalgesia or allodynia are the most commonly described pain-positive symptoms. It may present non-painful positive symptoms such as felting, drowsiness, feeling like wood and a feeling of walking on pebbles. The pain is usually localized distally to the lower extremity and exacerbates at night. Sleep disorders, anxiety, depressive symptoms, decreased appetite, weight loss, sexual dysfunction and difficulty in concentration, are often correlated by the severity of pain. The most common symptom of negative symptoms is the loss of sensation. Motor losses in DP are milder than sensory losses and are limited to the distal lower extremity. Depending on the severity of the involvement, atrophy and reflex losses may occur in the foot muscles; due to loss of pain sensation and deformities in the feet, prominent metatarsal head, claw-foot, hammertoe deformities are frequently observed. These deformations also increase the risk of callus

formation, ulcers and amputation. Neuropathic pain is the most important symptom that leads the patient to the physician in diabetic neuropathy cases with delayed diagnosis. Figure 2 and Table 2 describe different forms of diabetic neuropathy: different forms of DN, usually in the same patient (e.g., distal polyneuropathy and carpal tunnel syndrome), are important to be noted as that they may present together [14].

Diagnosis

A series of conferences have been organized aiming to redefine the minimum criteria for diagnosis, in order to overcome the existing problems arising from the differences in the methods used in the identification and diagnosis of neuropathy. The tests found in Table 3 and Table 4 are suitable for assessing each nerve fiber type and function. From distal symmetrical diabetic polyneuropathy, we can divide DP into 5 groups according to the Toronto classification [15].

1- Possible DP: Symptoms such as numbness, tingling, burning or symmetrical distal sensory loss against touch and vibration during the examination, one of the symptoms or signs such as pin prick and thermal sensory loss and/or allodynia/hyperalgesia, decreased or lost Achilles reflex.

2- Possible DP: Two or more neuropathic symptoms and examination findings (such as decreased sensory sensation, decreased or absent uneven Achilles reflex)

3- Proven DP: Abnormal neurophysiologic/ morphometric results accompanied by any symptom or finding

4- Subclinical DP: Abnormal neurophysiological/morphometric test results detected in the absence of signs or symptoms

5- Small fiber neuropathy (SFN): There is no commonly agreed definition yet. SFN is divided into 3:

a. Possible SFN: Presence of length-dependent symptoms and / or clinical signs of small fiber nerve injury

b. Possible SFN: Presence of length-dependent symptoms, clinical signs of small fiber damage and normal sural nerve conduction

c. Proven SFN: Presence of length-dependent symptoms, clinical signs of small fiber damage, normal sural nerve conduction, abnormal intraepidermal nerve fiber (IENF) density measurement and / or abnormal thermal cut-off measurements in ankle sural biopsy.

In diabetic polyneuropathy, the diagnosis should be made according to clinical and neurological examination findings. Neurological examination findings such as sensory loss, allodynia, hyperalgesia, motor weakness and absence of reflexes should be detected with positive and negative sensory and motor symptoms. Symptoms alone have low predictive value in the diagnosis of polyneuropathy. For polyneuropathy diagnosis, positive findings on examination are a better predictor than the patient's symptoms.

Differential Diagnosis

Non-diabetic factor should be considered in the etiology of neuropathy in 10% of diabetic cases. The non-diabetes causes of neuropathic pain should be questioned in the patients with asymmetric neurological deficits, predominant motor deficits, mononeuropathies, cranial nerve involvement, rapid and progressive neurological insufficiency, progressive neuropathy despite optimal glycemic control, those with onset of symptoms on upper limbs, familial history of non-diabetic neuropathy, in those with whom DN cannot be detected.

Types of Diabetic Neuropathy Associated with Pain

Table 5 lists the types of painful diabetic neuropathy. In this section, the clinical features of these painful neuropathy types in diabetes will be discussed in more detail.

I. Focal and multifocal neuropathies

Focal neuropathies consist of focal extremity neuropathies and cranial neuropathies. Focal extremity neuropathies are usually caused by entrapment neuropathies. Mononeuropathies are usually observed in the elderly population, are acute onset and tend to self-limit within 6-8 weeks. The involvement of the median nerve

Table2. Characteristic Differences Between Mononeuropathy, Trap Neuropathy, Distal Symmetrical Polineuropathy

Features	Mononeuropathy	Trap Neuropathy	Polyneuropathy
Starting	Fast	Progressive	Progressive
Pattern	Single nerve involvement sometimes multiple involvement	Single nerve exposure to trauma	Distal Symmetric Polyneuropathy
Nerve involvement	3, 4 and 7. cranial nerves, ulnar median, peroneal	Median, ulnar, peroneal median and lateral plantar	Mixt, motor, sensory autonomic
The natural course	Spontaneous remission	Progressive	Progressive
Distribution of sensory loss	Along the innervation pattern of the affected nerve	Area distal to the affected nerve that is trapped	Distal and symmetrical glove sock style scatter pattern

Table3. Sensory tests that can be performed at the bedside

Sensory Modality	Nerve Fiber	Instrument	Associated Sensory Receptors
Vibration	Aβ	128 Hz diapason	Ruffini mechanoreceptors
Pain (pinprick)	C	Free nerve endings termination	Heat and pain receptors
Pressure	AβAα	1 g and 10 g monofilament	Pacini receptors
Light touch	AβAα	Cotton touch	Meissner bodies
Cold	Aα	Skin contact with cold	Cold thermoreceptor

Table 4. Advanced objective tests for the diagnosis of diabetic neuropathy

Neurological Test	Types of neuropathy	Measurement	Advantages
Quantitative sensory tests	Thin and thick fiber neuropathy	Assessment of sensory loss	A measurable semiquantitative test
Skin biopsy and intraepidermal nerve fiber density (IENF)	Small fiber neuropathy	Somatic non-myelinated nerves, dermal myelinated nerve fibers, automatic nerve fibers consisting of small sensory fibers	Quantitative measurement of epidermal thin fibers by various antibody staining methods
Corneal confocal microscopy	Small fiber neuropathy	Detects small nerve fiber loss in cornea.	Non-invasive technique that correlates with the severity of neuropathy
Potential evoked by heat contact	Small fiber neuropathy	Heat-induced potentials are recorded with electroencephalography.	In the absence of other tests, it shows damage to thin fibers.
Evaluation of sudomotor functions	Distal small fiber neuropathy	A test for measuring electrochemical conduction between chlorine ions in hand and foot sweat	Early detection of neurophysiological abnormalities in peripheral autonomic functions
Nerve conduction studies	Thin and thick fiber neuropathy	Evaluation of conductivity of ner ve fibers by electrical stimulus	Objective, universally measurable and recordable method.

Table 5. Types of Painful Diabetic Neuropathy

I. Focal and multifocal neuropathies
a. Cranial
b. Focal extremity (Trap neuropathies)
c. Amyotrophy (Proximal motor neuropathy)
d. Truncal (Thoracolumbar) radiculoneuropathy
II. Generalized symmetrical polyneuropathies
Acute sensorial

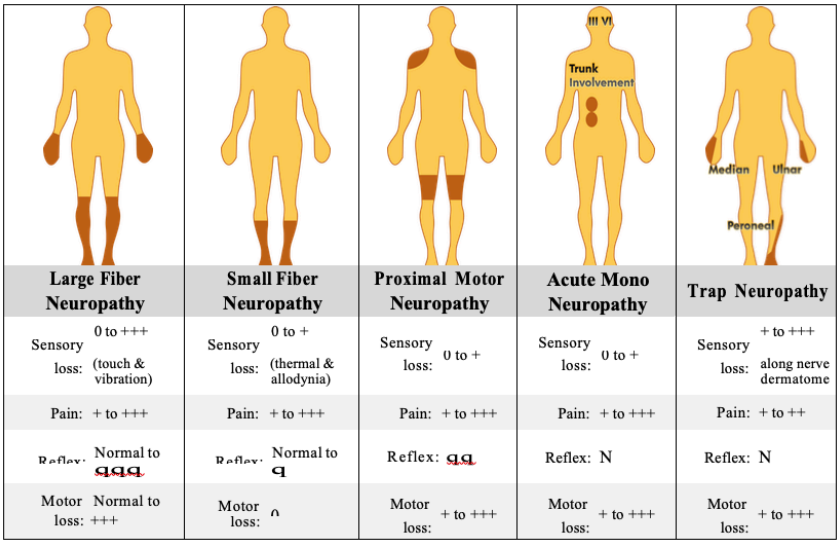

Figure 2. Clinical presentation of large fiber and small fiber neuropathy in diabetic polyneuropathy. N, normal. (16)

(5.8%), ulnar nerve (2.1%), radial nerve (0.6%) and common peroneal nerves are observed in mononeuropathies, respectively [14].

A. Cranial Neuropathies

1. Ocular Neuropathy

Ocular neuropathies are the most frequent microvascular complication of diabetes [16]. Ocular neuropathy usually occurs in older age and long-term diabetes, and most commonly 3, 4 and 6th cranial nerves are involved and symptoms specific to the involved nerve are observed. 3rd nerve involvement causes ptosis, diplopia and ipsilateral headache. Clinical findings usually improve within 3 months but may be recurrent.

2. Others

Involvement of facial nerve, optic and olfactory nerves, corneal myelinated and non-myelinated small fibers is more common in diabetic patients. Recurrent laryngeal nerve pathologies with trigeminal and vagal neuropathy are rare but can be seen.

B. Focal Extremity Mononeuropathies

1. Carpal tunnel syndrome

The prevalence of carpal tunnel syndrome (CTS) in diabetes is 3 times higher than in the normal population (20). In a recent study, it was reported that 65% (76) of the 117 diabetic patients had hand problems. 64 of these patients had CTS [17]. Neuropathy is characterized by symptoms of the median nerve trapped under the transverse carpal ligament. Resting splint, anti-inflammatory agents, cortisone injection under the ligament and decompression surgery in unresponsive cases,

are recommended for treatment.

2. Ulnar nerve neuropathies

Ulnar neuropathy at the elbow, known as cubital tunnel syndrome, is the second most common entrapment neuropathy. The most affected ones are the intrinsic muscles of the hand, and weakness and atrophy occur in the progressive stage. In the treatment of symptoms, conservative treatment is performed for unresponsive cases.

C. Proximal Motor Neuropathy

Proximal motor neuropathy of the lower extremity is often called diabetic amyotrophy. It is more common in Type 2 diabetic patients between the ages of 50-60. It is thought that vascular, metabolic and immune factors are involved in the etiology. Electrodiagnostic examinations show that the lesion is located in nerve roots and plexus. Pain is often an initial complaint and is worse at night, weakness becomes apparent a few weeks after the onset of pain and atrophy of quadriceps, iliopsoas, or the adductor muscles occur. Generally, recovery is completed over a period of 12-24 months and the prognosis is good. The first approach to treatment is strict pain control. It is important to ensure good glycemic control for the etiology. In addition, the benefits of high-dose corticosteroid use and IV immunoglobulin administration have been reported in selected cases [18, 19].

D. Truncal Radiculoneuropathy

Thoracoabdominal or truncal neuropathy or radiculopathy, occurs most commonly in diabetic patients over the age of 50 years. The onset is usually acute, it mainly involves T3-T12 and is generally unilateral. This condition may often be confused with myocardial infarction, an intraspinal pathological event, abdominal disease or malignancy, and differential diagnosis should be made. Sensory loss and allodynia may be present in the painful area. Symptoms tend to gradually improve within a few months. In addition, IV immunoglobulin and high dose corticosteroid administration may be beneficial.

II. Generalized Symmetrical Polyneuropathies

A. Acute Sensorial Neuropathy

It is a neuropathy that occurs following a period of high glucose levels or diabetic ketoacidosis in newly diagnosed diabetics. According to some authors, it is a characteristic variant of distal symmetrical polyneuropathy. The syndrome is characterized by severe pain, cachexia, weight loss, depression and erectile dysfunction in men. It is mostly seen in male patients. A physician should exclude such factors as Fabry disease, amyloidosis, HIV infection, heavy metal poisoning and excessive alcohol consumption. Rapidly normalizing blood glucose by insulin or even by oral anti-diabetic therapy in poor glycemic controlled cases, may trigger this condition. Although the exact mechanism is not known, blood glucose level alterations are thought to cause perineural ischemia [20]. Another group of researchers linked the disease to lumbosacral radiculoplexus neuropathy, and suggested that there are immune-mediated mechanisms in the pathogenesis of the disease. Symptoms usually regress spontaneously within weeks following glucose control.

B. Chronic Sensorimotor Neuropathy or Distal Symmetric Polyneuropathy

The most common type of DN is chronic sensorimotor neuropathy [21]. Although it is similar in type I and II diabetes, chronic sensorimotor neuropathy may accompany the condition even at the time of diagnosis of type II diabetes. A progressive decrease in peripheral nerve fibers has been demonstrated in skin and skin biopsies taken from patients diagnosed with diabetes or in the prediabetic phase [22]. Sensory symptoms in patients are more pronounced than motor symptoms and usually involve lower extremities.

Treatment approaches for pathogenesis in diabetic neuropathy

There is no treatment for pathogenetic mechanisms in diabetic neuropathy that completely eliminates neuronal damage. The purposes of treatment can be listed as follows:

1. Slow down the progression of the disease

2. Pain control

3. Preventing complications

4. Rapid treatment of occurred complications

5. Maintain the functional capacity of the patient

Importance of Glycemic Control

The most effective approach for the prevention of all microvascular complications of diabetes and neuropathic pain is undoubtedly to achieve good glycemic control. According to the data of the Diabetes Control and Complications Trial Research Group (DCCT) studies, a 60-69% risk reduction of neuropathy development was found in Type I diabetic patients who were followed up with intensive insulin therapy for nearly 8 years and were targeted to keep HbA1c close to normal [22]. This Epidemiology of Diabetes Intervention and Complications study, the continuation of the DCCT study, showed that this positive effect continued even 13-14 years after the end of the treatment. As a result, good blood sugar metabolic control achieved in the first 10 years of diabetes diagnosis may prevent neuropathy and this positive effect persists even after HbA1c increases [23]. The results of studies on the relationship between strict blood glucose control and neuropathy risk in patients with Type 2 diabetes are not as clear as in patients with Type 1 diabetes. In the ADVANCE study, which included 11,140 patients with type 2 diabetes, patients were divided into 2 groups. The first group consisted of patients receiving standard glucose therapy and the second group consisted of patients with strict sugar control. During the five-year observation period, new-onset neuropathy and neuropathic symptoms worsening in these patients were observed in similar rates [24]. Multiple daily insulin injections reduces neuropathy risk by 10-25% [25]. Although clinical course and treatment modalities are different in both Type 1 and Type 2 diabetes, metabolic targets are common. High blood glucose values are the triggering factor in the development of microvascular complications in both diabetes types. Near-normal glucose control is a prerequisite for the prevention and treatment of neuropathy. An individual with a blood glucose levels above ≥126 mg/dl for fasting, ≥200 mg/dl for postprandial (two hours after eating) is being diagnosed as diabetes [26]. In Type 2 diabetic patients with multiple risk factors and comorbidities, strict glycemic control alone is partially effective in preventing distal symmetric polyneuropathy. It causes a slight slowdown in the progression of neuropathy without preventing neuronal loss. Targets to change lifestyle should be set to prevent distal symmetric polyneuropathy in patients with prediabetic, metabolic syndrome or Type 2 diabetes.

Aldose Reductase Inhibitors

The major enzyme of the accelerated polyol pathway due to hyperglycemia is aldose reductase. They act by inhibiting the activity of this enzyme and reducing neural sorbitol levels. Epalrestat is the only aldolase reductase inhibitor currently licensed in Japan. In a randomized, placebo-controlled study for 3 years, it was reported that median motor nerve conduction rate and minimum F wave delay were prevented by administering 150 mg of epalrestat daily to 594 diabetic neuropathy patients. It has been shown that it significantly reduces symptoms such as numbness, sensory abnormalities, and cramping (30).

Antioxidants

Data suggest that oxidative and nitrosative stress is important in the pathogenesis of neuropathy and that antioxidants may be used in the treatment. Prolonged oral alpha-lipoic acid administration has been shown to cause clinical improvement and a slight delay in the progression of neuropathic deficits in patients with mild distal symmetric polyneuropathy. Due to its controversial results, its place in the pain treatment of diabetic neuropathy is not clear [27-30].

Growth Factors

Low levels of NGF induce peripheral nerve lesions in diabetic patients [31]. Increased neovascularization in nerve cells has been shown in diabetic neuropathic mice with IGF-1 supplementation [32]. Positive effects of VEGF on nerve functions have been shown in diabetic animal model studies [33]. VEGF gene therapy studies on humans are ongoing.

Immune treatment

The diabetes-related monosialoganglioside antibodies (anti-GM1 autoantibody) are

Table 6. 6 Drugs Used in the Treatment of Symptomatic Pain in Patients with Diabetic Polyneuropathy

Pharmaceutical group	Medication	Dose	Drug side effects
Tricyclic's	Amitriptyline, Nortriptyline, Imipramine, Desipramine	50-150mg, 50-150mg, 25-150mg, 25-150mg	Drowsiness, dizziness, dry mouth, tachycardia, orthostatic, hypotension, urinary retention, constipation, sweating, blurred vision
SSRI's	Paroxetine, Citalopram	40mg, 40mg	Drowsiness, dizziness, sweating, nausea, anorexia, diarrhea, impotence, tremor
SNRI's	Duloxetine	60-120mg	Nausea, dizziness, anorexia
Anticonvulsants	Gabapentin, Pregabalin, Carbamazepine, oxcarbazepine, Topiramate	900-3600mg, 150-600mg, 200-1200mg, 600-1800mg, 400mg	Drowsiness, dizziness, confusion, ataxia, drowsiness, confusion, edema, weight gain, drowsiness, dizziness, nausea, leukopenia, hyponatremia, drowsiness, ataxia, loss of appetite, tremor
Opioids	Tramadol, Oksikodon	200-400mg, 20-80mg	Nausea, constipation, drowsiness, nausea, constipation, addiction
Topical	Capsaicin, Lidocaine	0,0075 %-8 %, 0,04 %-5 %	Local irritation, Local irritation
Injection	Botilinum toxin	Max 200IU	None

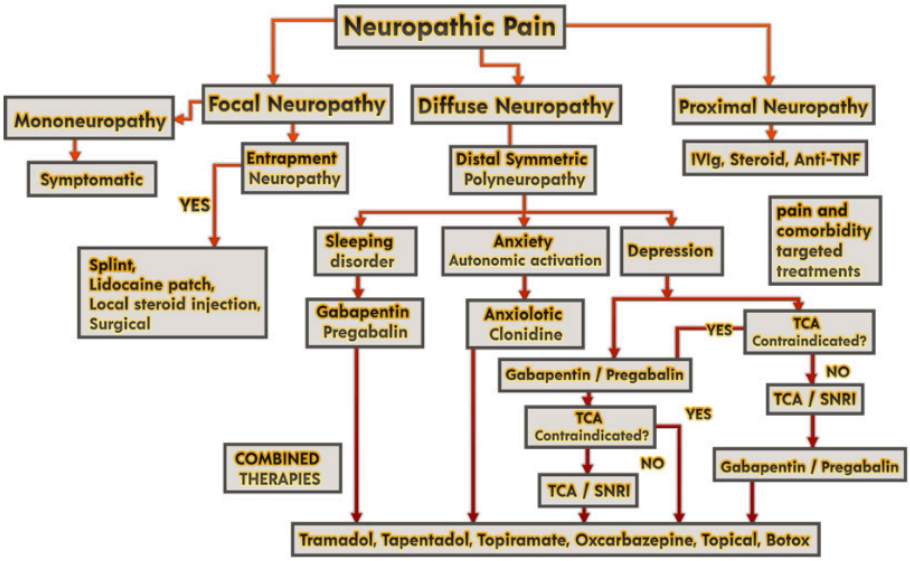

Figure 3. Diabetic neuropathy treatment scheme. (TCA, tricyclic antidepressants; SNRI, selective serotonin and noradrenaline reuptake inhibitors (36,37).)

detected in 12% of patients with proximal motor neuropathy. The relationship between autoimmunity and neuropathy is evident in patients with proximal motor neuropathy, chronic inflammatory demyelinating polyneuropathy, vasculitis, monoclonal gammopathy. Recent data has shown that even though autoantibodies against neuronal cells are not neurotoxic, they are not so innocent and have prognostic value in the development of neuropathy [34]. The use of high-dose corticosteroids and iv immunoglobulin in the treatment of these cases have been reported.

Pain Therapy

Drugs used in the treatment of diabetic neuropathy are used in painful diabetic neuropathy to reduce neuropathic pain, to control autonomic neuropathy symptoms and to improve quality of life. Figure 3 summarizes the treatment algorithm for neuropathy pain.

Tricyclic and Tetracyclic Antidepressants

Serotonin (5-HT) and norepinephrine (NE) reuptake inhibition is used for the treatment of neuropathic pain with mechanisms of action such as blocking of the Na and Ca channels. Imipramine, amitriptyline, clomipramine 5-HT and NE are reuptake inhibitors, desipramine is a relative NE reuptake inhibitor. In a Cochrane review it is reported that there is no unbiased evidence for a beneficial effect and there seems an overestimation of the treatment [35]. Antidepressant switch should be considered after a failure with one [35]. Side effects are summarized in Table 6.

Selective Serotonin-Noradrenaline Reuptake Inhibitors

This group includes venlafaxine, duloxetine and milnacipran. This group of drugs causes balanced inhibition of 5-HT and NA. SNRIs do not interact with adrenergic, muscarinic, histaminergic receptors, such as tricyclics. Therefore, they do not cause side effects such as drowsiness, weight gain, constipation and cardiotoxicity. SNRIs, like tricyclic antidepressants, act on neuropathic pain by creating inhibitory effects on interneurons in the spinal cord via pathways descending with balanced inhibition of 5-HT and NA. Duloxetine as an antidepressant, can be prescribed for neuropathic pain. In Turkey, diabetic neuropathic pain is indicated up to 120 mg/day. Duloxetine 60 and 120 mg showed significant pain relief for the first month but not continued [36]. Another advantage is that it does not cause weight gain like other antidepressants [37]. In a Cochrane review it is reported that there is no evidence to revise the guidelines to recommend the use of venlafaxine in neuropathic pain and placebo effects were notably strong in several studies [38].

Anticonvulsants

Antiepileptic drugs are effective drugs that have been used for the treatment of neuropathic pain for a long time. The most important known mechanisms of action of antiepileptics are blocking of sodium channels, inhibiting calcium conduction, activating the GABA system and reducing the effectiveness of glutamate. With a better understanding of the pathophysiology of diabetic polyneuropathy and the mechanisms of action of drugs used in treatment, a realistic choice of polytherapies emerges and a synergistic effect can be achieved by the combination of drugs with different mechanisms of action.

Ca Channel Modulators (Pregabalin, Gabapentin, Mirogabalin)

Five types of calcium channels were identified. Of these, N and L-type channels are involved in the modulation of sensory neurons of the spinal cord. Gabapentin and pregabalin (gabapentinoids) show the effects of voltage-dependent calcium channels by binding to α2 delta subunits. Unlike conventional calcium channel antagonists, it does not block calcium channels and modulates their activity. The mechanism of action of this drug group in neuromodulation has not yet been clearly elucidated.

Gabapentin

Gabapentin is a GABA receptor agonist. In a randomized, double-blind, placebo-controlled trial of 165 patients (67% in patients receiving 3600 mg/day gabapentin), 60% of patients had moderate pain relief, while the pain reduction in the placebo group remained at 30%. It shows effects on both central and peripheral nerve system. Gabapentin is the first line agent of diabetic neuropathic pain in the United Kingdom [39]. Chou et al. showed similar effects of gabapentin when compared with tricyclic antidepressants for pain relief of diabetic neuropathy [40]. Gabapentin also shows positive effects on sleep disorders that accompany pain [41].

Pregabalin

Pregabalin has a stronger efficacy with α2 delta affinity, which is six times higher than gabapentin. The analgesic efficacy of the drug increases in a dose-dependent manner and the analgesic effect starts faster than gabapentin. At a daily dose of 600 mg, the NNT score is 4 and 300mg is 5.9 [42-45]. Like gabapentin, pregabalin also has positive effects on symptoms associated with pain, sleep disturbance, mood changes and anxiety. Gabapentinoids are almost ideal drugs that are well tolerated due to their pharmacokinetic properties. Concomitant use of clozapine, opioids, and sedative drugs may increase side effects. In addition, they show little drug interaction. They do not metabolize and bind to proteins. The most

common side effects are dizziness, drowsiness, peripheral edema, headache and weight gain.

Although the research is promising, there has been increasing concern as the latest data address the abuse of and dependence on gabapentinoids. There are studies suggesting that euphoric effect might be abused by patients who previously had substance addiction. As in other potentially addictive gabamimetics (benzodiazepines, propofol, etc.), rapid tolerance to the euphoric effect develops. Gabapentinoids has potential of drug abuse [46]. For these reasons, gabapentinoids should not be considered as the first choice in patients with a history of multiple drug use (especially opioid) and substance use disorder. Gabapentin may be preferred in this group of patients, because gabapentin is relatively less likely to cause addiction than pregabalin. In patients without a history of substance use disorder, it is not necessary to take additional measures different from other drugs prescribed for the use of gabapentinoids [47].

Mirogabalin

Mirogabalin firstly developed by Daiichi Sankyo for the treatment of Fibromyalgia. When primary end point was not met (additionally pregabalin found to be more effective) trial was discontinued. Mirogabalin has a high affinity for the a2delta-1 subunit of voltage-gated calcium (Ca2+) channels (VGCCs) on the dorsal root ganglion. In 2019, oral mirogabalin (Tarlige®; 2.5, 5, 10 and 15mg) were approved in Japan for the treatment of peripheral neuropathic pain (PNP) on the basis of trials conducted in patients with diabetic peripheral neuropathic pain (DPNP). Half-life of Mirogabalin is about 2–3 h after a single dose and 2–5 h after sequential doses. Average daily pain score reduction was significantly greater with mirogabalin 30 mg/day. Adverse Events occurred in 31.3% in patients with DPNP which were reported as somnolence (12.5 and 19.9%), dizziness (9.0 and 11.8%) and weight gain (3.2 and 6.7%) [48].

Na Channel Blockers

Voltage-dependent sodium channels are crucial determinants of neuronal excitability and signaling. After nerve injury, hyper excitability and ectopic discharges occur at the site of injury and the body of

the dorsal root ganglion cell. Carbamazepine, oxcarbazepine, are the most effective blockers of sodium channels for pains in the form of lightning flashes produced by such ectopic discharges. Although carbamazepine is widely used in the treatment of neuropathic pain, it is not recommended for the treatment of painful diabetic neuropathy due to its limited data [49]. In a Cochrane review It is concluded that Lacosamide (200–600 mg/day) has limited efficacy in the treatment of peripheral diabetic neuropathy [50].

Opioid Agents

In a randomized controlled trial of tramadol use in the treatment of patients with diabetic polyneuropathy, it was found to be more effective than a placebo, and this effect lasted for at least six months. Although the side effect profile is similar to other opioid analgesics, the development of dependence and tolerance in long-term tramadol treatment is rare and the possibility of abuse is low [51]. The recommended maximum daily dose is 400-600 mg. In liver diseases or renal insufficiency, it is recommended to decrease the tramadol dose or to increase the dose range. It should be kept in mind that serotoninergic syndrome (myoclonus, rigidity, hyperreflexia, tremor, confusion, agitation, restlessness, coma) may occur if tramadol is used together with other serotoninergic drugs (especially SSRI). Oxycodone, one of the strongest opioids, may be used in severe patients resistant to other analgesic treatments. Although there is limited information about combination therapies, oxycodone may be considered as a combination therapy in patients who cannot achieve adequate pain palliation with monotherapy. When oxycodone treatment and placebo were compared in patients with diabetic polyneuropathy who could not achieve pain palliation with antidepressant and antiepileptic drugs, at the end of the 4-week observation, it was shown that pain was relieved and quality of life improved significantly [52]. Recent recommendations emphasize the importance of a physician's risk assessment (in terms of dependence and abuse) and clinical skills for the management of existing medicines before

the opioid is prescribed for the safe and effective use of opioids [53]. In a Cochrane review, it is reported that the studies provide very limited, very low-quality evidence of the efficacy and safety of methadone for chronic neuropathic pain. No conclusions can be made regarding the current status [54].

Tapentadol

Tapentadol is a novel, centrally acting analgesic molecule. It has two action mechanisms: one is inhibiting NE reuptake and the other one is activating μ-opioid receptors [55]. Tapentadol ER (100- 250 mg bid) was effective and well tolerated for the management of moderate to severe chronic pain associated with DPN [56]. It has been approved by the FDA for the treatment of painful diabetic polyneuropathy. Unfortunately, the drug is still not available in Turkey [4].

Cannabinoids

Cannabinoids should have a potential neuropathic pain treatment. With good toleration, flexible-dose of nabilone 1-4 mg/day should be effective for DPN symptom relief, improving sleep disturbances and a better quality of life [57].

Topical Capsaicin

The reduction of substance P on the axon ends of C fibers helps to relieve pain. By prolonged administration of capsaicin, substance P and other possible neurotransmitters released from the sensory nerve endings are depleted. Thus, the transmission of the painful stimulus from the peripheral nerve endings is reduced or completely eliminated. Capsaicin appears to have the potential to be the choice of neuropathic pain management [58].

Lidocaine

The use of topical lidocaine in painful neuropathy is associated with post-herpetic neuralgia. While 5% lidocaine was given to one group treated for 2 weeks, the other group was given pregabalin for 4 weeks and lidocaine was shown to be as effective as pregabalin in reducing pain without side effects. This treatment may be continued with oral mexiletine and superficial pain caused by overstimulation is targeted with oral mexiletine treatment [59].

Financial disclosure: The authors declared that this study has received no financial support.

Conflict of interest: No conflict of interest was declared by the authors.

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