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Pharmacology of Botulinum Toxins: From Poison to Remedy

Botulinum Toksinlerinin Farmakolojisi: Zehirden Çareye

Kansu BÜYÜKAFŞAR
0000-0003-4117-6013

Mersin University Medical Faculty
Department of Pharmacology, Mersin, Turkey

ABSTRACT

Botulinum toxin (BTX) is produced by autolysis of several strains of *Clostridium botulinum*, a gram-positive, spore-forming, rod-shaped, strictly anaerobic bacterium. However there are also non-clostridial microorganisms that are enable to produce the toxin. As some other beneficial poison, BTX also fits well the quotations by old scientists and philosophers like "Almost every substance can become a poison but only thing is the dose discriminating the difference" (Paracelsus, XVI century) or "Poisons can be employed as a means for the destruction of life or as agents for the treatment of the sick" (Claude Bernard, XIX century) or "Poison is a medicine, medicine is a poison" (Ahi Evran, XIII century). In the 1980's, Alan Scott first published articles on the use of BTX for the treatment of strabismus. The Food and Drug Administration of the USA (FDA) first approved botulinum toxin for the treatment of strabismus (crossed eye) blepharospasm (uncontrollable eye blinking) in 1989 and for glabellar rhytides in 2002, the first cosmetic indication. Since then BTX has been used for a verity of indications not only dermatological but also non-dermatological indications including on-labelled as well as off-labelled uses. In this review you will find the pharmacological profile of botulinum toxins, i.e., mode of action, pharmacokinetics, adverse effects, indications and contrindications, drug interactions, duration and site of action, etc. Furthermore, current commercial products and novel dosage forms as well as new perspective of BTX use will also be discussed.
Keywords: Botulinum toxin; pharmacology; poison; remedy; esthetic; muscle; pain.

ÖZ

Botulinum toksini (BTX) Gram (+), sporlu, çubuk şekilli ve mutlak anareobik bir bakteri olan *Clostridium botulinum*'un parçalanması sonucu üretilir. Ancak bu toksini üretebilen Clostridium-olmayan mikroorganizmalar da bulunmaktadır. Faydalı olabilen bazı toksinler gibi BTX de eski bilim insanları ya da filozofların "Hemen her şey zehir olabilmektedir, farkı yaratan dozdur" (Paracelsus, XVI yüzyıl) veya "Zehirler yaşamı tahrip etmek veya hastaları tedavi etmek için kullanılabilir" (Claude Bernard, XIX yüzyıl) ya da "Zehir ilaçtır, ilaç zehirdir" (Ahi Evran, XIII yüzyıl) gibi tanımlamalarına çok uymaktadır. 1980'lerde Alan Scott, şaşılık tedavisinde BTX'in kullanımı ile ilgili ilk makaleleri yayınlamıştır. Amerikan Gıda ve İlaç Dairesi (FDA), 1989'da strabismus (şaşılık) ve blefarospazm (kontrol edilemeyen göz kırpması) tedavisi için, 2002'de de ilk kozmetik indikasyon olarak kaş-arası dikey kırışıklıklarının tedavisi için ilk kez BTX'i onaylamıştır. O günden beri BTX, sadece dermatolojik değil aynı zamanda dermatolojik-olmayan pek çok indikasyonda, hem onaylı hem de onaysız olarak kullanılmaktadır. Bu derlemede botulinum toksininin etki mekanizmaları, farmakokinetiği, yan etkileri, indikasyon ve kontrindikasyonları, ilaç etkileşmeleri, etki yerleri ve etki süresi vb. pek çok açıdan farmakolojik profilini bulacaksınız. Ayrıca mevcut ticari ürünleri ve yeni dozaj formları ve aynı zamanda BTX'in gelecekteki kullanımı konusu da tartışılacaktır.
Anahtar kelimeler: Botulinum toksini; farmakoloji; zehir; çare; estetik; kas; ağrı.

Corresponding Author
Sorumlu Yazar
Kansu BÜYÜKAFŞAR
kansu23@yahoo.com

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INTRODUCTION

Botulinum toxin (BTX) is produced by autolysis of a gram-positive, spore-forming, rod-shaped anaerobic bacterium called as Clostridium botulinum. (1,2). BTXs have been known to cause food-borne poisoning for many years. The first cases were observed in eighteenth-century Europe, and the condition was termed "sausage poisoning" or botulism as "Botulus" means sausage in Latin (3). Depending on the type of illness caused by botulinum toxins, C. botulinum strains are divided into four different groups. Bacterial groups I and II are associated with the human illness, group III is associated with illness in animals, and group IV is not related to any illness (4). In chemical sense, BTX is a protein consisting of 7 related A-G toxins (serotypes). Each serotype can also be further divided into subtypes based on differences in amino acid sequence. BTX A, B, and F are produced by group I bacteria, and toxins B, E, and D are produced by group II bacteria (5). Botulinum toxin types A, B, and E have been identified as the most common neurotoxins causing human poisoning, whereas toxin types C and D are rarely associated with human toxicities; type F causes minimal human toxicity (2,6).

BTXs are ~150 kDa proteins composed of two major functional chains, i.e., a light chain (50kDa), and a heavy chain (100KaD), which are connected by a disulfide bond. The heavy chain is responsible for targeting the BTX to neuronal cell membrane. Each serotype binds via different mechanisms to different target receptors (4,7).

In the 1980's, Dr. Alan Scott first began publishing articles on the use of purified botulinum toxin for the treatment of strabismus (8). In 1989, the Food and Drug Administration of USA (FDA) first approved botulinum toxin for the treatment of blepharospasm. Then, in 2002, BTX received its first cosmetic indication for the treatment of dynamic rhytides of the glabella (9).

BOTULINUM TOXIN PRODUCTS

There are 4 most widely-used and commercially-available BTX products (Table 1): Botox® (OnabotulinumtoxinA, Allergan), Dysport® (AbobotulinumtoxinA, Ipsen), Xeomin® (IncobotulinumtoxinA, Merz) and Myobloc® (RimabotulinumtoxinB, Solstice Neuroscience).

All BTX-A clinical products currently available are dried-powder products that need to be reconstituted for use. Liquid formulation with different stabilizers and components, including the omission of excipients from animal origin and HSA is considered the next generation of toxin products (10).

PHARMACOLOGY OF BOTULINUM TOXIN

Mechanism of Action of Botulinum Toxin

Upon activation of nerve endings, acetylcholine-full vesicles move to the nerve membrane by the SNARE proteins (N-ethylmaleimide-sensitive factor attachment protein receptor) that modulate vesicular trafficking. These proteins are synaptobrevin, SNAP-25, and syntaxin (7,11), which form a complex followed by the fusion of synaptic vesicle and terminal membrane to lead acetylcholine release into the synaptic cleft. Then acetylcholine binds to its receptors in muscles to induce muscular contraction. Botulinum toxin irreversibly inhibits acetylcholine release at neuromuscular junctions. It binds to neuronal cell membrane and is internalized by receptor-mediated endocytosis. Acidification of the endosome induces a conformational change of BTX-A structure, resulting in the translocation of the enzymatic light chain into the cytosol, which acts like a protease and cleaves SNAP-25, (synaptosomal nerve-associated protein 25), a presynaptic protein that takes an essential role in exocytosis of neurotransmitters. All serotypes of botulinum toxin consist of a 150-kDa, single-chain progenitor toxin, which can be triggered by a protease to produce a 100-kDa heavy chain and a 50-kDa light chain. When the toxin is internalized into nerve cells, the interchain disulfide bond is broken, releasing the light chain possessing endopeptidase activity. This light chain specifically cleaves one of the three SNARE proteins involved in neurotransmitter release (2,12).

BTX blocks neuromuscular transmission in motor and autonomic nerve terminals by inhibiting the release of acetylcholine, resulting in a characteristic flaccid paralysis. BTX subtypes differ in cleavage SNARE complexes, e.g., BTX-A, BTX-C and BTX-E cleaves SNAP-25; however, BTX-B, BTX-D, BTX-F and BTX-G target synaptobrevin, the other important protein for exocytosis on the vesicle membrane. BTX-C also cleaves

Table 1. Some features of most widely-used commercially-available botulinum toxin preparations

	OnaA/BOTOX	AboA/Dysport	IncoA/Xeomin	RimaB/Myobloc
Approval	1989	1991	2005	2000
Pharmaceutical forms	Powder dissolved in injectable solution	Powder dissolved in injectable solution	Powder dissolved in injectable solution	Solution
Serotype	A1	A1	A1	B
Size	900 kDa	>500 kDa	150 kDa	700 kDa
Excipients	HSA (500 µg), NaCl	HSA (125 µg), Lactose	HSA (1 mg), Sucrose	HSA (500 µg/ml), NaCl, Na-succinate
pH value	≈7	≈7	≈7	5.6
Unit/vial	50, 100, 200	300, 500	100, 200	2500, 5000, 10000
Shelf life (months)	36	24	36	24
Toxin Quantity (ng/vial)	5	4.35	0.6	25, 50, 100
Re-constitution	0.9% NaCl	0.9% NaCl	0.9% NaCl	Prepared solution
Storage	2-8°C or <-5°C	2-8°C	Up to 25°C	2-8°C do not freeze

HSA: Human Serum Albumin, modified from Fonfria et al. (10).

syntaxin, another key protein to neurotransmitter release (Table 2, 13-18). Furthermore, a particular BTX types (i.e., BTX-C) could inactivate Rho proteins by ADP-ribosylation or by monoglycosylation. Hence BTX exerts diverse effects on biological system (19).

Site of Action of Botulinum Toxin

BTX mainly exerts its action on motor neurons innervating striated voluntary muscles. However, it has been demonstrated in cultured neuronal cells, that BTXs can also block the release of a wide variety of neurotransmitters but they are more potent in motor-neurons than in the other neuronal cell types (20). BTX-A also blocks ATP release from purinergic efferent nerves in the detrusor muscle. In afferent nerves, BTX-A injection markedly reduces the urothelial ATP release and increases nitric oxide (NO) release from the urothelium. Furthermore, BTX has also particular effects on smooth muscle reactivity and some other targets e.g., sensory afferents, whereby it induces an anti-nociceptive action via the inhibition of substance P release. Likewise, BTX can also attach to cell-surface proteins, such as E-cadherin, fibroblast growth factor receptor and vanilloid receptors. In addition to ATP, the toxin can also decrease the release of CGRP and NGF and, down-regulate TRPV1 expression. Through the inhibition of vasodilator substances such as acetylcholine, CGRP and substance-P, as well as the inhibition of muscle activity, BTX may cause the reduction of vasodilatation within the affected muscle. High dose (50 U/ml) of BTX-A has also been demonstrated to inhibit release of norepinephrine from sympathetic nerve endings (that's why it is effective in Raynaud syndrome) (21). Interestingly, the BTX receptors and intracellular targets are not unique for neurotransmission, as several of these receptors and targets have been found in non-neuronal cells. Affected structures by BTX are mainly:

1. The neuromuscular junction
2. Autonomic ganglia
3. Postganglionic parasympathetic nerve endings
4. Postganglionic sympathetic nerve endings that release ACh such as those in some of skeletal muscles vasculatures and eccrine sweat glands (Figure 1).
5. Non-neuronal targets

As for non-neuronal targets, BTX may also target SNAP-25-expressing non-neuronal cells such as epidermal keratinocytes, adipose-derived mesenchymal stem, nasal mucosal cells, urothelial cells, epithelial cells of intestine, prostate and alveoli, neutrophils, macrophages, dermal fibroblasts, sebocytes and vascular endothelial cells (6,15-17,22). Consequences of BTX action in these cells remain to be investigated in details.

Consequences of Action of Botulinum Toxin

Once the action of BTX prevails, the neurotransmission is blocked and the muscle may atrophy. BTX induces weakness of striated muscle. Transmission is also inhibited at neurons in muscle spindles, which may alter reflex overactivity. BTX also induces an anti-nociceptive action via the inhibition of substance P. In human skin, BTX is good for all wrinkles evoked by persistent muscular contractions. It also elicits antiaging activity on the skin by increasing skin collagen production, decreasing to slow down muscle shortening, improving skin barrier and hydration, reducing sebum production, so improves skin quality. BTX facilitate wound healing and decrease thickness of hypertrophic scars. It also decreases axillary or palmar hyperhidrosis (10). Recovery from the toxin effect can occur by sprouting of nerve endings, and formation of new synaptic clefts. Additionally, extrajunctional acetylcholine receptors have been reported to develop (6).

GENERAL INDICATIONS OF BOTULINUM TOXIN

BTXs are highly potent toxins responsible for a severe disease, called botulism. Nonetheless, these toxins are also efficient therapeutic tools with an increasing number of indications ranging from neuromuscular dysfunction to hypersecretion syndrome, pain relief, depression as well as cosmetic application. BTX was initially used for hyperactive movement disorders such as dystonia (24-26). There are several on-label uses of BTX (Table 3).

Table 2. Neuronal targets of BTX

BTX Type	Targets
BTX-A	SNAP-25
BTX-B	Synaptobrevin
BTX-C	SNAP-25 and Syntaxin
BTX-D	Synaptobrevin
BTX-E	SNAP-25
BTX-F	Synaptobrevin
BTX-G	Synaptobrevin

Table 3. On-label indications of BTX

Disorders	On-Label
Skeletal Muscles	Cervical dystonia Hemifascial spasm Blepharospasm Spasticity in adult and children
Smooth Muscles	Detrusor overactivity Neurogenic, idiopathic bladder overactivity
Exocrine Gland Hyperfunction	Sialorrhea Axillary hyperhidrosis
Pain-Related Disorders	Chronic migraine
Aesthetic	Glabellar lines Lateral canthal lines Front lines

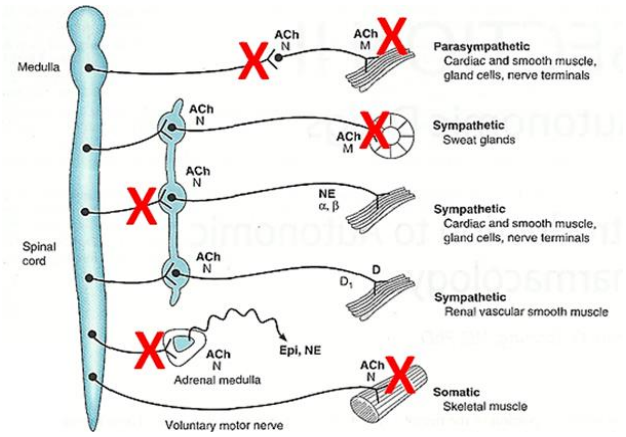

Figure 1. Site of action of botulinum toxin. BTXs act at neuromuscular junctions, autonomic ganglia, postganglionic parasympathetic nerve endings, postganglionic sympathetic nerve endings that release acetylcholine (e.g., eccrine sweat glands), modified from (23). **X** represents some of the potential inhibitory sites of BTX.

Reports of pain reduction in patients with dystonia led to studies of its use in treatment of chronic pain states (27). The pain reduction was initially assumed to be caused by muscle relaxation and a subsequent decompression of blood vessels and sensory nerves. Later experimental studies suggested that BTX may have a central effect on the nervous system (28-31). BTX has been used in the management of spasticity in the lower limbs (32). BTX serotype A (BTX-A) is now clinically used worldwide for the treatment of chronic migraine although the mode of action remains to be fully elucidated (33). In addition, intravesical BTX injection is effective in reducing urgency and urinary incontinence by inhibiting the detrusor muscle contraction through the blockade of acetylcholine from the preganglionic and postganglionic nerves in the efferent nerves. BTX-A also blocks ATP release from purinergic efferent nerves in the detrusor muscle. In afferent nerves, BTX-A injection markedly reduces the urothelial ATP release and increases nitric oxide (NO) release from the urothelium (34).

OFF-LABEL INDICATIONS OF BOTULINUM TOXIN

BTX can also be used in a wide variety of unapproved dermatological and non-dermatological indications such as, eye brow lifting, eye widening, gummy smile, jaw sculpting, Marionette lines, mental creases, dimpling of the chin, necklines and platysmal bands, perioral lines, masseter reduction, scarring, inflammatory skin diseases, alopecia, depression, chronic migraine, genodermatoses, pruritic diseases, hyperhidrosis, palmar hyperhidrosis, axillary bromhidrosis/chromhidrosis, other disorders of sweating, stump hyperhidrosis, pompholix, periorbital syringomas, eccrine angiomatosis, eccrine naevus, eccrine hydrocystoma, pruritic dermatoses, gustatory epiphora (crocodile tears), acantholytic disorders, genodermatoses (epidermolisis bullosa simplex and pachyonychia congenital), inflammatory dermatoses, pachydermoperiostosis pachydermia, wound healing, Raynaud phenomenon, persistent facial flushing/rosacea, spasticity, stroke, traumatic brain injury, cerebral palsy, multiple sclerosis, spinal cord injury, tics, chronic low back pain, disorders of localized muscle spasm and pain, tension headache, migraine headache, cervicogenic headache, achalasia cardia, Hirschsprung disease, Oddi sphincter dysfunction, chronic anal fissure, all types of dystonia, refractory vulvodynia, vestibulodynia, vaginismus, dyspareunia, menopausal hot flashes, rosacea nasal tip, anterior neck and chest flushing (6,10,35-39).

ADVERSE EFFECTS OF BOTULINUM TOXIN

Depending on its dose, site of injection, volume and inter-individual variations, there are numerous adverse effects of BTX. However, it seems to be quite safe in general when injected locally without excess dose. When it is used at excess doses it could, however, induce adverse events some of which would be serious. The following are common adverse effects of BTX: Allergic reactions, pain, edema, ecchymosis, and short-term hyperesthesia, bruising, infection (necrotizing fasciitis), delayed eyelid closure, brow ptosis, a decreased blink response, blepharoptosis, eye sensory disorders, excessive tearing and drooling, eyelid edema. In case of spreading of the toxin into the blood stream systemic side effects can be seen like generalized reactions such as nausea, malaise, flu-like symptoms and cutaneous eruptions. Headaches were the most frequent adverse events in the initial trial of BTX for the glabellar lines. Excess dose of onabotulinumtoxinA more than 400 U in a single session can be found safe and efficacious (40) but nevertheless caution must be taken according to the patient.

Some of the side effects of BTX seem likely due to the injection technique. When used at excessive doses, BTX may be expected to produce neuromuscular weakness with a variety of severe symptoms. In such cases, the affected individual should be medically supervised for several weeks for signs and symptoms of systemic muscular weakness which could be local or distant from the site of injection. Antitoxin raised against botulinum toxin is available but it will not reverse any botulinum toxin-induced effects already apparent by the time of antitoxin administration. (41,42).

CONTRINDICATIONS, PRECAUTIONS AND DRUG INTERACTIONS OF BOTULINUM TOXIN

Contrindications of BTX include hypersensitivity reactions to formulation (toxin or albumin), neuromuscular disease (myasthenia gravis, Lambert-Eaton syndrome, ALS, motor neuropathies), psychological instability, pregnancy, lactating and children under 12-years, existing inflammatory lesions, like acne or psoriasis at the injection site(s). Besides, the patients with unrealistic expectations, and those using too much daily facial expressions (public performers) can also be considered in the list of contrindications. Furthermore, BTX does not work for the wrinkles not caused by muscular contractions (41,42). Artists such as using wind instruments (e.g., side flute and clarinet etc.) or scuba-divers must be careful for the efficiency of lip contour muscles if they have been already applied by BTX to the lips-mouth area.

The use of some medications decreasing neuromuscular transmission should be avoided in patients treated with BTX. The drugs acting also on neuromuscular junction such as aminoglycoside-antibiotics, succinylcholine, curare-like neuromuscular-blockers and magnesium sulfate may increase effect of BTX. Some cholinergic medication (i.e., cholinesterase-inhibitors), penicillamine, quinine, chloroquine and hydroxychloroquine may reduce the toxin effect. Calcium channel blockers, antiplatelets and anticoagulants, NSAAI drugs (e.g., warfarin and aspirin) may cause bruising and delay in coagulation (41,42). In order to avoid any bruising effects of BTX application, gingko biloba extracts, red wine, supplements of vitamin E and fish oil need to be stopped 2 days before the BTX application. Furthermore, on the day of BTX-administration alcohol consumption should be avoided.

APPLICATION ROUTES OF BOTULINUM TOXIN

Although BTX is given by i.m. injection there are some intradermal, transdermal, intradetrusor, transurothelial, and transepithelial delivery forms. Furthermore liquid and slow-release BTX formulations have also been developed. Topical liposomal BTX cream has also been developed for axillary hyperhidrosis. The use of BTX iontophoresis enhanced its penetration (for axillary hyperhidrosis). Transdermal delivery of BTX via jet nebulization for

palmar, plantar, and axillary hyperhidrosis has also been tested. Topical formulation of BTX improves tolerability and adherence to therapy (10,43).

ACTION DURATION OF BOTULINUM TOXIN

The onset of action of BTX-A normally begins within 1-3 days; however, some individuals may necessitate as many as 5 days. However, peak effects could be generally obtained at around 10 days. The toxin's effects last about 3-4 months. Some subtypes of BTX have different action duration, e.g., BTX-E and BTX-F have significantly shorter duration of response (3-6 weeks), which are especially required, in orthopedics and rehabilitation-medicine. Much shorter duration may also be needed for some conditions like vaginismus. However, novel formulations and delivery techniques enabling longer action duration, such as transdermal delivery or other formulations with a longer duration of response (i.e., 6-9 months) are needed for clinical interest (6,10,44).

PHARMACOKINETICS OF BOTULINUM TOXIN

The effect of blood on the structure, function, and biologic half-life of the toxin was investigated but it was found that blood did not alter the structure, catalytic activity, and the neuromuscular blocking activity of the toxin. Experimental studies conducted on mice and rats have shown that the elimination half-life for native (non-metabolized) toxin in blood and serum was around 230-260 min when given with the doses that produced clinical poisoning. On the other hand binding of BTX to plasma albumin was not so high (25-30%) and majority of the circulating toxin is free (around 70-75%) and available for distribution to vulnerable nerve-endings. Binding of neutralizing antibodies to BTX has been demonstrated to enhance the clearance of toxin from the circulation and enhance the tissue accumulation of toxin, particularly in liver and spleen (45).

SPREADING OF BOTULINUM TOXIN FROM APPLICATION SITE

The observation that the reduction in the severity of spasticity may occur despite little neuromuscular blockade after the injection of BTX (46) and that the patients with blepharospasm or arm dystonia may experience a comfortable and sustained effect of BTX treatment in spite of the disappearance of local weakness (46-48) led the scientist implicate that the effects of BTX seems to be more than neuromuscular junction as the local action of BTX at the neuromuscular junction cannot explain whole effects of BTX (46). Accordingly, subsequent substantial evidence points out that i.m. BTX-A administration triggers alterations at the level of the central nervous system (13,46,49-53). One potential explanation for these findings is an indirect plasticity of central circuits following reduction of sensory input from the injected muscles (54). Presumably, similar to tetanus neurotoxin, BTX-A may be transported along nerve axons (53,55), followed by a process of cell-to-cell transfer by which the neurotoxin may gain access to second order neurons in the CNS (46,49). Several pathogens exploit this trafficking route to reach the CNS (56).

On the other hand BTX can also spread to surrounding and remote muscle from the injection site. It has been demonstrated that based on compound muscle action potential (CMAP) amplitude and/or cleaved SNAP-25 immunohistochemistry in remote muscles, all botulinum toxin products may spread from the area of injection. BTX-A has a potentially greater spread to nearby and remote non-injected muscles than BTX-B in mouse and primates. BTX-CD is the most prone to spread remotely; however, BTX-D is the least. Based upon safety index, the order was obtained: BTX-F > BTX-C > BTX-D > BTX-E > BTX-A > BTX-CD > BTX-B, with BTX-F being the safest. Some factors, e.g., volume, concentration, amount of toxin, formulation, serotype, needle-size, number of injections, precision of injection, biologic properties of the toxin, anatomy and tissue type of the target area, distribution of receptors, and SNARE proteins may influence spreading (Figure 2).

Interestingly, when ranked based upon safety index a different order was obtained: F > C > D > E > A > CD > B, with BTX-F being the safest. Therefore, some effects of BTX conflict with the expected benefits, hence, require further attention. The rank order of BTX serotypes based upon spread in a study in rats was CD > A > E > C > B > F > D. (10,36,37,57-59).

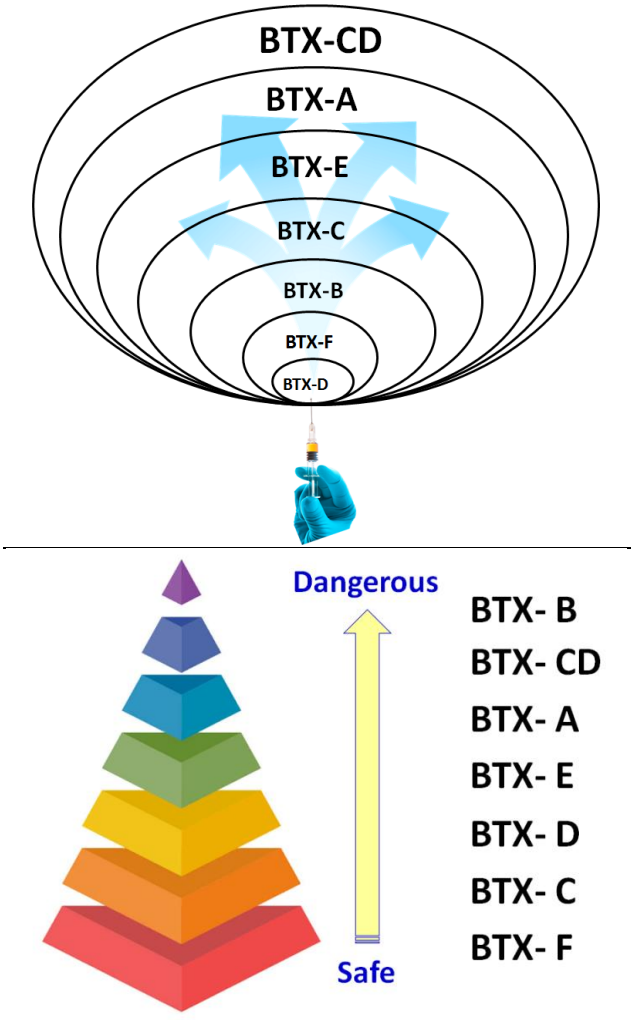

Figure 2. Spreading capacity (upper panel) and safety indexes (lower panel) of BTX. The most spreading BTX serotype is BTX-CD, the least BTX-D. As for safety, BTX-F seems to be the safest but BTX-B the most dangerous.

NOVEL BOTULINUM TOXIN PRODUCTS

Due to some disadvantageous features of BTX-A, more promising serotypes, e.g., biological and recombinant BTX-E products have already been developed for aesthetic and therapeutic indications. Another alternative to BTX-A, is serotype BTX-C, which induces similar efficacy and duration of action without secondary resistance after chronic use. This serotype was successfully tested in a pilot series of BTX-A-non-responsive patients with focal dystonia. The other promising subtype is BTX-A2, which seems to be more potent in vitro and in vivo than BTX-A1 due to its faster penetration to neuronal cells together with higher occupancy of the cellular receptors (SV2C). In addition, BTX-A2 has a less spreading and less immunogenic feature than BTX-A1, and it seems to be less susceptible to neutralization by human antisera raised to BTX-A1. However, accumulated data showing safety grade of BTX-A2 compared to BTX-A1 is needed (10).

NOVEL DELIVERY TECHNIQUES FOR BOTULINUM TOXIN

The injection of BTX can be painful for patients and needs the specific training. Therefore, novel formulations of needle-free delivery and slow or sustained release formulations would be of clinical interest. These may include transdermal delivery or other products with a longer duration of response. FDA approved BTX cream based on ionic nanoparticle technology. There are several advantages of the cream over conventional injection such as self-application comfort, elimination of painful and traumatic needle use. Especially for conditions such as hyperhidrosis, application can require multiple uncomfortable and painful subcutaneous injections in sensitive locations, such as the palm or axilla. Due to the biological barriers limiting successful penetration of the BTX preparations to the intended tissue, transdermal formulations will be for superficial delivery. However this kind of preparation is not suitable for movement disorders that requires deeper i.m. injections. However, physical or chemical permeabilization that enables to BTX preparations to penetrate deeper layers has been developed. Accordingly, Revance Therapeutics Inc have progressed this approach for transdermal delivery of BTX-A to the clinic for both lateral canthal lines and axillary hyperhidrosis (10,43).

ANTIBODY DEVELOPMENT AGAINST BOTULINUM TOXIN

One of the important issues in BTX use is antibody development against the toxin. Currently, in order to circumvent this problem several other serotypes of BTX than BTX-A or BTX-B have been explored as potential therapeutic agents in humans. BTX-E and BTX-F seem to be alternative serotypes in patients who had become resistant to BTX-A and found to be effective in dystonic patients (44,60,61).

CONCLUSIONS

Since the first approval of botulinum toxin for the treatment of strabismus blepharospasm in 1989, the use of BTX has been extending towards a wide variety of indications year by year. The pleiotropic effects of this toxin are likely due to the ubiquitous distribution of its targets within the body. Once the function and importance of these targets are further elucidated we most likely to see more common use of the toxin and the market size will be expanding.

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Can Hematological Parameters Predict the Severity of Acute Pancreatitis?

Hematolojik Parametreler Akut Pankreatitin Şiddetini Öngörebiliyor mu?

Nazım EKİN[1]
0000-0001-5302-8953
Eşref ARAÇ[1]
0000-0001-6041-3817
Songül ARAÇ[2]
0000-0001-6830-3639

[1]Gazi Yaşargil Education and Research Hospital Internal Medicine Clinic, Diyarbakır, Turkey

[2]Gazi Yaşargil Education and Research Hospital Emergency Medicine Clinic, Diyarbakır, Turkey

Corresponding Author
Sorumlu Yazar
Eşref ARAÇ
esrefarac@gmail.com

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ABSTRACT

Aim: Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), mean platelet volume (MPV) and erythrocyte distribution width (RDW) are considered to be associated with systemic inflammation. In this study, it was aimed to evaluate NLR, PLR, MPV and RDW as prognostic factors in acute pancreatitis (AP).

Material and Methods: A total of 315 patients admitted to Health Sciences University Gazi Yaşargil Training and Research Hospital between May 2016 and May 2019 and diagnosed with AP were included in the study. Data of the patients were analyzed retrospectively. Laboratory values of the patients at the time of admission to the hospital were recorded and the Ranson score was calculated. Patients were divided into two groups as Ranson score <3 (mild AP) and ≥3 (severe AP). NLR, PLR, MPV and RDW parameters were compared between these groups.

Results: Of the patients, 103 (32.7%) were males and 212 (67.3%) were females, and mean age was 57.2±19.5. According to the Ranson criteria, number of patients with a score below 3 was 274 (87.0%), and number of patients with a score of 3 or above was 41 (13.0%). In the severe AP group, NLR and PLR were significantly higher than in the mild AP group (16.2±14.3 vs. 8.2±7.7, p<0.001 and 283.7±223.0 vs. 195.5±139.3, p=0.004 respectively), but there was no statistically significant difference in terms of RDW-CV and MPV (13.7±1.0 vs. 13.9±1.9, p=0.849 and 9.7±1.3 vs. 9.5±1.1, p=0.201, respectively).

Conclusion: NLR and PLR are simple and safe tests that can be used to determine the severity of AP.

Keywords: Acute pancreatitis severity; Ranson criteria; neutrophil to lymphocyte ratio; platelet to lymphocyte ratio; erythrocyte distribution width; mean platelet volume.

ÖZ

Amaç: Nötrofil-lenfosit oranı (NLO), trombosit-lenfosit oranı (TLO), ortalama trombosit hacmi (MPV) ve eritrosit dağılım genişliğinin (RDW) sistemik inflamasyon ile ilişkili olduğu kabul edilir. Bu çalışmada, akut pankreatitte (AP) prognostik faktörler olarak NLO, TLO, MPV ve RDW değerlerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Mayıs 2016 ile Mayıs 2019 tarihleri arasında Sağlık Bilimleri Üniversitesi Gazi Yaşargil Eğitim ve Araştırma Hastanesine başvuran ve AP tanısı konulan toplam 315 hasta çalışmaya dahil edildi. Hasta verileri retrospektif olarak analiz edildi. Hastaların hastaneye başvuru zamanındaki laboratuvar değerleri kaydedildi ve Ranson skoru hesaplandı. Hastalar, Ranson skoru <3 (hafif AP) ve ≥3 (şiddetli AP) olanlar şeklinde iki gruba ayrıldı. Bu gruplar arasında NLO, TLO, MPV ve RDW parametreleri karşılaştırıldı.

Bulgular: Hastaların 103'ü (%32,7) erkek ve 212'si (%67,3) kadın olup yaş ortalaması 57,2±19,5 idi. Ranson kriterlerine göre skoru 3'ün altında olan hasta sayısı 274 (%87,0), skoru 3 ve üstünde olan hasta sayısı ise 41 (%13,0) olarak saptandı. Şiddetli AP grubunda NLO ve PLO, hafif AP grubuna göre anlamlı derecede yüksekti (sırasıyla 16,2±14,3'e karşı 8,2±7,7; p<0,001 ve 283,7±223,0 karşı 195,5±139,3; p=0,004), ancak RDW-CV ve MPV açısından istatistiksel olarak anlamlı bir farklılık yoktu (sırasıyla 13,7±1,0'e karşılık 13,9±1,9; p=0,849 ve 9,7±1,3'e karşı 9,5±1,1; p=0,201).

Sonuç: NLO ve PLO, AP şiddetini belirlemek için kullanabilecek olan basit ve güvenli testlerdir.

Anahtar kelimeler: Akut pankreatit şiddeti; Ranson kriterleri; nötrofil-lenfosit oranı; trombosit-lenfosit oranı; eritrosit dağılım genişliği; ortalama trombosit hacmi.

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease of the pancreas, which is characterized by abdominal pain and increased pancreatic enzymes and can cause local and systemic complications. It is one of the most common hospitalization reasons among gastroenterological diseases. It may course in a wide clinical range from mild pancreatitis with a mortality rate of 1.5% to severe pancreatitis with a mortality rate of 17%. The overall mortality rate is 5% (1). Therefore, determining the severity of the disease is very important in order to predict its prognosis. Many scoring systems, serum biomarkers and imaging methods are used in determining the severity and mortality of AP. Systemic Inflammatory Response Syndrome (SIRS), Ranson criteria, Bedside Index of Severity In Acute Pancreatitis(BISAP), Acute Physiology and Chronic Health Examination (APACHE) II, Harmless Acute Pancreatitis Score (HAPS), Glasgow score and BT severity indices are the scoring systems used (2-5). The fact that some of these scoring systems require at least 48 hours to complete, some scoring systems are complex and contain many parameters, and none of them have high precision in determining the severity of AP in a certain patient limit their usage. Among these scoring systems, APACHE II is recommended for the evaluation of AP and its use in patients other than intensive care patients is not very practical (6). Due to the insufficiency of these scoring systems, new biomarker searches have emerged that going to determine the severity and prognosis of AP. For this purpose, studies with platelet-lymphocyte ratio (PLR), mean platelet volume (MPV), neutrophil-lymphocyte ratio (NLR), red blood cell distribution width (RDW) and procalcitonin level have been performed in the literature. Severe pancreatitis with organ failure is thought to be caused by an uncontrolled systemic inflammatory response. The number of white cells, including neutrophil and lymphocyte count, is one of SIRS criteria and is a hematological test found in most AP scoring systems. While neutrophils advance SIRS and the inflammatory cascade in AP, lymphocyte reduction occurs in severe sepsis and is associated with poor results (7,8). The NLR may be more valuable than total White Blood Cell (WBC) or per se neutrophil and lymphocyte counts in predicting the course of inflammatory disease. PLR, RDW and MPV, which are also whole blood parameters, are associated with inflammatory diseases. In this study, we aimed to investigate the relationship of NLR, PLR, RDW and MPV with disease severity in patients diagnosed with AP.

MATERIAL AND METHODS

The data of patients who were admitted to Health Sciences University Gazi Yaşargil Training and Research Hospital between May 2016 and May 2019 and diagnosed with AP were retrospectively analyzed. Ethics committee approval was obtained from the ethics committee of Health Sciences University Gazi Yaşargil Training and Research Hospital with the number of 379 and dated 29.11.2019. Diagnosis of AP was made in the presence of two criteria among abdominal pain, an increase in amylase and/or lipase and imaging methods supporting the diagnosis. Patients aged sixteen and over were included in the study. Patients with concomitant cholangitis, cholecystitis, with an infection focus such as abscess, with chronic pancreatitis, pancreatic cancer history, hematological disease, who were receiving anti-inflammatory medication, antiviral medication, immunosuppressive medication, and who were not hospitalized while having diagnosed with AP in the emergency room, were not included in the study. The discharge reports, laboratory values and imaging tests of the patients were reached through the hospital information system. Whole blood, biochemistry parameters, and C-reactive protein (CRP) values at the time of appeal to the hospital of the patients were recorded. Patients' referral Ranson score [Biliary pancreatitis: WBC >18000 mm^3, age >70 years, lactate dehydrogenase (LDH) >400 U/L, glucose >220 mg/dL, aspartate aminotransferase (AST) >250 U/L; Non-biliary pancreatitis: WBC >16000 mm^3, age >55 years, glucose >200 mg/dL, AST >250 U/L, LDH >350 U/L] were calculated. Those with a Ranson score of <3 were considered as mild and those with ≥3 were considered as severe pancreatitis. NLR, PLR, RDW and MPV parameters were compared between these groups.

Statistical Analysis

Statistical analyses were performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) package program. In the evaluation of the data, as well as the descriptive statistics (mean, standard deviation, median, minimum, maximum, frequency and percentage), the distribution of variables were examined by Kolmogrov-Smirnov normality test, and Independent samples t-test was used for comparison of two groups of variables with normal distribution, and Mann Whitney U test was used for comparison of two groups of variables without normal distribution. Chi-square test was used for comparison of qualitative data, and Pearson and Spearman correlation tests were used as the correlation analysis. The results were evaluated at $p<0.05$ significance level.

RESULTS

A total of 315 patients diagnosed with AP were included in this study. Of these patients, 103 (32.7%) were male and 212 (67.3%) were female. The mean age of all patients was determined as 57.2±19.46 (range, 16-97) years. The etiological factors determined in this study are given in Table 1. A total of 4 patients (1.3%) died due to the disease, and the mean age of these patients was 75.7±5.5 years. In the mild AP group, the number of cases was 274 (87.0%, 184 female and 90 male) and the mean age was 54.8±19.0 years. In the severe AP group, the number of

Table 1. Distribution of the patients according to etiology of acute pancreatitis, n=315

Etiology	n	%
Gallstone	247	78.4
Idiopathic	53	16.8
PostERCP	6	1.9
Hyperlipidemia	3	1.0
Azathioprine	1	0.3
Fasiola Hepatica	1	0.3
Hypercalcemia	1	0.3
Ketoacidosis	1	0.3
Drug dependent	1	0.3
Weil's Disease	1	0.3

cases was 41 (13.0%, 28 female and 13 male) and the mean age was 72.4±14.2 years. In the severe AP group, the mean age was statistically significantly higher (p<0.001). In the mild AP group, necrosis was found in 7 cases and mortality was found in 3 cases. Mortality was detected in 1 patient in the severe AP group, whereas necrosis was not detected. There was no significant difference in necrosis and mortality between the two groups (p=0.301 vs p=0.473, respectively). Initial laboratory findings of mild and severe AP patients are summarized in Table 2. WBC (p=0.001), neutrophil (p<0.001), glucose (p<0.001), ALT (p<0.001), AST (p<0.001), LDH (p<0.001), bilirubin (p=0.008), NLR (p<0.001) and PLR (p=0.004) were significantly higher in the severe AP group than in the mild AP group. CRP1 (p=0.687), CRP2 (p=0.932), CRP3 (p=0.932), Hct(%) (p=0.693), Hgb (p=0.119), MCV (p=0.171), MPV (p=0.201), urea (p=0.619), creatinine (p=0.070), albumine (p=0.068), PLT (p=0.673) and RDW-CV (p=0.849) did not differ significantly between the groups. Lymphocyte was significantly higher in the mild AP group than in the severe AP group (p=0.001). Correlation analysis was performed between NLR, PLR, RDW, MPV and Ranson score (Table 3). While positive correlation was found between NLR and PLR and the Ranson score (r=0.236, p<0.001, and r=0.163, p=0.004, respectively), no correlation was found between MPV and RDW and the Ranson score (r=0.720, p=0.201 and r=-0.010, p=0.855, respectively).

DISCUSSION

Early detection of the severity of the AP and early therapeutic interventions to decrease morbidity and mortality rates are extremely important. Estimating the severity of AP is still difficult, especially at an early stage and poses a challenge for clinicians. It has been stated that there is a 4-fold increase in the risk of death in patients with a 24-hour delay in admission (9). Due to the stated causes, and deficiencies of existing scoring systems, new biomarker searches that going to determine the severity and prognosis of AP have emerged. In our study, AP severity and age, WBC, lymphocyte count, neutrophil count, NLR, PLR, glucose, AST, ALT and LDH levels were found to be associated. As is known, age, glucose, WBC, AST and LDH are the parameters used in Ranson scoring.

It has been shown in various studies that NLR which is obtained by dividing the number of neutrophils by the number of lymphocytes, increases in many diseases that have a course with inflammation and is effective in predicting the prognosis (7,8,10-14). In the study of Zahorec et al. (8), it has been reported that NLR is an inflammatory and stress parameter which is simple, fast, cheap and can be used routinely in critical patients. There are studies in the literature investigating the relationship between NLR and AP. In a performed study, it has been suggested that NLR is superior to total WBC in predicting the negative results of AP, and the value of NLR >4.7 should be used in determining the severity of the disease (15). In another study, it has been stated that the increase of NLR in patients appealed with AP can effectively distinguish between mild and severe AP. It also has been reported in this study that NLR represents a dynamic process, returns to normal in those whose clinic is stabilized, however, the highness continues in patients with complicated AP (16). In another study, NLR in the severe AP group was found to be significantly higher than in the mild AP group (17). In our study, we compared with the Ranson score of NLR and found that NLR was significantly higher in the severe disease group. Our conclusion supports the literature.

Platelets have an important role in coordinating inflammation and immune response. They affect inflammation by releasing cytokines, changing leukocyte and endothelial responses (18). PLR has been studied in various diseases (19-21). In some cancers, PLR has been shown to be a superior predictive factor compared to NLR (22). The predictive effect of PLR in AP has been less studied compared to NLR. In one study, it has been stated that PLR significantly increased in severe pancreatitis (23), while in another study, PLR rate in biliary pancreatitis has only been detected to show disease severity (24). In our study, regardless of etiology, we found PLR to be significantly higher in the severe pancreatitis group. However, there was no alcohol in etiology of the patients included in the study.

Another biomarker studied in AP is MPV. There are contradictory results in the literature regarding the place of MPV in AP. In some studies, MPV has been found to be significantly lower in patients with AP (25,26), while in another study they have reported that MPV was significantly higher in patients with acute edematous pancreatitis than in controls (27). However, in our study, the values of patients with AP were measured at the time of admission, and the control values were not examined. But, no significant difference in MPV level between mild and severe pancreatitis groups was detected.

One of the whole blood parameters investigated in AP is RDW. There are studies that provide opposing views regarding RDW. There are some studies stating that RDW is a biomarker associated with mortality and morbidity in AP (28), while in others it was found as unrelated (29). In our study, there was no significant difference found in RDW levels.

The facts that the study was retrospective, single-centered, and the Ranson score was not calculated after 48 hours by only taking the Ranson criteria as the basis, were the limitations of our study.

As a result, we determined that NLR and PLR are independent predictive parameters in determining the severity of AP. Also, we found that MPV and RDW could not determine the severity of the disease. We believe that NLR and PLR, which can be examined daily due to being easy, fast and having low cost and that obtained from the proportion of whole blood parameters, are acceptable for the clinician to use in determining the severity of AP.

Table 3. Correlation of parameters with Ranson score

	r	p
NLR	0.236	**<0.001**†
PLR	0.163	**0,004**†
MPV (fL)	0.720	0.201††
RDW-SD (%)	-0.039	0.496††
RDW-CV (%)	-0.010	0.855††

NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, MPV: Mean platelet volume, RDW: Erythrocyte distribution width, †: Spearman correlation, ††: Pearson correlation

Table 2. Comparison of patient groups according to acute pancreatitis severity

	Group	n	Mean±SD	Median	Q1-Q3	Min-Max	p
Age (years)	Mild AP	274	54.9±19.1	55.0	40-69	16-94	<0.001*
	Severe AP	41	72.4±14.2	75	67-81.5	32-92	
CRP 1st day (mg/L)	Mild AP	258	34.5±51.9	11.3	5.4-50.7	0.5-385.4	0.687†
	Severe AP	40	49.8±88.7	11.9	5.4-53.9	1-385.4	
CRP 2nd day (mg/L)	Mild AP	215	70.9±83.4	38.2	13.3-99.9	1.3-406.3	0.932†
	Severe AP	30	81.7±111.6	27.7	12.7-92.4	3.1-406.3	
CRP 3rd day (mg/L)	Mild AP	165	110.2±101.8	85.1	25.6-168.9	0.9-445	0.932†
	Severe AP	25	121.1±103.2	93.5	42-185.5	11.6-383.5	
WBC (10^3/uL)	Mild AP	274	12.6±5.1	11.5	9.2-15.0	3.2-45.4	0.001*
	Severe AP	41	15.5±5.1	14.5	10.8-19.9	7.6-27.9	
Htc (%)	Mild AP	274	42.0±5.3	41.5	38.5-45.5	22.2-61.1	0.693*
	Severe AP	41	42.3±5.6	43	38.8-46.8	26.8-50.1	
Hgb (g/dL)	Mild AP	274	13.6±1.9	13.4	12.3-14.9	7-19.3	0.119*
	Severe AP	41	14.1±2.7	13.7	12.7-15.3	10-26.8	
Neu (10^3/uL)	Mild AP	274	10.1±4.9	9	6.6-12.9	2.4-37.7	<0.001†
	Severe AP	41	13.5±5.2	13	8.7-17.7	6.3-25.8	
Lym (10^3/uL)	Mild AP	274	1.8±1.2	1.6	1.1-2.3	0.3-10.3	0.001†
	Severe AP	41	1.3±0.8	1	0.7-1.8	0.3-4.5	
MCV (fL)	Mild AP	274	88.2±7.1	88.8	84.7-92.3	60.2-128.6	0.171*
	Severe AP	41	89.8±4.4	89.3	87.2-92.5	78.6-98.6	
MPV (fL)	Mild AP	274	9.5±1.1	9.4	8.6-10.2	7.2-13	0.201*
	Severe AP	41	9.7±1.3	9.4	8.8-10.9	7.5-12.5	
PLT (10^3/uL)	Mild AP	274	265.1±75.9	256	207-314	101-563	0.673*
	Severe AP	41	258±73.8	255	203-288.5	127-492	
RDW-SD (%)	Mild AP	274	41.8±4.0	41.2	39.4-43.5	33.7-73.3	0.497*
	Severe AP	41	41.4±3.2	40.8	39.5-42.1	36.9-52.8	
RDW-CV (%)	Mild AP	274	13.9±1.9	13.6	13.1-14.5	11.9-21	0.849*
	Severe AP	41	13.7±1.0	13.4	13.2-14.1	12.1-17	
Glucose (mg/dl)	Mild AP	257	138.6±62.5	127	106-156	67-441	<0.001†
	Severe AP	39	197.3±101.1	146	125-221	100-532	
Urea (mg/dL)	Mild AP	257	38.9±37.5	31	24-41	11-207	0.619†
	Severe AP	40	40.1±27.1	32	25-43.8	19-160	
Creatinine (mg/dL)	Mild AP	258	0.9±0.5	0.8	0.7-1	0.1-3.7	0.070†
	Severe AP	40	1.1±0.7	0.8	0.7-1	0.6-3.7	
ALT (GPT) U/L	Mild AP	257	203.9±216.6	129	43-287	2-1503	<0.001†
	Severe AP	40	346.1±257.7	305.5	177.3-463.8	5-1177	
AST (GOT) U/L	Mild AP	257	200.6±199.3	151	57-274.5	2-1542	<0.001†
	Severe AP	40	430.4±283.8	378	291.3-517.3	1-1300	
LDH (U/L)	Mild AP	253	366.5±295.6	300	244-388	11-3129	<0.001†
	Severe AP	40	550.2±200.9	508.5	439-653.8	11-1056	
Albumine (g/L)	Mild AP	102	3.9±0.6	3.8	3.5-4.2	2.6-5.7	0.068†
	Severe AP	16	4.2±0.6	4	3.7-4.6	3.4-5.7	
Bilirubin (mg/dL)	Mild AP	262	1.7±1.8	1	0.6-2.2	0.2-15.5	0.008†
	Severe AP	39	2.0±1.5	1.81	0.9-2.7	0.3-6.5	
NLR	Mild AP	274	8.2±7.7	5.4	3.4-10.1	0.6-51.0	<0.001†
	Severe AP	41	16.2±14.3	10.8	5.7-25.5	2.7-63	
PLR	Mild AP	274	195.5±139.3	163.7	108.1-238.2	22.4-958.1	0.004†
	Severe AP	41	283.7±223.0	204.7	142.3-388.3	57.8-1142.9	

AP: acute pancreatitis, SD: Standard deviation, Q1: 1st Quartile, Q3: 3rd Quartile, Min: Minimum, Max: Maximum, *: Independent samples t-test, †: Mann Whitney U test, CRP: C-Reactive Protein, WBC: White blood cell, Htc: Hematocrit, Hgb: Hemoglobin, Neu: Neutrophil, Lym: Lymphocyte, MCV: Mean platelet volume, PLT: Platelet, RDW: Erythrocyte distribution width, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio

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Evaluation of the Indicators of Inflammation in Children and Adolescents with Attention Deficit and Hyperactivity Disorder: Effect of Sex and Subtype

Dikkat Eksikliği ve Hiperaktivite Bozukluğu Olan Çocuk ve Ergenlerde İnflamasyon Göstergelerinin Değerlendirilmesi: Cinsiyet ve Alt Tipin Etkisi

Gülen GÜLER AKSU[1]
0000-0001-9555-3916
Pelin DAĞ[2]
0000-0002-5051-3459

[1]Mersin University Medical Faculty Department of Child and Adolescent Psychiatry, Mersin, Turkey

[2]Mersin City Training and Research Hospital Department of Child and Adolescent Psychiatry, Mersin, Turkey

Corresponding Author
Sorumlu Yazar
Gülen GÜLER AKSU
dr.gulen@hotmail.com

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ABSTRACT

Aim: It was aimed to evaluate the hematological inflammatory markers in treatment-naive and comorbidity-free children and adolescents with attention deficit and hyperactivity disorder (ADHD) in this study.

Material and Methods: One hundred sixty-nine children aged 6-18, who were diagnosed with ADHD according to DSM-5 criteria were included in the study. Age and sex-matched 59 healthy children without any psychiatric and/or medical disorder were included as a control group. The children who had an intellectual disability and/or autism spectrum disorder, acute, chronic or inflammatory diseases were excluded from the study. Smoking, obesity and using psychotropic medications and lack of data in records were other exclusion criteria. ADHD and control groups were compared in terms of sociodemographic characteristics, inflammatory markers and hematological parameters.

Results: Mean platelet volume (MPV) and Basophil (BASO) levels were significantly higher in the ADHD group compared to the control group and this statistical difference was only observed for boys. In hyperactivity subtype, red cell distribution width (RDW), lymphocyte (LYMPH) and monocytes (MONO) were higher; in attention deficit subtype mean platelet volume-to-lymphocyte ratio (MPVLR) was higher than all other subtypes and control group. MPV was similar in three subtypes, and were higher in all of them than the control group.

Conclusion: This study revealed that MPV and BASO tend to be higher in the ADHD group especially in boys. Hematological biomarkers may be useful for diagnosis of ADHD and determination of ADHD subtypes but data on this subject are insufficient and more comprehensive studies are needed.

Keywords: ADHD; biomarkers; child; inflammation; sex.

ÖZ

Amaç: Bu çalışmada, tedavi almayan ve komorbiditesi olmayan dikkat eksikliği hiperaktivite bozukluğu (DEHB) tanılı çocuk ve ergenlerde hematolojik inflamatuar biyobelirteçlerin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışmaya DSM-5 kriterlerine göre DEHB tanısı alan 6-18 yaş arası 169 çocuk dahil edildi. Yaş ve cinsiyet açısından eşleştirilmiş psikiyatrik ve/veya tıbbi hastalığı olmayan 59 sağlıklı çocuk kontrol grubu olarak alındı. Anlıksal yeti yitimi ve/veya otizm spektrum bozukluğu, akut, kronik veya inflamatuar bir hastalığı olan çocuklar çalışma dışı bırakıldı. Sigara kullanımı, obezite, psikotrop ilaçların kullanımı ve kayıt bilgilerinin yetersiz olması diğer dışlama kriterleri idi. DEHB ve kontrol grupları sosyodemografik özellikler, inflamatuar belirteçler ve hematolojik parametreler açısından karşılaştırıldı.

Bulgular: Ortalama platelet volümü (MPV) ve bazofil (BASO) düzeyleri DEHB grubunda kontrol grubuna göre anlamlı düzeyde yüksek idi ve bu fark sadece erkeklerde görüldü. Hiperaktif grupta eritrosit dağılım genişliği (RDW), lenfosit (LYMPH)ve monosit (MONO) daha yüksekti; dikkat eksikliği grubunda ortalama platelet volümü/lenfosit oranı (MPVLR) diğer tüm alt gruplardan ve kontrol grubundan daha yüksek idi. MPV ise üç alt grupta benzerdi ve bu grupların tümünde kontrol grubundan daha yüksekti.

Sonuç: Bu çalışma MPV ve BASO düzeylerinin DEHB grubunda, özellikle erkek çocuklarda daha yüksek olma eğiliminde olduğunu ortaya koymaktadır. Hematolojik biyobelirteçler DEHB tanısında ve DEHB alt tiplerinin belirlenmesinde faydalı olabilir ancak bu konudaki veriler henüz yetersizdir ve daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: DEHB; biyobelirteçler; çocuk; inflamasyon; cinsiyet.

INTRODUCTION

Attention deficit and hyperactivity disorder (ADHD) is one of the neurodevelopmental disorders of childhood (1). The estimated worldwide prevalence of ADHD is 5.3% in children and adolescents. The frequency of ADHD declines with age but can persist into adulthood, affecting between 2.5 and 4.4% of adults (2). The prevalence of ADHD is %12.4 in a cross-sectional multicenter nationwide study in Turkey (3). Genetic, psychological, social, biochemical and environmental factors are known to be involved in the etiology of ADHD but the etiology has not yet been fully elucidated. In addition to very strong genetic evidence, the importance of gene-environment interaction in the etiology of ADHD is emphasized (4-6). Environmental factors are thought to cause risk for ADHD by causing inflammation in the prenatal brain and decreasing gray matter volume (7).

In recent years, the interaction between the immune system and the central nervous system has been one of the most important research topics. The immune system is known to have significant effects on learning, memory and neural plasticity (8). The effect of the immune system on neurobiology of ADHD may be due to the disruption of the balance between inflammatory and anti-inflammatory mechanisms (9). In the studies increased IL-6, IL-10 (10), anti-purkinje cell antibodies (10), dopamine transporter protein autoantibodies (11), increased pro-inflammatory cytokines such as TNF-β and decreased anti-inflammatory cytokines such as IL-2, IL-4, and INF-y (12) are some findings which indicate inflammation response in ADHD. Also, children whose mothers have immune system related diseases such as multiple sclerosis, type 1 diabetes, hypothyroidism, rheumatoid arthritis have an increased risk for ADHD (13). Inflammatory cytokines lead to ADHD by affecting prefrontal cortex maturation, neurotransmitter composition and peripheral inflammation also lead to ADHD by increasing excitability, and microglial activation through TNF-α (14-15).

While these inflammatory markers are investigated especially in studies, they cannot be used in clinical practice because they are expensive. Inexpensive, cost-effective, easily accessible hematological markers of inflammation derived from complete blood count test such as mean platelet volume (MPV), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), red cell distribution width (RDW), mean platelet volume-to-lymphocyte ratio (MPVLR) have become increasingly noticeable lately.

Although hematological markers of inflammation are studied in different psychiatric disorders, consistent data on ADHD is still insufficient. We aimed to evaluate the hematological markers of inflammation of treatment-naive and comorbidity-free children and adolescents with ADHD in this study.

MATERIAL AND METHODS

One hundred sixty-nine children aged 6-18, who admitted to Child and Adolescent Psychiatry Department of Mersin University Medical Faculty between 31.06.2018 and 31.12.2018 and diagnosed as ADHD according to Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5, 1) criteria participated in this study. Comorbid psychiatric disorders were screened and while oppositional defiant disorder (ODD) is included, all other psychiatric disorders are excluded.

Ethical approval was obtained for this study and that participation involved informed consent (Mersin University Institutional Review Board protocol approval date 23/01/2019, number 2019/39).

Exclusion criteria of the research were: 1) to have an intellectual disability and/or autism spectrum disorder according to DSM-5 criteria; 2) to have an acute, chronic or inflammatory disease; 3) smoking; 4) obesity; 5) to use psychotropic medication; and 6) to have missing data in digital system records.

The control group consisted of age and sex-matched 59 children who admitted to our clinic for advice and did not have any psychiatric disorder according to DSM-5. Sociodemographic data such as age, sex, and parental consanguinity, medical and mental psychopathology of parents, delivery time/type, and accompanying medical comorbidity were analyzed.

MPV, NLR, monocytes/lymphocyte ratio (MLR), PLR and RDW values and some other hematological parameters (hemoglobin (HGB), red blood cell (RBC), mean corpusculer volume (MCV), mean corpusculer hemoglobin (MCH), mean corpusculer hemoglobin concentration (MCHC), platelet (PLT), etc.) calculated from complete blood count in the digital record system of our hospital.

Sociodemographic characteristics, inflammatory markers, and hematological parameters were compared between the ADHD group and the control group.

Statistical Analysis

Normality assumption of continuous variables was evaluated with the Kolmogorov-Simirnov test, while Levene test was used for homogeneity of variances. Independent samples t test was used to compare two groups, while One-Way Analyze of Variance followed by Fisher LSD post hoc test was used for three or more groups. Receiver operating characteristic (ROC) curve analysis was used to determine the cut-off values for discriminate the groups. In the analysis of categorical variables, Pearson chi-square or Fisher's exact test in case of expected value less than 5, were used. The statistical significance level was taken as 0.05, and statistical analyses were done with SPSS v.20 statistical package.

RESULTS

This study consisted of 169 ADHD patients (39 girls, 130 boys) and 59 healthy children (11 girls, 48 boys) with a mean age of 9.68±2.98 and 10.33±3.15 years, respectively. There was no statistically significant difference between groups in terms of age (p=0.159), and sex (p=0.479). While the parental consanguinity was higher in the ADHD group (p=0.027), spontaneous vaginal delivery rate was higher in the control group (p=0.033) Sociodemographic characteristics and comparison of ADHD and control groups were given in Table 1.

Among inflammatory markers, MPV was found higher in the ADHD group than the control group (p<0.001); NLR, PLR, MLR, MPVLR did not differ between the groups. Among complete blood count parameters, only the basophil (BASO) level was higher in the ADHD group than the control group (p=0.037, Table 2).

Table 1. Sociodemographic characteristics of ADHD and control groups

	ADHD (n=169)	Control (n=59)	p
Age, years	9.68±2.98	10.33±3.15	0.159
Sex			
Girl	39 (23.1%)	11 (18.6%)	0.479
Boy	130 (76.9%)	48 (81.4%)	
Mother medical disease	19 (11.2%)	4 (6.8%)	0.327
Mother psychiatric disease	13 (7.7%)	3 (5.1%)	0.573
Father medical disease	10 (5.9%)	3 (5.1%)	0.999
Father psychiatric disease	4 (2.4%)	1 (1.7%)	0.999
Parental consanguinity	32 (18.9%)	4 (6.8%)	**0.027**
Spontaneous vaginal delivery	73 (43.2%)	35 (59.3%)	**0.033**
Delivery Time			
Term	148 (87.6%)	56 (94.9%)	0.413
Preterm	20 (11.8%)	3 (5.1%)	
Postterm	1 (0.6%)	0 (0.0%)	
Delivery Complication			
Absent	160 (94.7%)	58 (98.3%)	0.724
Breech delivery	1 (0.6%)	0 (0.0%)	
Cord entanglement	4 (2.4%)	0 (0.0%)	
Meconium aspiration	4 (2.4%)	1 (1.7%)	

ADHD: attention deficit and hyperactivity disorder

Table 2. Complete blood count parameters and inflammatory markers of ADHD and control groups

	ADHD (n=169)	Control (n=59)	p
HGB (g/dl)	12.85±0.99	12.97±1.16	0.446
HCT (%)	37.88±3.75	36.95±5.19	0.143
MCV (fL)	78.11±4.79	77.98±5.74	0.874
MCH (pg)	26.79±4.60	26.82±2.51	0.967
MCHC (gHb/dl)	33.86±1.29	34.26±1.45	0.064
RDW (%)	13.22±1.03	13.48±1.26	0.113
PDW (fL)	11.75±1.92	11.51±1.97	0.429
MPV (fL)	10.25±0.92	9.65±1.01	**<0.001**
WBC (10^3/uL)	7.79±2.18	7.71±2.01	0.804
RBC (10^6/uL)	4.95±0.83	4.87±0.43	0.466
PLT (10^3/uL)	328.05±71.60	332.02±87.56	0.731
NEUT (10^3/uL)	3.87±1.66	3.85±1.52	0.916
LYMPH (10^3/uL)	3.00±0.93	3.01±1.03	0.925
MONO (10^3/uL)	0.61±0.20	0.59±0.17	0.545
EO (10^3/uL)	0.25±0.19	0.24±0.18	0.526
BASO (10^3/uL)	0.06±0.08	0.04±0.03	**0.037**
NLR	1.39±0.75	1.38±0.65	0.864
PLR	117.06±34.40	118.19±45.85	0.842
MLR	0.22±0.09	0.21±0.10	0.732
MPVLR	3.75±1.22	3.56±1.23	0.312

ADHD: attention deficit and hyperactivity disorder, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, PDW: platelet distribution width, MPV: mean platelet volume, WBC: white blood cell, RBC: red blood cell, PLT: platelet, NEUT: neutrophil, LYMPH: lymphocyte, MONO: monocytes, EO: eosinophils, BASO: basophils, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, MLR: monocytes-to-lymphocyte ratio, MPVLR: mean platelet volume-to-lymphocyte ratio

MPV (p<0.001) and BASO (p=0.013) were significantly higher in boys of ADHD group than in boys of the control group, but these markers did not differ significantly in girl patients (Table 3).

Table 3. Complete blood count parameters and inflammatory markers of ADHD and control groups

	Sex	ADHD (n=169)	Control (n=59)	p
HGB (g/dl)	Girl	12.78±1.00	12.63±1.28	0.673
	Boy	12.87±0.99	13.05±1.12	0.309
HCT (%)	Girl	37.88±2.62	35.15±7.03	0.234
	Boy	37.88±4.04	37.37±4.66	0.470
MCV (fL)	Girl	79.59±3.84	76.35±9.26	0.281
	Boy	77.66±4.97	78.36±4.66	0.398
MCH (pg)	Girl	26.89±1.79	25.74±3.95	0.364
	Boy	26.76±5,16	27.07±2.03	0.691
MCHC (gHb/dl)	Girl	33.79±1.07	33.38±1.49	0.315
	Boy	33.88±1.36	34.46±1.38	**0.013**
RDW (%)	Girl	13.03±0.95	13.53±1.91	0.421
	Boy	13.27±1.05	13.47±1.08	0.275
PDW (fL)	Girl	11.78±1.96	12.39±2.02	0.372
	Boy	11.74±1.92	11.32±1.93	0.195
MPV (fL)	Girl	10.33±0.96	10.15±1.10	0.585
	Boy	10.22±0.91	9.54±0.97	**<0.001**
WBC (10^3/uL)	Girl	8.12±2.15	8.02±2.41	0.891
	Boy	7.69±2.19	7.64±1.93	0.886
RBC (10^6/uL)	Girl	5.02±1.55	4.96±0.35	0.903
	Boy	4.93±0.42	4.84±0.44	0.253
PLT (10^3/uL)	Girl	331.36±65.63	337.36±82.16	0.801
	Boy	327.06±73.51	330.79±89.54	0.778
NEUT (10^3/uL)	Girl	4.21±1.84	4.42±1.77	0.743
	Boy	3.77±1.60	3.72±1.45	0.835
LYMPH (10^3/uL)	Girl	2.93±1.02	2.72±0.85	0.534
	Boy	3.02±0.91	3.08±1.06	0.708
MONO (10^3/uL)	Girl	0.63±0.19	0.56±0.15	0.274
	Boy	0.60±0.20	0.59±0.18	0.892
EO (10^3/uL)	Girl	0.25±0.23	0.20±0.21	0.549
	Boy	0.26±0.18	0.24±0.18	0.694
BASO (10^3/uL)	Girl	0.07±0.14	0.03±0.02	0.413
	Boy	0.05±0.05	0.04±0.03	**0.013**
NLR	Girl	1.59±0.84	1.73±0.75	0.634
	Boy	1.34±0.72	1.30±0.61	0.733
PLR	Girl	122.30±34.01	135.39±58.93	0.349
	Boy	115.48±34.48	114.25±42.08	0.843
MLR	Girl	0.23±0.09	0.22±0.10	0.717
	Boy	0.21±0.09	0.21±0.10	0.903
MPVLR	Girl	3.95±1.40	4.13±1.49	0.706
	Boy	3.69±1.16	3.43±1.14	0.186

ADHD: attention deficit and hyperactivity disorder, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, PDW: platelet distribution width, MPV: mean platelet volume, WBC: white blood cell, RBC: red blood cell, PLT: platelet, NEUT: neutrophil, LYMPH: lymphocyte, MONO: monocytes, EO: eosinophils, BASO: basophils, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, MLR: monocytes-to-lymphocyte ratio, MPVLR: mean platelet volume-to-lymphocyte ratio

When ADHD subtypes and control group were compared, statistically significant differences were detected in terms of RDW (p=0.003), LYMPH (p=0.016), MONO (p=0.048), MPV (p<0.001) and MPVLR (p=0.004). According to the results of post hoc analyses RDW, LYMPH and MONO were higher in hyperactivity subtype than all other subtypes and control group, while MPV was similar in three subtype and three of them were higher than control group. Besides, MPVLR was higher in attention deficit subtype than all other subtypes and control group (Table 4).

ROC curve analysis was performed to assess the diagnostic value of MPV and BASO (Figure 1). The area under the curve for MPV was 0.662 (95% CI, 0.581 to 0.742, p<0.001), and a significant but weak cut off value was 9.75, with a sensitivity of 71.6% and specificity of 55.9%, respectively. The area under the curve for BASO was 0.656 (95% CI, 0.596 to 0.744, p<0.001) with a significant but weak cut-off value of 0.04 with a sensitivity of 70.4% and a specificity of 54.2%, respectively.

DISCUSSION

This study on the usability of hematological parameters as inflammatory markers obtained from the complete blood count indicated that MPV and BASO were significantly higher in the ADHD group compared to the control group and this difference was only valid for boys. This study indicated that RDW and MONO could be differentiating parameters for hyperactivity subtype and MPVLR for attention-deficit subtype.

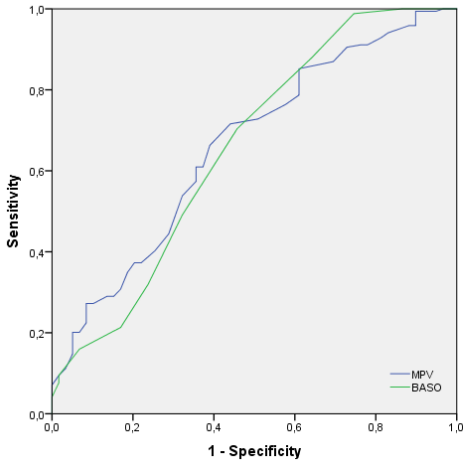

Figure 1. ROC curve analysis performed to assess the diagnostic value of MPV and BASO

Although there are studies in the literature on inflammatory hematological markers in ADHD, there is no study examining these markers according to sex and ADHD subtypes. There are different results in the studies investigating the relationship between ADHD and inflammation in the literature.

In cytokine studies, IL-6 levels were found to be higher in ADHD patients, but not correlated with ADHD severity (16). In another study, IL-6, IL-10, and anti-YO antibodies reported to be significantly higher in ADHD (10). On contrary to these findings, it suggested that ADHD symptomatology is not associated with pro-inflammatory cytokines such as IL-6, TNF-alpha and BDNF (17). Studies are on the rise about easy, inexpensive and suitable for routine use methods calculated from complete blood count parameters instead of expensive ones such as interleukins. MPV, NLR, PLR, RDW, MPVLR are hematological indicators predicting inflammation (18-20). These parameters obtained through a simple blood count, such as a hemogram, are shown as new markers in the evaluation of systemic inflammatory response.

MPV is a widely used marker that correlates with the platelet function and activation of inflammatory conditions (21). In a study on platelet and MPV levels in ADHD, MPV found to be higher than controls, but no difference found in platelet levels (22).

There are some studies which indicate NLR as an inflammatory marker can increase in depression, bipolar disorder and schizophrenia (19,23-26). PLR is a sensitive inflammatory marker and prognostic factor in some malignancies (27). While some studies showed that PLR increased in ADHD, some studies did not show any significant changes (19,23). RDW indicates the change in size and volume of red blood cells. Inflammatory and infectious conditions lead to an increase in RDW due to the peripheral flow of premature reticulocytes. It is associated with chronic inflammatory conditions rather than acute inflammation (28). There is not enough knowledge about RDW and MPVLR levels about ADHD in the literature.

Table 4. Complete blood count parameters and inflammatory markers in ADHD subtypes

	Attention deficit	Hyperactivity	Combined	Control	p
RDW (%)	13.16±0.92[a]	14.68±2.19[b]	13.16±0.94[a]	13.48±1.26[a]	**0.003**
MPV (fL)	10.50±0.74[a]	10.37±1.21[a]	10.18±0.94[a]	9.65±1.01[b]	**<0.001**
LYMPH (10^3/uL)	2.70±0.94[a]	4.02±1.22[b]	3.02±0.88[a]	3.01±1.03[a]	**0.016**
MONO (10^3/uL)	0.58±0.16[a]	0.81±0.29[b]	0.60±0.20[a]	0.59±0.17[a]	**0.048**
NLR	1.60±0.87	0.97±0.37	1.36±0.73	1.38±0.65	0.179
PLR	124.50±37.93	107.07±53.84	115.63±32.41	118.19±45.85	0.587
MLR	0.24±0.11	0.21±0.07	0.21±0.08	0.21±0.10	0.491
MPVLR	4.31±1.39[a]	2.70±0.54[b]	3.66±1.14[b]	3.56±1.23[b]	**0.004**

ADHD: attention deficit and hyperactivity disorder, RDW: red cell distribution width, MPV: mean platelet volume, LYMPH: lymphocyte, MONO: monocytes, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, MLR: monocytes-to-lymphocyte ratio, MPVLR: mean platelet volume-to-lymphocyte ratio, [a,b,c]: different superscript letters denote significant differences between the groups according to the results of post hoc tests

In high grade inflammatory disease, MPV levels negatively correlated with inflammation (29-31) however, MPV levels found to be increased in low grade inflammatory diseases such as embolism and infective endocarditis (21,32). MPV was also investigated in psychiatric disorders such as bipolar disorder, depression, anxiety disorder, panic disorder, suicide attempt, and ADHD (23,33-36). In this study, MPV and BASO levels were found to be high, especially in boys, but no significant difference was observed in other inflammatory and hematological markers. The exclusion of medical and psychiatric comorbidities except for ODD reinforces the relationship between ADHD and current findings. Although similar findings found in the literature, there are also opposite studies. Yorbik et al. (22) reported increased MPV as an indicator of the inflammatory response in ADHD. Avcil et al. (19) found MPV, NLR, and PLR levels were higher than the controls, Binici et al. (23) reported only MPV levels were higher in ADHD compared to the control group but this difference was not significant in the analyses performed with comorbidity and body mass index. In our study, we excluded all psychiatric disorders and obese patients except ODD and so body mass index was not evaluated as a cofactor.

In the literature, there is no information about the relationship between BASO levels and ADHD. In this respect, this study presents new information. ADHD is associated with allergic diseases such as asthma, allergic rhinitis and atopic dermatitis. The immune response to these allergic diseases are known to increase the risk of neurodevelopmental diseases by affecting the central nervous system (37,38). In a study about allergic disease and inflammation in ADHD, allergic diseases, Ig-E and eosinophil levels were higher in the ADHD group (39). In this study, there was no difference in eosinophil levels, but basophil levels were significantly higher in the ADHD group than in the control group. Eosinophil values in the normal range may be due to the exclusion of diseases such as comorbid asthma and allergy from this study.

Peripheral basophilia is known to be able to associate with allergic diseases. Although the mechanism of action of basophils is not known clearly, basophils are divided into two categories as thymic stromal lymphopoietin elicited basophil and IL-3 elicited basophil. Thymic stromal lymphopoietin elicited basophils are Ig-E independent, IL-3 elicited basophil have Ig-E dependent effect. Allergy mechanisms similarly classified as Ig-E dependent and independent (40). Although allergic diseases excluded from our study group, increased basophil levels found in the ADHD group. This suggests there may be a subclinical allergic inflammation in the etiopathogenesis of ADHD and BASO may be a marker for ADHD. There is a need for comprehensive studies on this subject.

There is no study showing RDW as an inflammatory marker in ADHD. In only one study about nutrient intake and hematological parameters in ADHD, only RDW levels were higher in the ADHD group without treatment compared to the ADHD in the treatment and control group (41). In our study, no difference found between the ADHD and the control group in RDW level, whereas RDW was significantly higher in the ADHD hyperactivity subtype than the other subtypes. RDW may be a significant marker for the differentiation of subtypes. Similarly, MONO levels for hyperactivity subtype and MPVLR for attention-deficit subtype may be a significant marker. In the literature, MONO levels did not differ between ADHD and controls, and did not investigate in ADHD subtypes (23). Another marker, MPVLR is a strong predictor of diabetic nephropathy (42), in the diagnosis of childhood appendicitis and the differentiation of appendicitis perforation (43) and early and late mortality in ST elevated myocardial infarction (44). While there are studies on MPVLR in different diseases, no studies found in psychiatric diseases.

This study has several limitations. First; it was designed as a retrospective and cross-sectional study. The causality relationship cannot establish because of the design of the study. Second; we did not evaluate other inflammatory markers such as cytokines in combination with these parameters. Third, we did not evaluate the severity of ADHD. So we could not see the variability of these parameters according to ADHD severity. On the other hand, there are many strengths of this study. The study groups consisted of newly diagnosed patients who do not use any medication and do not have any medical or psychiatric comorbidities except ODD.

CONCLUSION

To our knowledge, it is the first study evaluating the hematological inflammatory markers according to sex and ADHD subtypes. This study reveals that MPV and BASO tend to be higher in the ADHD group, especially in boys. Our findings suggest RDW, MONO and MPVLR may be a marker for the differentiation of subtypes. This study indicated the need for prospective study with a larger sample on complete blood count parameters in ADHD by sex and subtype.

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Clinicopathologic Features of Phyllodes Tumor in Breast

Memenin Filloides Tümörlerinin Klinikopatolojik Özellikleri

Saliha KARAGÖZ EREN[1]
0000-0003-4114-6578
Yunus DÖNDER[1]
0000-0002-0560-1708
Tamer ERTAN[1]
0000-0003-3721-2253
Ebru AKAY[2]
0000-0003-1190-1800
Alaettin ARSLAN[3]
0000-0002-1321-3465

[1]Kayseri City Training and Research Hospital General Surgery Clinic, Kayseri, Turkey

[2]Kayseri City Training and Research Hospital Pathology Clinic, Kayseri, Turkey

[3]Kayseri City Training and Research Hospital Radiation Oncology Clinic, Kayseri, Turkey

Corresponding Author
Sorumlu Yazar
Saliha KARAGÖZ EREN
salihakaragozeren@hotmail.com

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ABSTRACT

Aim: Phyllodes tumors are rare breast neoplasms comprising less than 1% of all breast neoplasms. The objective of this study was to consider the clinicopathological features of phyllodes tumors that underwent surgery in our hospital.
Material and Methods: We retrospectively analyzed the medical records of 16 patients who had histologically diagnosed phyllodes tumors over 9 years.
Results: There were 16 female patients with a mean age of 45.6±15.3 years. Magnetic resonance imaging was performed in 5 cases for preoperative diagnosis, and 4 were reported as phyllodes tumors, all of these patients were having high-grade phyllodes tumors (borderline or malignant) histopathologically. Preoperative core biopsy was performed in 14 patients, and histopathologically phyllodes tumor was diagnosed in two patients. In six patients, differentiation between hypercellular fibroadenoma and phyllodes tumor could not be performed. Breast-conserving surgery was the most common type of operation performed in 10 (62.5%) patients, three patients with positive margins were underwent reoperation. The pathological diagnoses were benign, borderline, and malignant in seven (43.8%), five (31.3%) and four (25.0%) patients, respectively. The median diameter of the tumors was measured as 6 cm after the postoperative pathological analysis. The median follow-up time was 36 months. During this time, there was no local or systemic recurrence.
Conclusion: Wide excision with a clear margin appears to be the most important factor in the management of these tumors and in the prevention of local recurrence. The preoperative diagnosis of phyllodes tumors contributes to decreasing the necessity for secondary surgical intervention avoiding border positivity.
Keywords: Phyllodes tumor; breast; surgery.

ÖZ

Amaç: Memenin filloides tümörleri nadir görülen meme tümörleridir ve tüm meme neoplazmlarının %1'inden daha azını oluştururlar. Bu çalışmanın amacı filloides tümör nedeniyle hastanemizde opere edilen olguların klinikopatolojik özelliklerini irdelemektir.
Gereç ve Yöntemler: Histopatolojik olarak 9 yıllık süre içerisinde filloides tümör tanısı konulan 16 olgu retrospektif olarak hastane tıbbi kayıtlarından incelendi.
Bulgular: Yaş ortalaması 45,6±15,3 yıl olan 16 kadın hasta vardı. Preoperatif tanı için 5 olguya manyetik rezonans görüntüleme yapıldı ve 4'ü filloides tümör olarak raporlandı, bu hastaların tümü histopatolojik olarak yüksek gradeli filloides tümörlerdi (borderline veya malign). Preoperatif dönemde tanı amaçlı kor biyopsi 14 hastaya yapıldı ve iki hastaya histopatolojik olarak filloides tümör tanısı konuldu. Altı hastada hipersellüler fibroadenom ve filloides tümör arasında ayrım yapılamadı. Meme koruyucu cerrahi 10 (%62,5) hastada en sık yapılan operasyon tipi olup, meme koruyucu cerrahi yapılan ve cerrahi sınır pozitif gelen üç hasta tekrar ameliyat edildi. Patolojik tanı sırasıyla yedi (%43,8), beş (%31,3) ve dört (%25,0) hastada benign, borderline ve malign idi. Postoperatif patoloji sonuçlarına göre ortanca tümör çapı 6 cm olarak ölçüldü. Ortanca takip süresi 36 aydı. Bu sürede sistemik metastaz ya da lokal nüks görülmedi.
Sonuç: Sağlam cerrahi sınırla geniş eksizyon, hastalığın cerrahi yönetimi ve lokal rekürrensin önlenmesi için en önemli faktör olarak görünmektedir. Preoperatif dönemde tanı koymak sınır pozitifliğini önleyerek sekonder cerrahi müdahale ihtiyacını azaltmaya katkıda bulunur.
Anahtar kelimeler: Filloides tümör; meme; cerrahi.

INTRODUCTION

Phyllodes tumor of the breast is rare fibro epithelial breast tumor, comprising less than 1% of all breast neoplasms (1,2). Phyllodes tumors can occur at all ages, but the mean age is 37-52 (3,4). Based on histological criteria, phyllodes tumors are classified as benign, borderline, and malignant. These are including the degree of stromal cellularity, stromal cytologic atypia, mitotic activity, stromal overgrowth, and status of tumor margins; permeative/circumscribed (5).

Surgical resection is still the primary treatment modality for these lesions. However, which patients will benefit from adjuvant radiotherapy and which type of surgery should be performed, are unclear (6). Also patients' outcomes were affected by clinical factors such as age, delay in diagnosis or misdiagnosis and inappropriate and inadequate management. We aimed to present patient and tumor characteristics, the clinicopathological findings, preoperative diagnostic modalities and to evaluate treatment outcomes of the patients diagnosed with phyllodes tumors.

MATERIAL AND METHODS

This study was approved by the clinical research ethics committee of Erciyes University Faculty of Medicine with the number of 2019/880 and dated 25.12.2019. Sixteen patients with pathological diagnosis of phyllodes tumors who underwent surgery at Kayseri City Hospital and Kayseri Training and Research Hospital between 2010 and 2019 were included in the study and clinicopathologic properties, the treatment modality, and radiological and pathological diagnoses were retrospectively analyzed. Patients who were diagnosed as having phyllodes tumor by radiological modalities or core biopsy but not operated in our hospital were not included in the study.

Statistical Analysis

To summarize data obtained in the study, descriptive statistics were given as mean±standard deviation or median with the interquartile range (IQR), minimum maximum [min-max] depending on the distribution of the continuous variables, while categorical variables were summarized as numbers and percentages. The normality test of the numerical variables was controlled by the Sahapiro-Wilk test. In a comparison of more than two independent groups, the Kruskal Wallis H test was used for the numerical variables without normal distribution, and the Dwass-Steel-Critchlow-Fligner test was applied for the differences between the groups.In a comparison of categorical variables in the groups, Fisher-Freeman-Halton test was used for RxC tables. For statistical analysis and figures, Jamovi Project (2020), Jamovi (Version 1.1.9.0), [Computer Software] (Retrieved from https://www.jamovi.org) and JASP Team (2019), JASP (Version 0.11.1) programs were used, and the significance level was taken into account as 0.05 in statistical analysis.

RESULTS

The demographic and clinical features of the patients with phyllodes tumors are given in Table 1. There were 16 female patients with a mean age of 45.6±15.3 (range, 17-74) years. The median diameter of the tumors was 7 (range, 2-22) cm using imaging findings. Most (n=7, 43.8%) of the lesions were located at the central locations.

Table 1. Demographic and clinical features of the patients with phylloides tumor (n=16)

Variables	
Age (year)¶	45.6±15.3 [17-74]
Radiological tumor size (cm)‡	8.5±6.2 / 7 (6.5) [2-22]
Site†	
Right	9 (56.3)
Left	7 (43.8)
Quadrant†	
Upper outer	2 (12.5)
Upper inner	1 (6.3)
Lower outer	2 (12.5)
Lower inner	4 (25.0)
Central	7 (43.8)
Preoperative diagnostic modality†	
US	3 (18.8)
MG+US	8 (50.0)
US+MRI	1 (6.3)
MRI+MG+US	4 (25.0)
Clinical/radiological diagnosis*†	
Fibroadenoma	2 (16.7)
Phylloides tumor	4 (33.3)
Breast cancer	1 (8.3)
None	5 (41.7)
Preoperative pathological diagnosis*†	
Fibroadenoma	2 (14.3)
Phylloides tumor	2 (14.3)
Benign	3 (21.4)
Mesenchymal tumor	1 (7.1)
Hypercellular fibroadenoma / phylloides tumor	6 (42.9)
BIRADS category*†	
3	2 (25.0)
4	4 (50.0)
5	2 (25.0)
Surgery†	
BCS	10 (62.5)
Simple mastectomy	2 (12.5)
NSM	1 (6.3)
MRM	3 (18.8)
Pathological tumor size (cm)‡	7.8±4.5 / 6 (7) [3-18]
Surgical margin (cm)‡	2.3±1.5 / 2 (3) [1-4]
Pathological diagnosis†	
Benign	7 (43.8)
Borderline	5 (31.3)
Malignant	4 (25.0)
Positive surgical margins*†	
Posterior	1 (33.3)
Anterior	1 (33.3)
More than one side	1 (33.3)
Coexisting diagnosis*†	
Cystic disease of the breast	1 (6.7)
In-situ ductal carsinoma	1 (6.7)
Simple ductal hyperplasia	1 (6.7)
Chondorsarcoma-osteosarcoma	1 (6.7)
None	11 (73.3)
Local recurrence†	0 (0.0)
Systemic recurrence†	0 (0.0)
Postoperative radiotherapy†	1 (6.3)
Length of follow-up (month)‡	42.9±29.1 / 36 (38) [4-110]

¶: mean±standard deviation [minimum-maximum], ‡: mean±standard deviation / median (interquartile range) [minimum-maximum], †: n (%), *: less than 16 patients, US: Ultrasound, MG: Mammography, MRI: Magnetic Resonance Imaging, BIRADS: Breast Imaging And Reporting Data System, BCS: Breast Conserving Surgery, NSM: Nipple Sparing Mastectomy, MRM: Modified Radical Mastectomy

Phyllodes tumor was diagnosed preoperatively in four of 12 patients (33.3%) using clinical and imaging findings. However, there were two (16.7%) fibroadenomas and one (8.3%) breast cancer diagnosis. Preoperative core biopsy was performed in 14 (87.5%) patients. In six (42.9%) patients, differentiation between hypercellular fibroadenoma and phyllodes tumor could not be performed. Fibroadenoma and phyllodes tumor was diagnosed in two (14.3% for both) patients. Core biopsy results of 4 patients did not reach in patients' records.

Breast-conserving surgery was the most common type of operation performed in 10 (62.5%) patients. The median diameter of the tumors was measured as 6 (range, 3-18) cm after the postoperative pathological analysis. The pathologically negative margins after the surgery were recorded in 13 (81.3%) patients. Three patients who underwent breast-conserving surgery with positive margins were reoperated; in two patients mastectomy was performed and one performed re-excision. The final margin status is clear. The pathological diagnoses were benign, borderline, and malignant in seven (43.8%), five (31.3%) and four (25.0%) patients, respectively. Although the median diameter of the tumors was found to be statistically higher in the borderline group, most of the tumors were >5 cm in size, both in malignant and borderline groups (Table 2).

In terms of histopathological features of phyllodes tumors, mild stromal atypia and moderate stromal hyperplasia were seen in 11 (67.5%) and seven (43.5%) patients, respectively. Stromal overgrowth was absent in 11 (68.8%) patients. Although the mitotic counts were between 0 and 4 in seven (43.8%) patients, ≥10 mitoses were detected in four (25.0%). In general, diffuse involvement of the margins (p=0.008), marked stromal atypia (p<0.001) and hyperplasia (p<0.001), presence of stromal overgrowth (p=0.001), and more mitoses (p<0.001) were more likely to be associated with malignant phyllodes tumors (Table 2). There was a significant association between ≥10 mitosis and malignant phyllodes tumor (p<0.001). All counts of ≥10 were seen only in patients with malignant pathology.

The median follow-up time was 36 (range, 4-110) months. During this time, there was no local or systemic recurrence. Postoperative radiotherapy was needed in one patient who underwent breast conserving surgery, because of pathological diagnosis also includes ductal carcinoma in situ.

DISCUSSION

Phyllodes tumors are rare fibroepithelial lesions, and the mean age is 37-52 in different studies with high patient numbers (3,4,7). In our series, the mean age was 45.6±15.3 (range, 17-74), two cases were 17 years old; all others were older than 38 years old. Fibroadenomas are widely accepted as the most common tumors in young ages (8). Clinically to differentiate fibroadenoma from phyllodes tumor is difficult without histological confirmation. Juvenile fibroadenoma often has a size larger than 5 cm, but that can reach giant sizes; therefore, the size of the lesion is important for differentiation, but it is not a clear parameter (9,10). In literature, borderline and malignant phyllodes tumor is uncommon in adolescent girls and young women but seems to be occurring with increased frequency (11). One of our 17 years old patients was

Table 2. Comparison of histopathological features of phylloids tumor

Variable	Overall (n=16)	Benign (n=7)	Borderline (n=5)	Malignant (n=4)	p
Pathological size‡	7.8±4.5 6 (7) [3-18]	4.8±1.4 5 (2.5) [3-7]	11.2±4.5 12 (3) [5-18]	9.3±1.4 7 (9) [6-15]	**0.027**
Pathological size group†					
≤5 cm	6 (37.5)	5 (71.4)	1 (20.0)	0 (0.0)	0.058
>5 cm	10 (62.5)	2 (28.6)	4 (80.0)	4 (100)	
Margin status†					
Not-involved	11 (68.8)	5 (71.4)	5 (100)	1 (25.0)	
Focal involvement	2 (12.5)	2 (28.6)	0 (0.0)	0 (0.0)	**0.008**
Diffuse involvement	3 (18.8)	0 (0.0)	0 (0.0)	3 (75.0)	
Stromal atypia†					
Mild	11 (68.8)	7 (100)	4 (80.0)	0 (0.0)	
Moderate	2 (12.5)	0 (0.0)	1 (20.0)	1 (25.0)	**<0.001**
Marked	3 (18.8)	0 (0.0)	0 (0.0)	3 (75.0)	
Stromal hyperplasia†					
Mild	5 (31.3)	5 (71.4)	0 (0.0)	0 (0.0)	
Moderate	7 (43.8)	2 (28.6)	5 (100)	0 (0.0)	**<0.001**
Marked	4 (25.0)	0 (0.0)	0 (0.0)	4 (100)	
Stromal overgrowth†					
Absent	11 (68.8)	7 (100)	4 (80.0)	0 (0.0)	**0.001**
Present	5 (31.3)	0 (0.0)	1 (20.0)	4 (100)	
Microscopic border†					
Circumscribed	8 (50.0)	6 (85.7)	2 (40.0)	0 (0.0)	**0.024**
Permeative/infiltrative	8 (50.0)	1 (14.3)	3 (60.0)	4 (100)	
Mitotic activity†*					
0-4	7 (43.8)	6 (85.7)	1 (20.0)	0 (0.0)	
5-10	5 (31.3)	1 (14.3)	4 (80.0)	0 (0.0)	**<0.001**
≥10	4 (25.0)	0 (0.0)	0 (0.0)	4 (100)	

‡: mean±standard deviation / median (interquartile range) [minimum-maximum], †: n (%), *: 10 high power fields, IQR: interquartile range

having borderline phyllodes tumor with a size of 12 cm, and the other one was having benign phyllodes tumor and with a 5 cm lesion.

The primary approach in the treatment of phyllodes tumors is surgery, and as a result of the high recurrence rate, it requires at least 1 cm intact surgical margin in all cases (12). Therefore, preoperative diagnosis of the cases is important to determine the surgical approach. However, in clinical practice, fibroadenoma-phyllodes tumor differentiation is not always possible with preoperative pathological diagnostic methods. Therefore, it is important for patients to have a preoperative suspicion of phyllodes tumor, to plan surgery to provide a wide surgical margin and to reduce the local recurrence rates.

The median follow-up time was 36 (range, 4-110) months. Three cases were followed up with the diagnosis of fibroadenoma for 1.5 years, 6 months, and 3 years. In one of the cases, the cystic component was observed in the US, which was performed due to the sudden increase in size, contained hemorrhagic fluid in aspiration and was operated on for cellular fibroadenoma/phyllodes after the tru-cut biopsy performed 6 months later. If heterogeneous hypoechoic internal echoes and lobulation are present, and calcifications are absent, a diagnosis of phyllodes tumors should be considered (13). However, sonography cannot distinguish among malignant, borderline, and benign phyllodes tumors. Among the ultrasonography findings of phyllodes tumors, in a study examining 84 cases, the presence of macrocysts was reported as the most common finding of 5 malignant cases (14). In mammography, they are seen as hyperdense, large, round/lobulated, well-circumscribed masses, and it is difficult to distinguish these tumors from fibroadenoma with similar mammography findings (14,15). Recently, magnetic resonance imaging uses for phyllodes tumors have been shown to determine for benign and malignant, especially silt-like patterns in enhanced images and signal changes from T2-weighted to enhanced images correlated significantly with the histologic grade (15). Magnetic resonance imaging was done in five patients, and 4 were reported as phyllodes tumor, and all of them were high-grade phyllodes tumor (borderline or malignant) as histopathologically. These patients had a clear surgical margin and there was no need for a secondary operation.

Although fine-needle aspiration biopsy is not preferred among the pre-operative pathological diagnostic methods of phyllodes tumors due to high false negativity, tru-cut biopsy results are more reliable in diagnosis. In the series including ninety-one patients, they have shown the sensitivity of fine-needle aspiration cytology, tru-cut biopsy, and imaging for diagnosing phyllodes tumors to be 40%, 63% and 65% respectively (16). Preoperative diagnosis is important for extensive excision planning; local recurrence rates range from 3-15% (7,17) for benign phyllodes tumors and 3-50% (7,12) in malignant cases. The differences between the rates may be related to the surgical margin positivity rates in different series. In addition, delay in diagnosis and increase in size may result in increased mastectomy rates. In our series, core biopsy was performed in 14 patients, and phyllodes tumor was diagnosed in two patients. In six (42.9%) patients, differentiation between hypercellular fibroadenoma and phyllodes tumor could not be performed. Our patients with delayed diagnosis and treatment did not have a tru-cut biopsy or were not compatible with phyllodes.

Preoperative core biopsy was performed in 14 (87.5%) patients. In six (42.9%) patients, differentiation between hypercellular fibroadenoma and phyllodes tumor could not be performed. Phyllodes tumor was diagnosed in two (16.7% for both) patients. Core biopsy results of 4 patients did not reach in patients' records.

Malignant phyllodes tumors mostly spread by the hematogenous way, and therefore axillary dissection is not recommended in most of the cases (3). Chen et al. (3), in their series of 172 cases, performed modified radical mastectomy in 42 patients due to recurrence of primary phyllodes tumors; no patients had lymph node metastasis. In our series, one patient underwent modified radical mastectomy six years ago, and it seems related to surgeons' experience and choice. The other two patients had suspected metastatic axillary lymph nodes. None of these patients had axillary node metastases.

The treatment is completely surgical in cases with benign phyllodes. The role of adequate postoperative adjuvant therapy in high-risk patients diagnosed with malignant phyllodes is controversial. Radiotherapy is only recommended for selected patients whose surgical margins are positive or that close and advanced surgery cannot be applied (18). In cases where the tumor is removed with wide excision, there is no consensus about whether radiotherapy provides additional benefit and whether it offers additional survival advantage. In our series, all patients had a clear surgical margin at the final pathological examination (including pathological examination of the second operation); none of these patients received radiotherapy except for ductal carcinoma in a patient having breast conserving surgery with in situ focus.

Pathologically, phyllodes tumors are classified as benign, borderline, and malignant according to the degree of stromal cellularity and atypia, mitotic count, stromal overgrowth, and the nature of their tumor borders (19). A benign phyllodes tumor shows mildly increased stromal cellularity and has minimal nuclear atypia, pushing borders, and mitoses of ≤4/10 high-power fields (HPFs). A malignant phyllodes tumor has marked stromal cellularity and atypia, has permeative margins, and has the mitotic activity of at least 10/10 HPFs. Stromal overgrowth is usually easily identified. Phyllodes tumors with intermediate properties are accepted as to be involved in the border category. In our series, there was a significant association between ≥10 mitosis and malignant phyllodes tumor; all counts ≥10 were seen only in patients with malignant pathology. Malignant phyllodes tumors may be confused with primary or metastatic sarcomas and metaplastic carcinoma. In such cases, the diagnosis of phyllodes tumor hinges on finding residual epithelial structures in the first and immunohistochemical demonstration of diffuse epithelial differentiation in the latter help to confirm the diagnosis (20). Three patients with malignant phyllodes tumor who had diffuse involvement were undergone the second operation, and a robust surgical margin was achieved over 1 cm.

Limitations of this study, in addition to its retrospective design, include the low number of patients and no events (recurrences and deaths) observed and also no patient

received radiotherapy for phyllodes tumor. This situation limited the ability to evaluation of treatment approaches.

CONCLUSION

As a result, phyllodes tumors are generally clinically and pathologically benign, and it is important to ensure that the surgical margin is negative in all patients. There was no recurrence in our series, all patients had clear margins, especially patients with a positive margin and malignant histology should undergo further surgery to obtain clear margins. Preoperative diagnosis and careful management are important because of the high local recurrence rate and their malignant potential. Magnetic resonance imaging can be used to contribute to the diagnosis. The preoperative diagnosis of phyllodes tumor contributes to decreasing the necessity for secondary surgical intervention avoiding border positivity.

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Evaluation of Surgical Techniques in Gynecomastia Treatment: Analysis of 65 Cases

Jinekomasti Tedavisinde Cerrahi Tekniklerin Değerlendirilmesi: 65 Olgunun Analizi

Muhammed Beşir ÖZTÜRK
0000-0002-8977-3741
Cengiz ERTEKİN
0000-0003-4610-3219
Tolga AKSAN
0000-0002-3328-2652
Uğur Kaan KALEM
0000-0002-6291-3978
Mustafa TEZCAN
0000-0002-3609-3312

Göztepe Training and Research Hospital Department of Plastic, Reconstructive and Aesthetic Surgery, Istanbul, Turkey

Corresponding Author
Sorumlu Yazar
Cengiz ERTEKİN
drcengizertekin@gmail.com

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ABSTRACT

Aim: Gynecomastia is a benign enlargement of the breast in males. Surgical treatment options include liposuction, glandular excision and the combination of liposuction and glandular excision. In this study, it was aimed to evaluate 65 consecutive gynecomastia patients who were operated using different techniques and to present the treatment approach, and results and complications related to surgical techniques.
Material and Methods: Sixty five patients who underwent gynecomastia operation in our clinic between June 2016 and January 2019 were included in this study. Demographic data, preoperative and postoperative photographs, clinical classification, perioperative details, postoperative results and complications were evaluated retrospectively.
Results: Fifty five (84.6%) patients had bilateral gynecomastia and 10 (15.4%) patients had unilateral gynecomastia. Of the 120 breasts operated, 91 (75.8%) were Grade II, 20 (16.7%) were Grade III and 9 (7.5%) were Grade I, according to the Simon classification. Twenty-eight (43.1%) patients underwent liposuction and glandular excision, 35 (53.8%) patients underwent liposuction alone, and two (3.1%) patients underwent glandular excision only. Skin excision was performed for two patients at the first operation. Only two patients, one for inadequate reduction and the other for skin excess, were demanded revision surgery.
Conclusion: Surgical treatment options for gynecomastia patients can be determined according to clinical evaluation of breast tissue and skin excess. In young patients with good skin quality, skin excision can be left for a second session. Although there was no significant difference between the methods used in this study, more complications were found with the excisional technique.
Keywords: Gynecomastia; liposuction; surgery.

ÖZ

Amaç: Jinekomasti erkeklerde görülen iyi huylu meme büyümesidir. Cerrahi tedavi seçenekleri arasında liposakşın, glandüler eksizyon ve liposakşın ve glandüler eksizyon kombinasyonu yer almaktadır. Bu çalışmada farklı teknikler kullanılarak ameliyat edilen ardışık 65 jinekomasti hastasının değerlendirilmesi, tedavi yaklaşımının ve cerrahi tekniklerle ilgili sonuçların ve komplikasyonların sunulması amaçlanmıştır.
Gereç ve Yöntemler: Bu çalışmaya Haziran 2016 ve Ocak 2019 arasında kliniğimizde jinekomasti ameliyatı yapılan 65 hasta dahil edildi. Demografik veriler, preoperatif ve postoperatif fotoğraflar, klinik sınıflandırma, perioperatif detaylar, postoperatif sonuçlar ve komplikasyonlar retrospektif olarak değerlendirildi.
Bulgular: Elli beş (%84,6) hastada iki taraflı jinekomasti ve 10 hastada (%15,3) tek taraflı jinekomasti vardı. Simon sınıflamasına göre, ameliyat edilen toplam 120 memenin 91 (%75,8)'i Evre II, 20 (%16,7)'si Evre III ve 9 (%7,5)'u Evre I idi. Yirmi sekiz (%43,1) hastaya liposakşın ve glandüler eksizyon uygulandı, 35 (%53,8) hastaya sadece liposakşın yapıldı ve 2 (%3,1) hastaya sadece glandüler eksizyon uygulandı. İlk ameliyatta iki hastaya deri eksizyonu yapıldı. Biri yetersiz küçültme ve diğeri ciltte fazlalığı için olmak üzere sadece iki hasta revizyon cerrahisi talep etti.
Sonuç: Jinekomasti hastalarında meme dokusunun klinik değerlendirilmesine ve cilt fazlalığı derecesine göre cerrahi tedavi seçeneği belirlenebilir. Genç hastalarda deri kalitesi iyi ise cilt fazlalığı olsa dahi cilt ekzisyonu ikinci bir seansa bırakılabilir. Bu çalışmada kullanılan yöntemler arasında anlamlı bir fark olmamasına rağmen, eksizyonel teknikte daha fazla sayıda komplikasyon görülmüştür.
Anahtar kelimeler: Jinekomasti; liposakşın; cerrahi.

INTRODUCTION

Gynecomastia is the benign growth of glandular breast tissue and skin in men (1). Its global prevalence ranges from 32% to 65% (2). The course of the disease is associated with unsatisfactory body perception as well as severe anxiety, stress and reduced quality of life (3). Although it is often idiopathic, it can be secondary to various metabolic and endocrine diseases, such as alcoholic cirrhosis, hypogonadism, adrenal cortex hyperplasia and hypothyroidism, drugs and acquired or congenital hypogonadal conditions, including Klinefelter syndrome (4). Histologically, it is classified as fluoride, fibrous and intermediate forms (5). Clinically, gynecomastia forms have been defined as glandular, fatty and composite (fatty-glandular). Simon grading is one of the mostly used classification systems for gynecomastia patients. Grade I includes patients with mild enlargement with no skin excess, Grade II, moderate enlargement with or without skin excess, Grade III marked enlargement with skin excess (4). Treatment is determined according to excess skin and clinical classification of gynecomastia (6). Treatment options include follow-up, medical treatment and surgical intervention (7). Surgical intervention is the gold standard in primary idiopathic gynecomastia (8). In recent years, surgical treatment has shown significant changes from the open approach towards liposuction assisted therapies and the use of open approach without liposuction is reserved only for limited cases (9,10).

This study was aimed to investigate the patients who underwent surgical treatment in the form of glandular excision, liposuction plus glandular excision or liposuction only.

MATERIAL AND METHODS

This study included 65 patients who underwent gynecomastia surgery in our clinic between June 2016 and January 2019. The study was approved by the Istanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethical Committee with the number of 2020/0239. Demographic data, imaging studies, preoperative and postoperative photographs, grading data (Simon Classification), ultrasonography results, clinical classification, comorbidities, postoperative results and complications were collected retrospectively.

Surgical Technique

Preoperative drawings were made while the patients were standing. All operations were performed under general anaesthesia. A solution with a 1/500000 concentration of epinephrine-containing Ringer's lactate was used as a tumescent solution. It was applied to the lateral side of the infra-mamarian fold using 0.4 cm incisions made at the level of the anterior axillary line. In cases requiring an additional incision, a second incision was made on the midclavicular line on the infra-mamarian fold. Liposuction performed in radial pattern, first in deep fatty plane, subsequently in superficial fat. Three holed liposuction cannulas with the diameter of 3 mm and 4 mm were used. Patients were evaluated for glandular and skin excision after liposuction. If glandular excision was planned, a 2-4 cm incision was made in the inferior semi-areolar region. Areola was elevated as a superior-based flap with a minimum thickness of 0.5 cm. Anterior attachments of glandular tissue were cut, subsequently posterior attachments were incised over pectoral fascia from inferior to superior to excise glandular tissue. Hemovac drains were used in patients who underwent excision. The drains were inserted from the lateral incision which is used for liposuction and kept for 24-48 hours. Compression corset were recommended for the first month after the operation. The heavy activity was restricted for 1.5 months.

RESULTS

Sixty five patients included in the study, of which 55 (84.6%) had bilateral gynecomastia, and 10 (15.4%) had unilateral gynecomastia. Our patients' ages ranged from 13 to 67 (mean of 25) years. A total of 120 breasts were classified according to Simon classification; 91 (75.8%) breasts were at Grade II, 20 (16.7%) breasts were at Grade III and 9 (7.5%) breasts at Grade I (Table 1). Twenty six (40.0%) of the patients had fatty pattern breasts, 19 (29.2%) of the patients had a glandular type and 20 (30.8%) of them had a mixed pattern. The clinical examinations were consistent with the ultrasonography examinations. Twenty-eight (43.1%) patients underwent liposuction and glandular excision, 35 (53.8%) patients underwent liposuction alone and 2 (3.1%) patients underwent glandular excision only (Figure 1). 33.3% (n=15) of Grade IIa and 37.0% (n=17) of Grade IIb and 50.0% (n=10) of Grade III breasts were treated using both liposuction and glandular excision (Figure 2). Liposuction was performed alone on 66.7% (n=6) of Simon Grade I breasts, this percentage was 66.7% (n=30) in Grade IIa, 63.0% (n=29) in Grade IIb and 50.0% (n=10) in Grade III breasts (Figure 3). Two breasts were successfully treated

Table 1. Gynecomastia grades of patients and surgical techniques in each grade (n=120 breast)

	Grade I (n=9)	Grade IIa (n=45)	Grade IIb (n=46)	Grade III (n=20)
Liposuction	6 (66.7)	30 (66.7)	29 (63.0)	10 (50.0)
Glandular Excision	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)
Liposuction + Glandular Excision	1 (11.1)	15 (33.3)	17 (37.0)	10 (50.0)

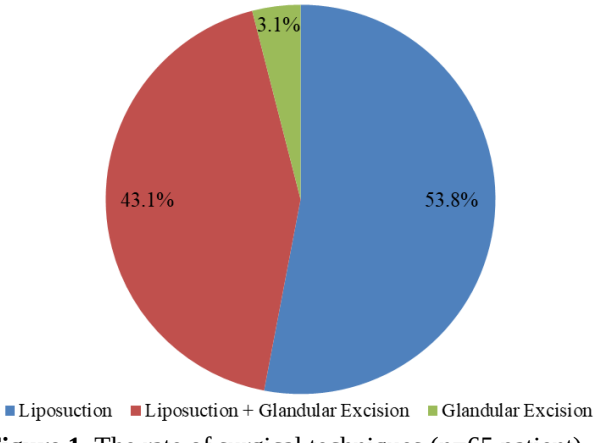

Figure 1. The rate of surgical techniques (n=65 patient)

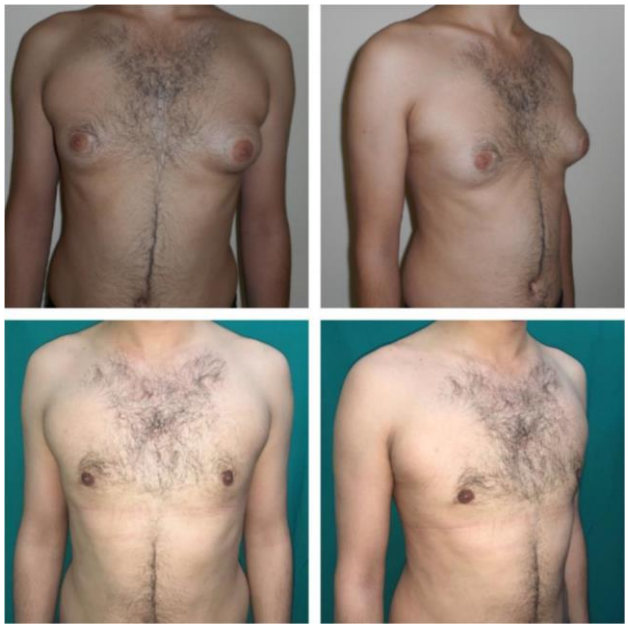

Figure 2. Bilateral Simon Grade IIa gynecomastia, liposuction and glandular excision, preoperative and nine months after surgery, 300 cc lipoaspirate for each breast

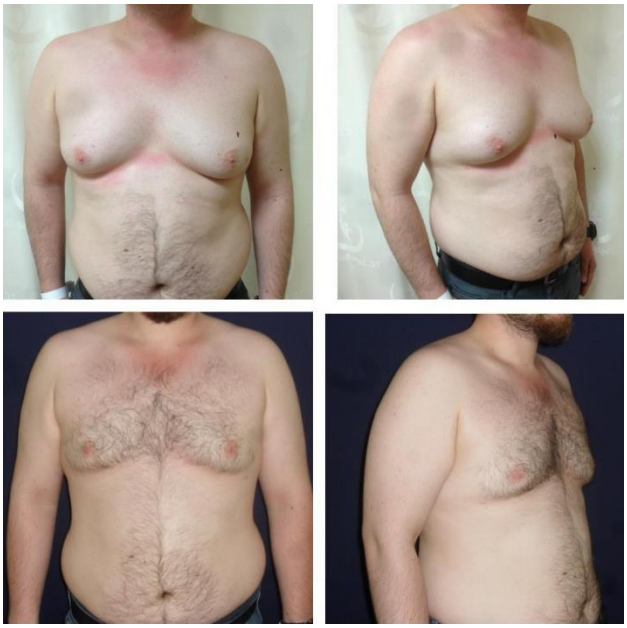

Figure 3. Bilateral Simon Grade IIb gynecomastia, bilateral liposuction only, preoperative and five months after surgery, 400 cc lipoaspirate for each breast

with glandular excision only, these 2 patients were in Grade I group. A 13-year-old patient who presented with severe gynecomastia, underwent reduction mammoplasty surgery for excess skin removal. In that case bilateral nipple jugulum distances were 29 cm. Another case that we had excised skin was a 40 years old post-bariatric patient. Five patients underwent additional circumferential areola reduction during surgery. The mean surgical time was 100 (range, 45-185) min. The mean follow-up period was 20 months.

The pathologic examination was performed for glandular excision group. There were no findings other than fibrous gynecomastia. Unilateral gynecomastia patients were examined separately by the means of pathology results and additional diseases. There were no additional pathology finding except from fibrous gynecomastia in their specimens. One patient had history of cryptorchidism and another patient had history of prolactinoma. Preoperative routine ultrasonography was obtained from all the patients in this study and ultrasonography examinations of the breasts did not reveal any pathological masses. We have performed skin excision in two patients at the first session of the operation. There was only one patient in Grade III who requested reoperation in long term follow-up. Among all groups, there was no other patient that required a second intervention for skin removal.

We have compared the mean volume of lipoaspirate in all patients. We have found that the volume increased proportionally with Simon grades. The mean volume values of lipoaspirate were 221.4 ml (Grade I), 305.2 ml (Grade II), and 391.2 ml (Grade III) in order.

A hematoma developed in one patient with glandular excision group in the early postoperative period. The patient was re-operated at the same day and bleeding control was achieved. There were no postoperative seroma cases in patient groups. None of the patients developed an infection, areola necrosis or nipple retraction. The inadequate reduction occurred in five patients. Only one of the patients with inadequate reduction requested re-operation. Two patients required debridement and scar revision in early postoperative period due to thermal burns at cannula access areas.

DISCUSSION

Although gynecomastia may occur for physiological, pathological and pharmacological reasons, the majority of cases are idiopathic (11). Pathophysiological studies state that the disease is caused by an imbalance of the hormones synthesized in the zona reticularis of the adrenocortical gland (12). Increased levels of oestrogen or deficiency of testosterone cause breast tissue growth in men (1). Obesity contributes to the process by increasing the oestrogen level proportional to fatty tissue (13). Feminine-looking breast tissue can cause serious psychosocial stress in men, and this is the main factor leading the patient to seek a surgical procedure (2).

Surgical options include nipple-sparing subcutaneous mastectomy, liposuction and combinations of these two options (14). The surgical approach is superior to medical treatment because it is faster and more effective with better aesthetic results and less recurrence (1).

In the last 20 years, the open technique has been abandoned, and the trend towards minimally invasive techniques has increased (15). The current literature supports ultrasound-assisted liposuction (UAL) alone or a combination of liposuction with glandular excision using periareolar incision and the pull-through technique (7). The type of surgery is decided based on clinical and ultrasonographic examination of the breasts. Periareolar glandular excision is mostly combined with liposuction in glandular and composite pattern breasts whereas in fatty breasts mostly liposuction was performed alone (4).

Except for two cases, conventional vacuum-assisted liposuction (VAL) before glandular excision was used for all

of the patients in this study. In previous studies, it was reported that UAL produced more effective fat emulsification than VAL and is more effective in dense fibroconnective tissue areas (14). Although conventional and ultrasonic liposuction was not compared in this retrospective study, in patients with the minimal glandular component, sufficient results were achieved with the only VAL.

Rochrich et al. (6) used UAL only in 85% of their patients and reported good results without performing additional incisions or resections. They recommended re-evaluating patients for skin redraping about six months after UAL. Although we used classical liposuction in all cases, satisfactory results were also achieved in Grade IIb and Grade III patients without skin excisions. Among Grade IIb, 29 (63.0%) patients were treated with liposuction only and 17 (37.0%) patients were treated with liposuction and glandular excision. In Grade III, 50.0% (n=10) of patients were treated with liposuction and in other half combined technique was used. Patients were followed for six months for skin redraping after liposuction, and only one patient in the Grade III group had required reoperation for skin excess, an inverted T pattern skin excision was used in this case. Generally, mean age of the patients requiring gynecomastia surgery is low. In young patients, skin redraping is usually sufficient after liposuction and skin excisions can be planned later if needed. One of the patients that we performed skin removal in first operation was a postbariatric patient who had poor skin quality and elasticity. The other patient had severe skin excess and his nipple-jugulum distances were 29 cm bilaterally.

Fodor et al. (16) and Scuderi et al. (17) compared power-assisted liposuction (PAL) with traditional liposuction. They concluded that the use of PAL provided faster fat aspiration and caused less fatigue. In the present study, the mean surgical time was 100 minutes. A review of the literature found that shorter operative times were reported in similar studies (18). We believe that the use of conventional liposuction in our cases contributed to this outcome.

Petty et al. (15) compared four groups of gynecomastia patients using liposuction, excision, combined methods and arthroscopic shavers. They reported that they did not detect a significant difference between the groups in terms of complications. In their study, seromas were the most common complication in all groups. Courtiss et al. (9) reported a higher rate of complications (18.7%) in their study that covered 156 patients treated with the excisional technique, and the most common complications were reported as over/under resection and hematoma. In the present study, the inadequate reduction was determined in five patients, and hematoma developed in three patients. In this study, none of the patients developed areolar necrosis or seroma that requires intervention. When surgical techniques were compared in different groups (excisional, combined and liposuction alone), no significant differences in terms of complications were found.

Lista et al. (19) reported that they achieved satisfactory results in 96 patients with combined liposuction and pull-through glandular excision techniques. They reported that only two patients developed seromas in the postoperative period. Hammond et al. (20) reported satisfactory results in their study covering 15 patients in which they used UAL with the pull-through technique. In their study, Bracaglia et al. (21) used pull through technique with liposuction on 45 patients and reported good results. Hematoma occurred in one patient, and inadequate reduction occurred in one patient.

In the present study, the pull-through technique was used in seven patients. No additional complications occurred, and no re-operations were required.

The main limitation of our study was the inadequate sample of patients in glandular excision group. Also, we have not weighed the glandular excision materials in our patients so this may cause difficulty when comparing with other studies.

CONCLUSION

Gynecomastia is the persistent enlargement of breast tissue in men, and surgical treatment is the most effective option. Surgical options can be classified as liposuction, excision and combined techniques. Surgical treatment options can be determined according to clinical evaluation of breast tissue and skin excess. In young patients with good skin quality, skin excision may be left for a second session. Although there was no significant difference between the methods used in this study, more complications were found with the excisional technique.

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Short Term Outcomes of Endarterectomy to Asymptomatic Extracranial Carotid Artery Disease

Ekstrakranial Karotis Arter Hastalığına Uygulanan Endarterektomi Kısa Dönem Sonuçları

Mehmet KALENDER[1]
0000-0002-8984-4028
Oğuz UĞUR[2]
0000-0003-0433-4270
Hayat GÖKMENGİL[3]
0000-0002-7308-0887
Ahmet Nihat BAYSAL[3]
0000-0002-8779-4784
İpek YAKIN DÜZYOL[4]
0000-0003-0374-8121
Hakan PARLAR[1]
0000-0003-0982-9766
Okay Güven KARACA[5]
0000-0002-7749-9706

[1]Kocaeli Derince Research and Training Hospital Cardiovascular Surgery Clinic, Kocaeli, Turkey

[2]Aksaray University Medical Faculty Department of Cardiovascular Surgery, Aksaray, Turkey

[3]Konya Research and Training Hospital Cardiovascular Surgery Clinic, Konya, Turkey

[4]Kocaeli Derince Research and Training Hospital Anesthesiology and Reanimation Clinic, Kocaeli, Turkey

[5]Duzce University Medical Faculty Department of Cardiovascular Surgery, Düzce, Turkey

Corresponding Author
Sorumlu Yazar
Mehmet KALENDER
ka97084@yahoo.com

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ABSTRACT

Aim: Recently published papers regarding the treatment of carotid artery stenosis are in contradiction with previous trials. Some experts have argued that this evidence supports a conservative approach to carotid revascularization (carotid endarterectomy or carotid stenting) in asymptomatic patients. The objective of this study is to evaluate outcomes of carotid endarterectomy based on preoperative symptom status.

Material and Methods: This retrospective study included patients underwent carotid endarterectomy to internal carotid artery between August 2008 and August 2015. Patients were divided into two groups according to preoperative symptoms. Asymptomatic group consisted of 41 patients with no preoperative neurological symptoms, and symptomatic group of 62 patients with preoperative neurological symptoms (vertigo, amaurosis fugax, transient ischemic attack and ischemic stroke). Postoperatively, all patients received standard therapy in line with the recommendation of the guidelines.

Results: One hundred and three patients were enrolled in this study. The mean age of patients was 68.20±9.79 (range, 41-86) years, and 27 (26.2%) of them were female. There were no statistically significant difference in terms of demographic characteristics between the groups except risk groups; asymptomatic group had more high risk grouped patients (p=0.001). Asymptomatic group was associated with statistically significantly more postoperative mortality compared with symptomatic group (p=0.028). None of the preoperative variables were related to postoperative stroke. In symptomatic group, postoperative stroke was seen in the patients who had preoperative transient ischemic attack and major stroke.

Conclusion: Time of surgical treatment in asymptomatic carotid artery disease should be planned according to patients' cardiac risk factors.

Keywords: Carotid artery stenosis; endarterectomy; stroke.

ÖZ

Amaç: Yakın zamanda yayınlanan karotis arter darlığı tedavisindeki çalışmalar eski sonuçlarla çelişmektedir. Bazı uzmanlar, bu bulguların asemptomatik hastalarda karotis revaskülarizasyona (karotis endarterektomi veya karotis stentleme) konservatif yaklaşımı desteklediğini ifade etmektedir. Bu çalışmanın amacı karotis endarterektomi sonuçlarının preoperatif semptom durumuna dayalı olarak incelenmesidir.

Gereç ve Yöntemler: Bu retrospektif çalışmaya Ağustos 2008 ve Ağustos 2015 tarihleri arasında internal karotis arter darlığına karotis endarterektomi uygulanan hastalar dahil edilmiştir. Hastalar preoperative semptomlarına göre iki gruba ayrıldı. Asemptomatik grup preoperatif nörolojik semptomu bulunmayan 41 hasta içermekte ve semptomatik grup preoperatif nörolojik semptomu (vertigo, amorozis fugax, geçici iskemik atak ve iskemik inme) olan 62 hasta içermekte idi. Postoperatif dönemde tüm hastalara kılavuzların önerisine uygun olarak standart tedavi uygulandı.

Bulgular: Bu çalışmaya 103 hasta dahil edildi. Hastaların ortalama yaşı 68,20±9,79 (aralık, 41-86) yıl ve 27 (%26,2)'si kadın idi. Gruplar arasında, risk grubu dışında demografik özellikler açısından istatiksel anlamlı farklılık yoktu; asemptomatik grupta yüksek riskli olarak gruplanan hasta daha fazlaydı (p=0,001). Asemptomatik grupta, semptomatik grup ile karşılaştırıldığında postoperatif mortalite istatistiksel olarak anlamlı şekilde daha fazla görüldü (p=0,028). Preoperatif değişkenlerinin hiç biri postoperatif inme ile ilişkili değildi. Semptomatik grupta, postoperatif inme preoperatif geçici iskemik atak ve majör inme olan hastalarda görüldü.

Sonuç: Asemptomatik karotis arter hastalığında cerrahi tedavinin zamanlaması hastanın kardiak risk faktörlerine göre yapılmalıdır.

Anahtar kelimeler: Karotis arter darlığı; endarterektomi; inme.

INTRODUCTION

Recently published papers regarding the treatment of carotid artery stenosis are in contradiction with previous trials. Transient ischemic attacks (TIA) occurred in nearly 70 percent of patients as the qualifying event for entry in the NASCET trial cohort, and observational data suggest that ischemic stroke due to extracranial carotid disease is preceded by TIA in 50 to 75 percent of patients (1-4). However, these data may be subject to selection bias as large disabling or lethal strokes were systematically excluded from NASCET and most studies assessing prestroke symptoms due to the inability to obtain a direct patient history. Some experts have argued that this evidence supports a conservative approach to carotid revascularization (carotid endarterectomy, CEA or carotid stenting) in asymptomatic patients (5,6). The objective of this study is to evaluate the outcomes of CEA based on preoperative symptom status.

MATERIAL AND METHODS

The institutional local ethics committee approved the study (Konya Education and Research Hospital, dated 14.01.2016 and approval number: 04-21). This retrospective study included the patients who had surgically treated internal carotid artery (ICA), in the period from August 2008 to August 2015 in Konya Education and Research Hospital. The end points of study were postoperative stroke and mortality. Given the involvement of ICA stenosis, patients were divided into two groups. Asymptomatic group of patients consisted of 41 patients with no preoperative neurological symptoms, and symptomatic group of 62 patients with preoperative neurological symptoms (vertigo, amaurosis fugax, TIA and ischemic stroke). Patients had either deep or superficial cervical plexus block anesthesia which was decided by anesthesiology, none needed to switch general anesthesia. Bupivacaine and lidocaine were used as the anesthetic agent. Shunt used in the absence of sufficient back flow to the internal carotid artery, or contralateral carotid artery with severe stenosis or in patients with neurologic deficits observed after clamping during the operation. Carotid arteriotomy closed either primary or by way of a patch. Postoperatively all patients had standard medication dictated by guidelines such as antithrombotic, antiplatelet, statin, antihypertensive- if needed-, and antibiotic. Cardiac risk groups were categorized as high risk group (myocardial infarction, MI in 6 weeks, USAP and/or CCS III-IV angina, congestive heart failure, valvular heart disease (intervention needed) and left ventricular ejection fraction lower than 30%), moderate risk group (MI in more than 6 weeks, stable angina pectoris and/or CCS I-II angina, medication required arrhythmia and left ventricular ejection fraction 30-50%) and low risk group (diabetes mellitus, hypertension, chronic obstructive pulmonary disease, obesity, chronic renal insufficiency, peripheral arterial disease, dyslipidemia and left ventricular ejection fraction more than 50%). Stenosis detected with Doppler ultrasound and confirmed by MRI, CT or conventional angiography.

Statistical Analysis

Distribution of the numerical data was examined using Shapiro-Wilk and Kolmogorov-Simirnov tests. Continuous variables were displayed by the mean and standard deviation if normally distributed and by the median, interquartile range and range if there was no normal distribution. Categorical variables were displayed as counts and percentages. Continuous variables were analyzed with Independent samples t test or Mann-Whitney U test according to the normality assumption. Group comparison was done using the Pearson chi-square and Fisher exact tests, where appropriate. All tests were two-sided, and a p-value of 0.05 or lower was considered statistically significant. All statistical analyses were performed using IBM SPSS v.21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

RESULTS

Totally 103 patients were enrolled in this study. The mean age of patients was 68.20±9.79 (range, 41-86) years, and 27 (26.2%) of them were female. Table 1 depicts baseline patient characteristics and there were no significant difference among groups except risk groups (Asymptomatic group had more high risk grouped patients, p<0.001).

Asymptomatic patients' group was associated with significantly more postoperative mortality compared with symptomatic patients' group (p=0.028, Table 2).

High-risk cardiac grouped patients were found to be associated with postoperative MI. Hospital mortality was seen in 9 patients. Two patients on the postoperative 3rd day had CABGx3 and 2 had CABGx3+AVR. One patient was waiting for CABG, and all five died due to MI. Two patients had additional morbidity (chronic obstructive pulmonary disease), and two patients died due to postoperative major stroke.

Table 3a depicts demographics of only postoperative stroke observed patients and as seen none of the preoperative variables were related with postoperative stroke (Table 3a). On the other hand, among symptomatic patients, preoperative TIA, and major stroke were associated with higher postoperative stroke rates (Table 3b). Comparison of postoperative stroke observed patients according to preoperative symptom revealed relationship (Table 3c).

Table 1. Demographics of study groups

	Asymptomatic (n=41)	Symptomatic (n=62)	p
Sex			
Male	30 (73.2%)	46 (74.2%)	0.908
Female	11 (26.8%)	16 (25.8%)	
Age, years	68.66±9.42	67.90±10.09	0.704
CEA side			
Right	20 (48.8%)	39 (62.9%)	0.156
Left	21 (51.2%)	23 (37.1%)	
Carotid lesion			
Right	60 (60) [10-100]	70 (60) [10-100]	0.851
Left	60 (50) [10-100]	50 (55) [10-100]	0.374
Risk group			
High	15 (36.6%)	4 (6.5%)	
Moderate	6 (14.6%)	9 (14.5%)	**<0.001**
Low	20 (48.8%)	49 (79.0%)	
Primary closure	13 (31.7%)	18 (29.0%)	0.772

CEA: Carotid endarterectomy

Table 2. Postoperative outcomes of study groups

Outcome	Asymptomatic (n=41)	Symptomatic (n=62)	p
CVE	1 (2.4%)	4 (6.5%)	0.646
MI	4 (9.8%)	1 (1.6%)	0.080
Bleeding	2 (4.9%)	1 (1.6%)	0.562
Mortality	7 (17.1%)	2 (3.2%)	**0.028**

CVE: Cerebrovascular event, MI: Myocardial infarction

Table 3a. Demographics of postoperative stroke (cerebrovascular event) observed patients

	Non-CVE (n=98)	CVE (n=5)	p
Symptomatic	58 (59.2%)	4 (80.0%)	0.646
Sex			
Male	72 (73.5%)	4 (80.0%)	0.999
Female	26 (26.5%)	1 (20.0%)	
Age, years	68.09±9.93	70.40±6.80	0.610
CEA side			
Right	54 (55.1%)	5 (100%)	0.070
Left	44 (44.9%)	0 (0.0%)	
Carotid lesion			
Right	85 (20) [10-100]	80 (20) [70-90]	0.680
Contralateral	30 (40) [10-99]	50 (30) [30-60]	0.232
Left	70 (25) [10-100]	---	---
Contralateral	30 (40) [10-100]	---	---
Risk group			
High	18 (18.4%)	1 (20.0%)	
Moderate	13 (13.3%)	2 (40.0%)	0.194
Low	67 (68.4%)	2 (40.0%)	
Primary closure	29 (29.6%)	2 (40.0%)	0.636

CVE: Cerebrovascular event, CEA: Carotid endarterectomy

Table 3b. Comparison of postoperative stroke observed patients according to the complaints at admission

	Non-CVE (n=98)	CVE (n=5)	p
Asymptomatic	40 (40.8%)	1 (20.0%)	
Symptomatic			
Dizziness	17 (17.3%)	0 (0.0%)	
Left-sided hemiparesis	6 (6.1%)	0 (0.0%)	
Right-sided hemiparesis	11 (11.2%)	0 (0.0%)	
TIA	2 (2.1%)	2 (40.0%)	0.100
Uneventful CVE	1 (1.0%)	0 (0.0%)	
Syncope	2 (2.1%)	0 (0.0%)	
Amaurosis fugax	1 (1.0%)	0 (0.0%)	
Numbness in the arm	2 (2.1%)	0 (0.0%)	
Major stroke	16 (16.3%)	2 (40.0%)	

CVE: Cerebrovascular event, TIA: Transient ischemic attack

Table 3c. Comparison of postoperative stroke observed patients according to the preoperative symptom

	Non-CVE (n=58)	CVE (n=4)	p
Dizziness	17 (29.3%)	0 (0.0%)	
Left-sided hemiparesis	6 (10.3%)	0 (0.0%)	
Right-sided hemiparesis	11 (19.0%)	0 (0.0%)	
TIA	2 (3.4%)	2 (50.0%)	
Uneventful CVE	1 (1.7%)	0 (0.0%)	0.144
Syncope	2 (3.4%)	0 (0.0%)	
Amaurosis fugax	1 (1.7%)	0 (0.0%)	
Numbness in the arm	2 (3.4%)	0 (0.0%)	
Major stroke	16 (27.6%)	2 (50.0%)	

CVE: Cerebrovascular event, TIA: Transient ischemic attack

DISCUSSION

Carotid artery stenosis is a common presentation of atherosclerotic disease. A total of 9% to 12% of patients with the known atherosclerotic disease has high-grade carotid artery stenosis (7). CEA is a widely accepted method of treating patients with significant carotid artery stenosis.

In our study, we detected higher mortality rate (8.7%) comparing to the literature. In larger trials and reviews, mortality rate reported ranging from 1% to 6.7% (1,8). Medical complications following CEA were associated with myocardial infarction history or angina and hypertension (9). During NASCET trial patients with recent myocardial infarction or unstable angina pectoris or heart failure were excluded and thus causing low peri-procedural medical complication rate. On the other hand, trials concluding high risk grouped patients had worse outcome parallel to our findings (10,11). Kumamaru et al. (12) and O'Neill L et al. (13) had showed that surgeon reported case-volume and 30-day mortality are inversely proportional and our results support this two reports.

Interestingly Galyfos et al. (14) reported that low to moderate risk grouped patients had increased cardiac troponin I levels associated with myocardial ischemia comparing to high risk grouped patients. Asymptomatic carotid atherosclerosis is also a marker of increased risk for myocardial infarction and vascular death (11,15,16). Thus, asymptomatic carotid atherosclerosis is considered a risk equivalent for cardiovascular disease. In our study, groups had statistically significant difference according to cardiac risk groups, and mortality observed patients were mostly in high cardiac risk group.

Dodick et al. (5) and Shanik et al. (6) argued that large trials may have selection bias due to as large disabling or lethal strokes were systematically excluded. Natural history studies advocate majority of strokes caused by carotid artery disease are preceded by TIA, and the incidence of unprotected stroke is low (5). There are papers advocating best medical treatment alone for asymptomatic carotid artery disease patients (15-19). On the other hand major trials as VA, ACAS, and ACST I-II encourage intervention for asymptomatic carotid artery disease (20-23).

The NASCET described five baseline variables for increased risk factor: hemispheric TIA as qualifying event, contralateral carotid occlusion, ipsilateral ischemic lesion on CT scan and irregular or ulcerated ipsilateral plaque. Also, ECST confirmed findings of NASCET and added age and sex as predictive risk factors (24). In our study we observed increased postoperative stroke in preoperatively symptomatic patients (TIA and CVE) parallel to the literature.

Interestingly we have observed postoperative stroke in patients with right-sided procedure applied in contrast to NASCET's finding.

This study had some limitations. Because this study was retrospective and randomization between groups was not performed, it is conceivable that there are differences in patient characteristics among the groups and that a selection bias. Furthermore, this study enrolled in a multi-surgeon center which also may contribute to selection bias. Additionally, the study was limited by the number of patients with CEA. Finally, our long-term follow-up was limited to 30 days.

CONCLUSION

Asymptomatic carotid artery disease patients' surgery time should be planned according to their cardiac risk factors.

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Healing Effects of Single-Dose Triptolide in Rats with Severe Acute Pancreatitis

Şiddetli Akut Pantreatitli Ratlarda Tek-Doz Triptolitin İyileşmeye Etkisi

Hüseyin ÖZDEN[1]
0000-0002-2786-3805
Asuman KİLİTÇİ[2]
0000-0002-5489-2222
Yaşar ŞAHİN[3]
0000-0001-5936-4210
Gökhan KARACA[4]
0000-0002-5107-5999
Haldun UMUDUM[5]
0000-0002-4550-1971
Ahmet YILDIZ[4]
0000-0002-6889-4528
Erkan DULKADİROĞLU[6]
0000-0001-7665-7453

[1]Ahi Evran University Faculty of Medicine Department of General Surgery, Kırşehir, Turkey

[2]Ahi Evran University Faculty of Medicine Department of Pathology, Kırşehir, Turkey

[3]Kırıkkale University Faculty of Veterinary Medicine Department of Pharmacology and Toxicology, Kırıkkale, Turkey

[4]Kırıkkale University Faculty of Medicine Department of General Surgery, Kırıkkale, Turkey

[5]Ufuk University Faculty of Medicine Department of Pathology, Ankara, Turkey

[6]Kırşehir Training and Research Hospital Internal Medicine Clinic, Kırşehir, Turkey

Corresponding Author
Sorumlu Yazar
Hüseyin ÖZDEN
huseyinozden@ahievran.edu.tr

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ABSTRACT

Aim: Severe acute pancreatitis (SAP) carries high morbidity and mortality risk. If the proinflammatory response phase of SAP cannot be controlled, it may result in multiorgan failure (MOF). Nuclear factor-kappa B (NF-κB) activation plays an important role in the development of MOF. In this study, it was aimed to investigate the healing effects of triptolide, an anti-inflammatory and immunosuppressive agent in rats with SAP.
Material and Methods: A total of 20 Wistar-Albino rats were divided into two groups as the SAP and triptolide treatment (TT) groups. SAP was induced by intraperitoneal injection of cerulean (50 mg/kg) in both groups. TT group was administered a single dose (0.2 mg/kg) triptolide 24 hour after the induction of SAP. Serum ALT, AST, GGT, Lipase, Glucose, ALP and amylase levels and pancreatic tissue samples were examined.
Results: Serum glucose and amylase levels were found to be significantly lower in the TT group (p=0.011 and p=0.035, respectively). There was no significant difference between the groups in terms of other biochemical parameters. Pancreatic edema, acinar cell degeneration, fat necrosis, intrapancreatic&perivascular inflammation, inflammation in the peripancreatic fat tissue were common histopathological findings in both groups. There was no significant difference between the groups in terms of histopathologic changes.
Conclusion: Cerulein-induced pancreatitis is a successful method for experimental SAP. The healing effects of single-dose triptolide treatment are not evident in the early phase of SAP. The therapeutic effects of triptolide on inflammatory and oxidative stress were not significantly approved by histopathological and biochemical parameters by the pancreatic tissue.
Keywords: Severe acute pancreatitis; cerulean; triptolide.

ÖZ

Amaç: Şiddetli akut pankreatit (SAP) yüksek morbidite ve mortalite riski taşır. SAP'nin proenflamatuar yanıt fazı kontrol edilemezse, çoklu organ yetmezliği (ÇOY) ile sonuçlanabilir. Nükleer faktör-kappa B (NF-κB) aktivasyonu ÇOY'un gelişiminde önemli bir rol oynar. Bu çalışmada, bir anti-enflamatuar ve immünsüpresif ajan olan triptolidin SAP'lı sıçanlarda iyileştirici etkilerinin araştırılması amaçlanmıştır.
Gereç ve Yöntemler: Toplam 20 Wistar-Albino sıçanı SAP ve triptolide tedavi (TT) grubu olmak üzere iki gruba ayrıldı. SAP her iki grupta da intraperitoneal cerulein (50 mg/kg) enjeksiyonu ile indüklendi. TT grubuna, SAP indüksiyonundan 24 saat sonra tek bir doz (0.2 mg/kg) triptolid uygulandı. Serum ALT, AST, GGT, Lipaz, Glikoz, ALP ve amilaz düzeyleri ve pankreatik doku örnekleri incelendi.
Bulgular: Serum glukoz ve amilaz düzeyleri TT grubunda anlamlı olarak daha düşük bulundu (sırasıyla p=0.011 ve p=0.035). Diğer biyokimyasal parametreler açısından gruplar arasında anlamlı bir fark yoktu. Pankreas ödemi, asiner hücre dejenerasyonu, yağ nekrozu, intrapankreatik&perivasküler inflamasyon ve peripankreatik yağ dokusunda inflamasyon her iki grupta da sık görülen histopatolojik bulgulardı. Gruplar arasında histopatolojik değişiklikler açısından anlamlı bir fark yoktu.
Sonuç: Cerulein kaynaklı pankreatit, deneysel SAP için başarılı bir yöntemdir. Tek doz triptolid tedavisinin iyileştirici etkileri SAP'nin erken evresinde belirgin değildir. Pankreatik doku histopatolojik ve biyokimyasal parametreler açısından incelendiğinde, triptolidin enflamatuar ve oksidatif stres üzerindeki terapötik etkileri yeterli düzeyde değildi.
Anahtar kelimeler: Şiddetli akut pankreatit; cerulein; triptolid.

INTRODUCTION

The clinical spectrum of acute pancreatitis ranges from mild and treatable with supportive measures to severe diseases with life-threatening complications. Severe acute pancreatitis (SAP) is the most serious type of pancreatitis and has a high morbidity and mortality risk. SAP occurs in two phases. Initially, proinflammatory response develops during the first 1-2 weeks and results in systemic inflammatory response syndrome (SIRS). After the first 1-2 weeks, the anti-inflammatory response occurs. In the presence of severe SIRS, proinflammatory mediators may lead to early multiple (respiratory, cardiovascular, renal and hepatic) organ failure (1,2).

Activation of transcription factor nuclear factor-kappa B (NF-κB) in acinar cells during the early stage of pancreatitis triggers the expression of multiple proinflammatory genes. It has been postulated that there is a positive correlation between NF-κB levels and the severity of acute pancreatitis. Experimental studies have reported NF-κB activation to be an early and central event in the progression of inflammation in AP (3-5).

Triptolide is extracted from the roots of Chinese herb *Tripterygium wilfordii* and is known as an NF-κB inhibitor. Previous studies have shown that triptolide has a broad-spectrum therapeutic potential due to its immunosuppressive, anti-inflammatory and anti-tumor properties (6-8).

Our experimental study aimed to investigate the healing effects of single dose-triptolide treatment in the early phase of cerulein-induced severe acute pancreatitis in rats.

MATERIAL AND METHODS

This study was conducted with the permission of Kırıkkale University's Local Ethics Committee for Animal Experiments (08.01.2019 and decision no: 11). A total of twenty male Wistar albino rats weighing 280±20 g were randomly divided into two groups (n=10 each group) as the SAP and triptolide treatment (TT) groups. All rats were fasted for 12 hours before the experimental procedure, but were given free access to ad libitum and food, and also kept to standard animal care conditions (22±2°C) with a 12:12-h light: dark cycle. Severe pancreatitis was induced by intraperitoneal injection of cerulein (50 mg/kg) to both groups. The TT group was administered a single dose (0.2 mg/kg) of intraperitoneal triptolide 24 hours after the development of severe pancreatitis. All surgical procedures were performed using 10 mg/mL xylazine (Rompun; Bayer) and 50 mg/mL ketamine (Ketalar; JHP), given intramuscularly into the leg of the rats at a dose of 0.25 mL/100 g of rat mass prior to the application. All rats were sacrificed 48 hours after the development of severe pancreatitis. Rats were sacrificed by cardiac puncture. Biochemical and histopathological tissue samples were obtained from all rats by laparotomy. Blood samples were separated immediately by centrifugation at 4000 rpm for 5 minutes and stored at -20°C for biochemical analysis. About 4 ml was drawn from the left ventricle, centrifuged and observed by an auto biochemistry analyzer for ALT, AST, GGT, Lipase, Glucose, ALP and amylase levels. Pancreatic tissues of all rats were sampled for histopathologic examination for the severity of acute pancreatitis.

Histopathological Evaluation

Pancreatic-tissue samples fixed in 10% formalin were embedded in paraffin, cut into 4 μm sections, placed on slides and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope by two pathologists who were blinded to the experimental procedure. A modified semi-quantitative scoring was performed for the microscopic evaluation of the pancreatitis and four categories (Grade 0: none, 1: low, 2: moderate, 3: severe) were defined. To grade the damage to the pancreas, edema, acinic cell degeneration, acinar necrosis, fat necrosis, hemorrhage, intrapancreatic and perivascular inflammation, inflammation in the peripancreatic fat tissue were included as the parameters of the scoring system.

Statistical Analysis

SPSS v.21.0 was used for the statistical analysis of the study. Assumption of normality was tested by Shapiro-Wilk tests. Explanatory statistics for variables were given as mean±standard deviation or median, interquartile range (IQR), minimum-maximum, and number and percentage. Group comparisons were made using the Independent samples t-test, Mann-Whitney U test, Fisher's Exact test and Fisher-Freeman-Halton test according to the type of variables and the state of the verification of assumptions. A p-value <0.05 was considered statistically significant.

RESULTS

The mean ALT, AST, GGT, lipase, and ALP levels of the groups were not significantly different. The mean serum glucose levels of the SAP group were significantly higher compared to the TT group (p=0.011). The mean serum amylase levels were also significantly higher in the SAP group compared to the TT group (p=0.035). The statistical comparisons of the groups in terms of blood parameters including ALT, AST, GGT, lipase, glucose, ALP and amylase levels are presented in Table 1 in detail.

Table 1. Comparison of the groups in terms of pancreatic and liver enzyme levels

	SAP group (n=10)	TT group (n=10)	p
ALT	53.5±7.84	56.0±13.86	0.626[a]
ALP	265.3±81.55	272.4±72.52	0.839[a]
Glucose	137.6±4.16	128.7±8.99	**0.011**[a]
AST	121.0 (30.25) [117-151]	151.0 (48.25) [114-173]	0.315[b]
GGT	1.0 (1.0) [1-2]	1.0 (1.0) [1-6]	0.912[b]
Lipase	3.0 (1.0) [2-3]	3.0 (0.0) [2-4]	0.190[b]
Amylase	1813.0 (490.5) [1493-2588]	1682.0 (367.5) [1017-1838]	**0.035**[b]

SAP: Severe Acute Pancreatitis, TT: Triptolide Treatment, [a]: Independent samples t test, [b]: Mann-Whitney U test, Descriptive statistics were given as mean±standard deviation or median (interquartile range) [minimum-maximum]

A statistically significant difference was found only in fat necrosis. However, there was no significant difference in other parameters. Histopathologically, there was no significant difference between the groups in terms of edema, acinar cell degeneration, or intrapancreatic&perivascular inflammation findings. Also, the fat necrosis, hemorrhage, inflammation in the peripancreatic fat tissue outcomes of both treatment groups were superior, but this difference was not statistically significant. Table 2 presents in detail the comparisons of the groups in terms of histopathological changes. The difference between the histopathologic changes in the groups was not statistically significant.

DISCUSSION

Severe acute pancreatitis comprises about 20% of all acute pancreatitis cases and may lead to local and systemic complications. Organ failure is commonly seen in the early period of acute pancreatitis (9). About 35%-50% of hospital deaths seen within the first week after admission are due to single or multiple organ failure. The pathogenic mechanism, pathologic process and management of SAP have yet to be completely clarified. Thus, the incidence of serious complications of SAP and morbidity rates could not be substantially decreased (8,10-12).

Previous studies showed that NF-κB plays a critical role in the clinical course of pancreatitis. Experimental studies showed that the activation of NF-κB occurs within pancreatic acinar cells in the early phase of acute pancreatitis. NF-κB is widely accepted as a key modulator in the regulation of inflammation due to its ability to control the expression of inflammatory mediators. The relationship between the level of NF-κB activation and the severity of acute pancreatitis has been well-defined. Thus, treatment strategies aimed to inactivate NF-κB seem to be reasonable and promising (13-16).

Gukovsky et al. (14) showed that the NF-κB is activated in pancreatic acinar cells within 30 min after cerulein-induced pancreatitis. A cerulein-induced experimental acute pancreatitis model was performed in our study. This model is the most widely preferred experimental animal model of acute pancreatitis as it is highly reproducible and cost effective (17-19). Intravenous or intraperitoneal injection of overdose (0.5 μg/kg/hr in rats) cerulein induces pancreatic enzyme activation within 30 minutes. Findings in cerulein-induced experimental acute pancreatitis are similar and comparable to those of acute pancreatitis in humans. Besides that hyperamylasemia, histopathological findings such as the infiltration of inflammatory cells within the pancreas, pancreatic edema, acinar cell degeneration and the presence of activated pancreatic enzyme in pancreatic tissue are similar in experimental pancreatitis models and humans with pancreatitis (17).

Histopathological changes in the pancreatic tissues of the animals in our study groups were consistent with the outcomes in the literature. A single-dose intraperitoneal cerulein injection resulted in edema, acinar cell degeneration and inflammatory cell infiltration in the pancreatic tissue of rats. In our study, there was no statistical difference between histopathological assessment results. However, the hemorrhage and fat necrosis outcomes of the treatment groups were better. We believe the reason that our results were not statistically significant may be because of the small number of experimental rats in the study groups.

Triptolide is reported as a strong anti-inflammatory and immunosuppressive agent in the literature. These properties of triptolide are explained by the inhibition of the production of cytokines such as TNF-alpha, IL-1, IL-6, IL-8 and phagocytosis of phagocytes. It has been shown that these effects of triptolide are closely related to NF-κB activity (7,8,20). Gray et al. (21) reported that lung and liver damage occur due to the activation of NF-κB in the presence of pancreatitis-induced SIRS. Zhao et al. (8) showed that the activation of NF-κB occurs in Kupffer cells in the liver tissue of rats with SAP and leads to an increase in serum TNF-alpha, IL-6 and ALT levels. They also showed that triptolide treatment inhibits NF-κB activity and considerably decreases serum TNF-alpha, IL-6 and ALT levels.

Our main limitation in this study was the lack of sham group of animals. We also could not examine the cytokine (TNF-alpha, IL-1, IL-6) levels due to our limited budget.

Table 2. Comparisons of the groups in terms of histopathologic changes

	SAP group (n=10)	TT group (n=10)	p
Edema			
1	4 (40.0)	6 (60.0)	
2	6 (60.0)	3(30.0)	0.370[a]
3	0 (0.0)	1 (10.0)	
Acinar Cell Degeneration			
0	6 (60.0)	3 (30.0)	
1	2 (20.0)	7 (70.0)	0.103[a]
3	2 (20.0)	0 (0.0)	
Fat Necrosis			
1	6 (60.0)	3 (30.0)	
2	4 (40.0)	2 (20.0)	**0.045**[a]
3	0 (0.0)	5 (50.0)	
Hemorrhage			
0	10 (100)	6 (60.0)	
1	0 (0.0)	2 (20.0)	0.087[a]
2	0 (0.0)	2 (20.0)	
Intrapancreatic and perivascular inflammation			
0	6 (60.0)	5 (50.0)	1.000[b]
1	4 (40.0)	5 (50.0)	
Asinar Necrosis			
0	10 (100)	10 (100)	---
Inflammation in the peripancreatic fat tissue			
0	0 (0.0)	1 (10.0)	
1	6 (60.0)	3 (30.0)	0.370[a]
2	4 (40.0)	6 (60.0)	

SAP: Severe Acute Pancreatitis, TT: Triptolide Treatment, [a]: Fisher-Freeman-Halton test, [b]: Fisher's Exact test

CONCLUSION

While triptolide used in the treatment of acute pancreatitis suggests an improvement in the clinics of acute pancreatitis by lowering amylase levels, one of the most important indicators of acute pancreatitis, we do not think it is suitable for use in the treatment of acute pancreatitis due to its negative effect on liver function tests.

Our results showed that triptolide treatment significantly decreases serum glucose and amylase levels in rats with

SAP. However, there was no significant difference between the groups in terms of serum ALT, AST, GGT, lipase and ALP levels. The present study shows that triptolide cannot be used to treat severe pancreatitis relying on its antioxidant and pancreatoprotective feature.

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Current Approach to the Prognostic Parameters of Testicular Germ Cell Tumors Accompanied by Our Cases

Testiküler Germ Hücreli Tümörlerin Prognostik Parametrelerine Olgularımız Eşliğinde Güncel Yaklaşım

Ganime ÇOBAN[1]
0000-0002-5779-6797
Pelin YILDIZ[1]
0000-0002-7709-7264
Zeynep SEZAL[1]
0000-0002-0112-0229
Adila ADILLI[1]
0000-0002-2743-930X
Mehmet BEŞİROĞLU[2]
0000-0002-1171-8320
Muzaffer AKÇAY[3]
0000-0001-6716-3830
Zuhal GUCİN[1]
0000-0001-5330-2158

[1]Bezmialem Vakif University Faculty of Medicine Department of Pathology, İstanbul, Turkey

[2]Bezmialem Vakif University Faculty of Medicine Department of Oncology, İstanbul, Turkey

[3]Bezmialem Vakif University Faculty of Medicine Department of Urology, İstanbul, Turkey

Corresponding Author
Sorumlu Yazar
Ganime ÇOBAN
drgcoban@hotmail.com

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ABSTRACT

Aim: Testicular germ cell tumors (TGCT) are solid neoplasms common in young adult men and an important cause of cancer-related deaths during this period. Revisions in histopathological classification and staging affect prognosis and treatment. The aim of this study was to analyze our TGCT cases, to review prognostic parameters, and their relationship between germ cell neoplasia in situ (GCNIS), intratubular and intertubular tumors.
Material and Methods: In this study, Hematoxylin&Eosin-stained sections of 77 TGCTs were re-evaluated. The presence of GCNIS, intratubular and intertubular germ cell tumors were recorded. Histopathological classification and staging were revised based on the changes in the 8th edition of American Joint Committee on Cancer (AJCC).
Results: The majority of the patients were diagnosed as seminoma (n=42), followed by mixed germ cell tumors (n=33) and spermatocytic tumors (n=2). Rete testis invasion in 30 cases, epididymal invasion in 6 cases, hilar soft tissue invasion in 10 cases, tunica vaginalis invasion in 1 case, spermatic cord invasion in 4 cases, and lymphovascular invasion in 22 cases were detected. Intertubular seminoma in 25 cases, intratubular carcinoma in 16 cases, and GCNIS in 73 cases were detected.
Conclusion: The major criteria to determine treatment choices are histopathological diagnosis, pathological tumor stage, serum tumor markers and presence of metastasis. According to AJCC 8th edition, addition of hilar soft tissue invasion to staging has increased the number of our pT2 cases. Moreover, assuming discontinuous tumor invasion of spermatic cord by vascular invasion as pM1 has also increased the number of metastatic testis tumors.
Keywords: Intratubular; intertubular; seminoma; mixed germ cell tumor.

ÖZ

Amaç: Testiküler germ hücreli tümörler (TGHT), genç yetişkin erkeklerde sık görülen ve bu dönemdeki kanser ilişkili ölümlerin önemli bir sebebi olan solid neoplazmlardır. Histopatolojik sınıflandırma ve evrelemedeki değişiklikler, prognoz ve tedaviyi etkilemektedir. Bu çalışmanın amacı, TGHT tanısı almış olguların analizini yapmak, prognostik parametreleri ve bu parametrelerin insitu germ hücreli neoplazi (İGHN), intratubuler ve intertubuler tümörlerin varlığı ile ilişkilerini gözden geçirmektir.
Gereç ve Yöntemler: Bu çalışmada TGHT tanısı almış 77 olgunun Hematoksilen&Eozin boyalı preparatları yeniden değerlendirildi. İGHN, intratubuler ve intertubuler germ hücreli tümör varlığı kaydedildi. Amerikan Birleşik Kanser Komitesi (American Joint Committee on Cancer, AJCC) 8. basımındaki değişiklikler temel alınarak yeniden histopatolojik sınıflama ve evreleme yapıldı.
Bulgular: Olguların büyük çoğunluğu (n=42) seminom tanısı aldı, bunu mikst germ hücreli tümör (n=33) ve spermatositik tümör (n=2) izledi. Olguların 30'unda rete testis invazyonu, 6'sında epididim invazyonu, 10'unda hiler yumuşak doku invazyonu, 1'inde tunika vaginalis invazyonu, 4'ünde spermatik kord invazyonu ve 22'sinde ise lenfovasküler invazyon görüldü. İntertubuler seminom 25, intratubuler karsinom 16, İGHN ise 73 olguda saptandı.
Sonuç: Histopatolojik tanı, patolojik tümör evresi, serum tümör belirteçleri ve metastaz olup olmaması tedaviyi belirleyen başlıca kriterlerdir. AJCC 8. basıma göre, hiler yumuşak dokuya invazyonun evrelemeye eklenmesi ile pT2 olgularımız arttı. Ayrıca, spermatik kord içerisinde devamlılık göstermeksizin damar trombüsünden yumuşak doku invazyonun pM1 olarak kabul edilmesi de metastatik testis tümörlerinin sayısını artırmıştır.
Anahtar kelimeler: İntratubuler; intertubuler; seminoma; mikst germ hücreli tümör.

INTRODUCTION

Testicular germ cell tumors (TGCT), which make up about 98% of all testicular neoplasms, are the most common solid neoplasms in young adult men aged between 15-45 years (1,2). Genetic and environmental factors play an important role in the etiology. The World Health Organization (WHO) divided TGCTs into two subgroups as germ cell neoplasia in situ (GCNIS)-derived tumors and non-GCNIS-derived tumors (3). Clinical behavior and treatment methods of the tumors are effective in this differentiation (Figure 1). TGCTs are also divided into two groups as seminoma and non-seminomatous tumors (NST) according to their histological features (4). Changes in the pathological staging of T1 and T2 tumors were made in the 8th edition of the American Joint Committee on Cancer (AJCC), and hilar soft tissue invasion and epididymal invasion were accepted as pT2. Additionally involvement of the spermatic cord soft tissue via a vascular thrombus without continuous path is better to be classified as a metastatic deposit, pM1 (5). Approximately two-thirds of the patients are in stage 1 at the time of diagnosis. Although TGCTs are malignant, they respond well to most treatments. They metastasize especially to retroperitoneal lymph node, lung, liver, bone and central nervous system (6).

The aim of this study was to analyze the cases diagnosed with TGCT in our hospital, to review the prognostic parameters, to detect GCNIS, intratubular and intertubular tumors and changes of staging of the tumors.

MATERIAL AND METHODS

Ethics committee approval was received for this study from the ethics committee of Bezmialem Vakif University (03.12.2019 and 22/411). The cases diagnosed with TGCT from the specimens of orchiectomy performed in our center between January 2012 and June 2019 were included in the study. Non-germ cell tumors were excluded. Hematoxylin&Eosin (HE)-stained sections of the cases were re-evaluated. Histopathological diagnosis of the tumors and prognostic parameters including tumor diameter, tumor histology, rete testis, tunica vaginalis, hilar soft tissue and spermatic cord involvement and lymphovascular invasion were re-examined considering new classification and staging systems (4,5). The presence of GCNIS, intratubular and intertubular germ cell tumors was recorded. Clinical findings and demographic data of the cases were also presented.

Statistical Analysis

Statistical evaluation was calculated with Microsoft Office 2010 Excel programme. Mean, standard deviation, median, min-max, frequency and percentage values were given as descriptive statistics.

RESULTS

Between January 2012 and June 2019, there were 77 cases diagnosed with germ cell tumors in our department. The mean age was 35.7±10.2 (range, 17-64) years. The most common symptom was painless swelling in the testis. The laterality was; on the left testis in 43 (55.8%) and in the right testis in 34 (44.2%) of the cases. The mean diameter of the tumors was 4.3±1.9 (range, 0.4-12) cm. Forty two (54.5%) of the cases had seminoma, 33 (42.9%) had mixed germ cell tumors (MGCTs), and 2 (2.6%) had spermatocytic tumors. GCNIS was observed in 73 (94.8%) of the cases, while it was not observed in 4 (5.2%) of the cases. There were no intratubular germ cell tumors in 61 (79.2%) cases, while they were present in 16 (20.8%) of the cases. One of these cases had intratubular embryonal carcinoma and the others had intratubular seminoma. Intertubular germ cell tumor was not observed in 52 (67.5%) of the cases, while it was observed in 25 (32.5%) of the cases (Figure 2). The stage of tumors was as follow: 51 (66.2%) patients of T1, 23 (29.9%) patients of T2, 2 (2.6%) patients of T3, and 1 (1.3%) patient of T4 was recorded. Thirty (39.0%) of the cases had rete testis involvement. Pagetoid spread was observed in 4 cases with rete testis involvement, and two of them also had stromal invasion (Figure 3). Lymphovascular invasion (LVI) was detected in 22 (28.6%) of the cases. Two of them had LVI in the spermatic cord. Epididymal invasion was detected in 6 (7.8%) cases, hilar soft tissue invasion in 10 (13.0%) cases, tunica vaginalis invasion in 1 (1.3%) case, spermatic

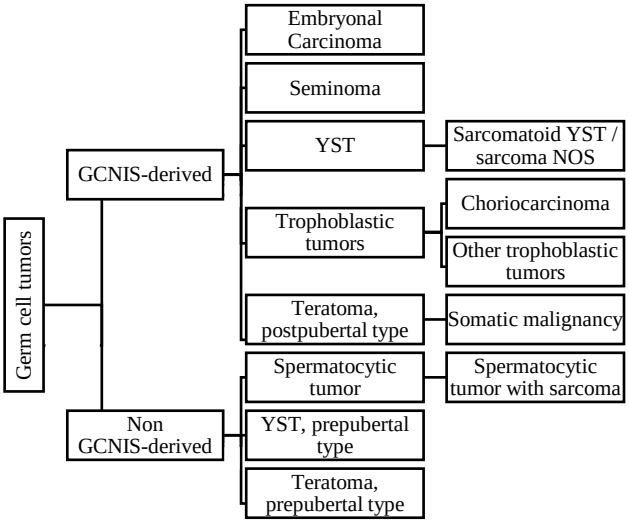

Figure 1. Classification of germ cell tumors (4). GCNIS: Germ cell neoplasia in situ, YST: Yolk Sac Tumor, NOS: Not Otherwise Specified

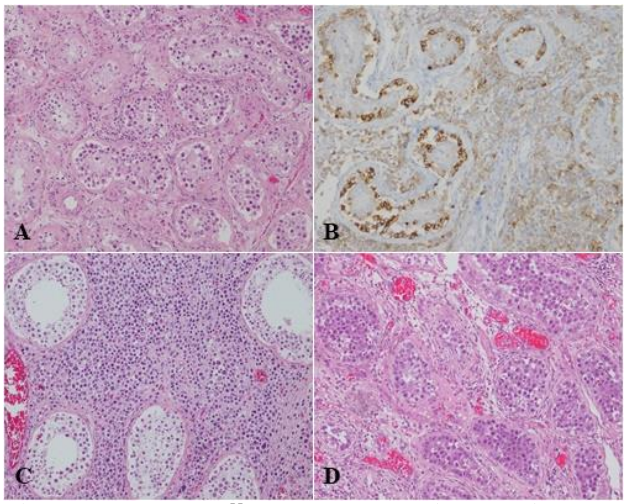

Figure 2. Germ cell tumor in situ (A, x100) PLAP immunohistochemistry with positive staining of germ cell tumor in situ (B, x100), intertubular and intratubular seminoma (C,D, x100).

cord invasion in 4 (5.2%) cases and scrotal invasion in 1 (1.3%) case (Table 1). Spermatic cord metastasis was detected in only one case. Retroperitoneal lymph node (RPLN) involvement was present in 19 cases. The mean follow up was 28.0±22.9 (range, 1-91) months. Two cases (2.6%) had lung metastasis, two cases (2.6%) had liver metastasis, and one case (1.3%) had central nervous system metastasis. The case with spermatic cord metastasis was followed for 38 months, and no other metastasis focus was observed. Intertubular seminoma was detected in 3 of 22 metastatic cases.

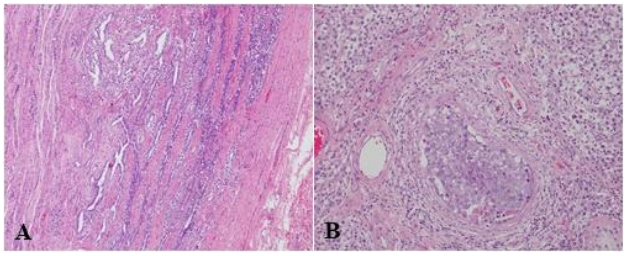

Figure 3. Rete testis stromal invasion and pagetoid spread (A, x40), lymphovascular invasion in the hilar soft tissue (B, x100)

Table 1. Histopathological diagnosis and pathological tumor stages of the cases

	Seminoma (n=42)	MGCT (n=33)	Spermatocytic Tumor (n=2)
Age (years), mean±SD (min-max)	38.0±9.8 (21-64)	31.7±8.1 (17-52)	57.0±4.0 (53-61)
Diameter (cm), mean±SD (min-max)	3.9±2.0 (0.4-12)	4.6±1.5 (2.5-9.2)	6.5±1.5 (5-8)
Localization, n (%)			
Right	21 (50.0%)	12 (36.4%)	1 (50.0%)
Left	21 (50.0%)	21 (63.6%)	1 (50.0%)
GCNIS, n (%)	41 (97.6%)	32 (97.0%)	0 (0.0%)
Intertubular seminoma, n (%)	20 (47.6%)	5 (15.2%)	0 (0.0%)
Intratubular seminoma, n (%)	8 (19.1%)	7 (21.2%)	0 (0.0%)
Intratubular embryonal carcinoma, n (%)	0 (0.0%)	1 (3.0%)	0 (0.0%)
Pathological Stage, n (%)			
Rete testis invasion	12 (28.6%)	18 (54.5%)	0 (0.0%)
Hilar soft tissue invasion	6 (14.3%)	4 (12.1%)	0 (0.0%)
Epididymal invasion	3 (7.1%)	3 (9.1%)	0 (0.0%)
Spermatic cord invasion	0 (0.0%)	4 (12.1%)	0 (0.0%)
Tunica vaginalis invasion	1 (2.4%)	0 (0.0%)	0 (0.0%)
Lymphovascular invasion	7 (16.7%)	15 (45.5%)	0 (0.0%)

MGCT: Mixed Germ Cell Tumor, GCNIS: Germ cell neoplasia in situ

DISCUSSION

GCNIS-derived tumors are seen in the postpubertal period between the ages of 18-45 years, on average, while non-GCNIS-derived tumors are usually seen in the prepubertal period. In our study, the most common tumors were GCNIS-derived tumors, and there were 4 non-GCNIS-derived germ cell tumors.

TGCTs are also divided into two groups as seminoma and NST, considering prognosis and different treatment options. Seminoma is seen at the rate of 55%, while NST is seen at the rate of 45% (7,8). In this study, seminoma was detected at the rate of 54.5%, spermatocytic tumor was observed at the rate of 2.6%, and MGCT was observed at the rate of 42.9%. Embryonal carcinoma and teratoma were the most common MGCTs. Seminomas may sometimes be accompanied by syncytiotrophoblasts, which should not be considered as MGCTs. The term "unclassified intratubular germ cell neoplasia", which were precursor lesions of TGCTs was replaced by the term "GCNIS" in the 2016 WHO classification. GCNISs are the precursor lesions of invasive germ cell tumors, and 70% of these lesions develop invasive cancer 7 years after diagnosis (9). GCNISs are seen 72-98% of peritumoral area, whereas they can be detected incidentally in 0.4-0.8% of the patients without an invasive tumor (10). GCNIS was seen in 94.8% of our cases, which was consistent with the rates reported in the literature. OCT3 / 4 and placental alkaline phosphatase (PLAP) are most commonly used in detecting GCNIS, although numerous immunohistochemical markers have been investigated (11) in addition to HE sections. Among non-invasive germ cell neoplasms, there are specific forms of intratubular germ cell neoplasia called intratubular seminoma (ITS) or intratubular embryonal carcinoma (ITEC) (3). Intratubular carcinoma was observed in 20.8% of our cases. All of these cases had invasive tumors. ITS is located in a separate focal area, between the seminiferous tubules next to the mass-forming seminoma area, without disturbing the pattern of the tubules. This tumor, also called microinvasive germ cell tumor, can be detected in cases operated due to cryptorchidism (12). The presence of concomitant distant metastases at the time of diagnosis in two ITS cases of Henley (13) suggests that these in situ lesions may be associated with prognosis.

Histopathological diagnosis, pathological tumor stage, serum tumor markers and the presence of metastasis are the main criteria that determine treatment. Both seminoma and NSGCT have similar criteria for pathological staging. However, unlike NSGCT, pT1 is subclassified as T1a and T1b according to tumor diameter in seminoma (5). Tumor diameter is known to be important in adjuvant RT or carboplatin-based therapy. There is no criterion for diameter in other germ cell tumors. Tumor stage of seminomas were reported as following: pT1a (28.6%),

pT1b (71.4%). Rete testis invasion is clinically important, although it is not involved in pathological staging. The tumor should be screened carefully with serial sections, since a tumor showing pagetoid spread throughout the rete testis may also show stromal invasion. Yilmaz et al. (14) stated that the tumor showing rete testis stromal invasion had a direct relationship with advanced clinical stage, but there was no relationship between pagetoid spread and clinical stage. Rete testis invasion increases the risk of recurrence with increasing tumor diameter, especially in seminomas (15). In addition, Lobo et al. (16) found a relationship between rete testis invasion and LVI and that rete testis invasion increased in seminomas over 3 cm. Rete testis stromal invasion was observed in 39% of our cases, and two of them also had pagetoid spread. There were also two cases showing only pagetoid spread. LVI was seen in 46.7% of the cases with rete testis invasion. There were 33 cases with seminoma over 3 cm, 33.3% of them had rete testis invasion, which was consistent with the literature reported by Lobo et al. (16).

Pathological tumor stage is T2 in the presence of tunica vaginalis, LVI, epididymal invasion and hilar soft tissue invasion (17). Hilar soft tissue invasion is most common localization for extratesticular spread in both seminoma and NSGCT (1,18). Hilar soft tissue invasion rates of our cases (13.0%) was consistent with the literature. Interestingly, hilar soft tissue invasion is staged as T2, while rete testis invasion, which is the border in transition of tumor to this area and found to be associated with high metastasis especially in seminomas, and is not involved in pathological staging. LVI can be found in the intraparenchymal area, tunica and spermatic cord, and it is considered as pT2 (19). LVI is effective in recurrence. Divrik et al. (20) detected relapse in 75.5% of those with LVI and 17.9% of those without LVI in 211 Stage 1 NSGCT cases. McCleskey et al. (21) showed that the first one had a more advanced clinical stage in their study comparing LVI seen in NSGCTs without spermatic cord invasion with LVI identified in the testis. They did not detect this relationship in seminomas. Gordetsky et al. (22) found that LVI observed in the spermatic cord and T3 were in similar clinical stages in NSGCTs, and there was no difference in terms of recurrence rates. One of our cases had LVI in the spermatic cord and liver metastasis.

Another noteworthy point is whether spermatic cord invasion is continuous or not. Continuous spermatic cord stromal invasion with or without LVI is considered as pT3, while spermatic cord stromal invasion, which is discontinuous along the spermatic cord, with or without LVI is considered as pM1 (17,19). This may require a complete sampling of the spermatic cord by numbering from the hilar soft tissue to the surgical margin of the spermatic cord in testicular tumors.

In addition to histopathological diagnosis and pathological staging, the presence of microlithiasis, atrophy and sertoli cell nodule in the non-tumor testicular tissue are other risk factors that should be stated in the report (23). In our reports, we record the findings such as microlithiasis, atrophy and sertoli cell nodules in non-tumoral testicle tissue. TGCTs may be accompanied by lymphoplasmacytic inflammation and granulomas. Sharma et al. (24) found that CD66b-positive neutrophils were an independent prognostic factor in their study.

Cisplatin-based chemotherapy and surgery provide high cure rates, while resistance to treatment and poor prognosis were seen at the rate of 15% in germ cell tumors. Martinelli et al. (25) found that CALCA and MGMT were associated with poor prognosis, and CALCA methylation was associated with refractory disease in germ cell tumors. While microsatellite analysis of mismatch repair genes is not associated with clinical course, epididymal invasion and EGFR expression are associated with recurrence in stage 1 tumors (26). Aurora-B, serine-threonine kinases, GPR30 and HMGAs are the new molecules that can be used in targeted therapy in resistant cases (27).

CONCLUSION

In this study, we discussed the important criteria in the staging of testicular germ cell tumors, accompanied by our cases. Both histopathological diagnosis rates and pathological stages are consistent with the literature. We observed that the number of pT2 tumors increased with the addition of hilar soft tissue invasion to the staging. Additionally a case with spermatic cord invasion was previously regarded as pT3 was carried to pM1 group. We think that it is necessary to examine the whole cord by coding in macroscopic evaluation if possible, since spermatic cord involvement may be associated with T3 or M1. The number of spermatic cord metastasis was detected in only one case. More studies with large number of patients would be helpful for making comparison with prognostic parameters.

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Comparison of Type I Diabetes Frequency in Children with Cesarean and Normal Vaginal Delivery

Sezaryen ve Normal Vajinal Doğan Çocuklarda Tip 1 Diyabet Sıklığının Karşılaştırılması

İbrahim Ethem ŞAHİN[1]
0000-0003-3745-7015
Ceyhan HACIOĞLU[1]
0000-0002-0993-6118
Merve ALPAY[1]
0000-0002-8782-9561
Önder KILIÇASLAN[2]
0000-0002-9311-006X

[1]Düzce University Faculty of Medicine Department of Medical Biochemistry, Düzce, Turkey

[2]Düzce University Faculty of Medicine Department of Child Health and Diseases, Düzce, Turkey

Corresponding Author
Sorumlu Yazar
İbrahim Ethem ŞAHİN
ibrahimethem71@yahoo.com

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ABSTRACT

Aim: The effect of type 1 diabetes mellitus over the world is rising day after day. There are controversial results that may be related to cesarean delivery that has become widespread in recent years and the risk of type 1 diabetes mellitus. In this study, we aimed to investigate the frequency of type 1 diabetes mellitus in children born by cesarean delivery or normal vaginal delivery, considering that there may be an association between mode of birth way and diabetes mellitus.

Material and Methods: The study was organized with 368 children who were diagnosed as type 1 diabetes mellitus between 8-16 ages that applied to the diabetes outpatient clinic in 2019. The patients were grouped according to the mode of delivery. Descriptive data such as age, gender, HBA1c, and BMI were evaluated retrospectively.

Results: The children with type 1 diabetes mellitus, cesarean delivery show 33.2% more than normal vaginal delivery ($p<0.001$). While 9.0% of children with cesarean delivery had mother's diabetes mellitus, this rate was 5.7% in children with normal vaginal delivery. Similarly, 5.3% of children with cesarean delivery had diabetes mellitus in their father, while this rate was 8.9% in children with normal vaginal delivery.

Conclusion: According to the results of this study, cesarean delivery may have a significant effect on the risk for type 1 diabetes mellitus in childhood either adolescence. Even if parents with diabetes were not included, it was found that cesarean delivery have meaningful relation by diabetes mellitus stimulation. Regarding this, further studies are needed.

Keywords: Cesarean delivery; normal vaginal delivery; type 1 diabetes.

ÖZ

Amaç: Tip 1 diyabetin dünya üzerindeki etkisi her geçen gün artmaktadır. Son yıllarda yaygınlaşan sezaryen doğum ve tip 1 diyabet riski arasında ilişkili olabilecek tartışmalı sonuçlar vardır. Bu çalışmada, doğum şekli ve tip 1 diyabet arasında bir bağlantı var olduğunu düşünerek sezaryen ve normal vajinal yolla doğan çocuklarda tip 1 diyabet görülme sıklığının araştırılması amaçlandı.

Gereç ve Yöntemler: Araştırmaya 2019 yılında diyabet polikliniğine başvuran 8-16 yaşları arasında olan tip 1 diyabet tanısı konmuş 368 çocuk dahil edildi. Hastalar doğum şekline göre gruplandırıldı. Hastalardan elde edilen yaş, cinsiyet, HBA1c ve BMI gibi tanımlayıcı veriler geriye dönük olarak değerlendirildi.

Bulgular: Tip 1 diyabet tanısı olan çocuklarda sezaryen doğumun normal vajinal doğuma göre %33,2 daha fazla olduğu görüldü ($p<0,001$). Sezaryen ile doğan çocukların %9,0'unun annesinde diyabet tanısı bulunurken, normal vajinal yolla doğan çocuklarda bu oran %5,7 idi. Benzer şekilde, sezaryen ile doğan çocukların %5,3'ünün babasında diyabet tanısı bulunurken, normal vajinal yolla doğan çocuklarda bu oran %8,9 idi.

Sonuç: Bu çalışmadan elde edilen sonuçlara göre, sezaryen doğum çocukluk veya ergenlik döneminde tip 1 diyabet riski üzerinde önemli bir etkiye sahip olabilir. Bununla birlikte, diyabetli ebeveyn olguları dahil edilmese bile, sezaryen ile doğum ve tip 1 diyabet arasında önemli bir ilişki olduğu bulunmuştur. Bununla ilgili olarak, daha ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: Sezaryen doğum; normal vajinal doğum; tip 1 diyabet.

INTRODUCTION

Diabetes mellitus (DM) is one of important metabolic diseases defined as hyperglycemia caused by secretion or activity of impaired insulin (1). Some patients with diabetes might life-threatening experience as acute hyperglycemic attacks, while others may life-threatening experience as acute hypoglycemic attacks for outcome diabetes treatment (2). During disease progression, some complications such as retinopathy, nephropathy, neuropathy, atherosclerosis and gangrene might be seen (3). Diabetes is a common disease whose true prevalence is unknown and the population of patients have diabetes are increasing day by day. While the estimated number of patients were around 380 million in 2014, this number is expected to reach 592 million by 2035 (4). In the national health and nutrition analysis conducted using fasting blood sugar and oral glucose tolerance test at 2005/2006, and it was determined that the prevalence of diabetes was 12.9% in individuals under 20 years of age in the United States. This rate of 12.9% corresponds to approximately 40 million people (5). In a study on pre-diabetes prevalence in adults with diabetes in 2010 in China (6), diabetes was 11.6% (effected 113.9 million people), and pre-diabetes was 50.1% (effected 493.4 million people). For classification made in 1979, two main kinds of diabetes were identified: type 1 (insulin dependent, IDDM) and type 2 (non insulin dependent, NIDDM). Among cases of total diabetes, 5-10% patients with DM are in the type 1 group approximately (7).

Patients often have a sudden onset such as polyuria and rapid weight loss. Patients have insulin deficiency as a result of a problem caused by pancreatic beta cells. The peak incidence occurs in childhood and adolescence. Type 2 group accounts for 90% of all patients with DM (8). The symptoms in patients are minimal and not prone to ketosis. In this group of patients, insulin concentrations may be normal, decreased or increased. In this form of diabetes, the effect of insulin is generally impaired. It is commonly associated with obesity and weight loss alone can control hyperglycemia. However, many individuals with type 2 diabetes may need dietary intervention, antihypertensive agents, or insulin therapy to control hyperglycemia (9). Although the disease can be seen at a young age, it usually occurs after the age of 40 (8).

Worldwide cesarean delivery is increasing day by day. Scientific progress, social, cultural and legal changes have led to a fundamental cesarean change between patients and doctors (10). Changing risk profiles and previous cesarean births in old primipas may contribute to the increase of the number of cesarean people (11). Despite all these perspectives, cesarean birth has numerous potential complications (infection, organ injuries, blood transfusion need) for mother and baby (12). Apart from the risks during the operation, there are many postpartum complications such as thromboembolism (10). There are various risks (such as uterine rupture, placenta previa) in women who have delivered by cesarean (13). In recent years, it has been observed that there may be some risks (neonatal respiratory distress, allergic rhinitis, pulmonary hypertension, type 1 DM) in babies born by cesarean section (14-16).

In 1991, 15.3% of all births in Germany were performed by cesarean, while in 2012 this rate was 31.7% (17). The cesarean rate in Sub-Saharan Africa is 3%, 31% in Central America and 32.2% in the USA (18,19). This elevation can be explained by a rising tendency to avoid risk, obstetric practice adaptation and increased media caution. It was carried out in the European Union in 2000 with 221 cesarean per 1000 live births, this rate increased to 268 per 1000 live births in 2011 (17).

Many studies have investigated cesarean section regarding the threating of type 1 diabetes in childhood. A meta-analysis results involved 16 observational studies showed that there is a potential relationship among higher frequency of cesarean section and an increased risk of type 1 DM (20). In the meta-analysis included 20 retrospective studies, there was a 20% increasement at childhood-onset type 1 diabetes risky in children giving birth by cesarean section (21). Many studies have researched cesarean and type 1 diabetes, however in some studies, the findings have been incoherent, possibly as a cause of insufficient sampling and limited strength. The aim of this study is to determinate whether a relationship with the risk of type 1 diabetes in children born with cesarean section and normal vaginal delivery.

MATERIAL AND METHODS

The literature review was done using PubMed and using the keywords "cesarean delivery", "normal vaginal delivery", "mode of delivery", "diabetes mellitus, type 1", "IDDM". The researches carried out mostly belonged to 2007 and before. The data in the patient files of all children who were between 6-18 ages who applied to the Endocrinology outpatient clinic of Düzce University in 2019, were diagnosed with type 1 DM, and their HbA1c value was above 6%, were analyzed retrospectively. Children who have type 1 DM between the ages of 8 and 16, having a mode of delivery in epicrisis, maternal body mass index (BMI) 20-30 and children with elective cesarean section and normal vaginal delivery were included in the study. The criteria for exclusion are children under the age of 8 and over 16, lack of natal knowledge in epicrisis, children with urgent cesarean delivery and the mother has any chronic conditions. According to the information obtained from the patient files, the patients were separated with two groups as normal vaginal birth and elective cesarean birth. 368 of 1574 patients who contain the inclusion criteria were included in the study. Of the 368 patients, 205 (55.7%) were male and 163 (44.3%) were female. This study was approved by Düzce University Non-Interventional Health Research Ethics Committee (02.03.2020 and 2020/29) prior to any data collection. In accordance with the Helsinki Declaration Principles, “informed consent” was taken from the patients who participated in the study.

Statistical Analysis

Descriptive statistics for categorical variables were used as numbers and percentages, and mean and standard deviation (SD) for numerical variables. Pearson chi-square test was used to examine between type of birth and the proportion of children with type 1 DM as well as obstetric features such as the number of parents with diabetes or non-diabetes and sex of the child. Kolmogorov-Smirnov tests were used to determine the suitability of variables for normal distribution. For univariate analyzes between

cesarean section and normal vaginal birth groups, Student's t-test was used. SPSS 20.0 software was used for statistical analysis, and p values <0.05 were regarded as statistically significant.

RESULTS

Amongst 368 children with birth way data, 245 were born by cesarean delivery and 123 by normal vaginal delivery. The most cases (66.6%) of children with type 1 DM were born by cesarean section. Besides that, 33.4% of children with type 1 DM were born by normal vaginal delivery. The children who have type 1 DM, cesarean section births show 33.2% more than normal vaginal delivery (p<0.001). There was no statistically significant difference in gender, maternal age and BMI of children with diabetes between cesarean delivery and normal vaginal delivery groups; thus, homogeneity between the groups is provided.

The number of mothers and fathers with diabetes are shown in Table 1. The number of mothers with diabetes in cesarean birth group (n=22, 9.0%) is higher compare to normal vaginal birth group (n=7, 5.7%). However, the number of fathers with diabetes in normal vaginal birth group (n=11, 8.9%) is higher than in cesarean birth group (n=13, 5.3%). When it is evaluated separately according to the type of birth and the diabetes status of the parents, children with type 1 diabetes, whose parents non-diabetes, were common in the cesarean section delivery group (n=210, 85.7%) according as vaginal normal delivery group (n=105, 85.4%).

DISCUSSION

We evaluated all children between the ages of 6 and 18 who were admitted to the pediatric type 1 DM outpatient clinic in 2019 year which diagnosis of type 1 diabetes using hospital automation throughput. In accordance with results, the frequency of type 1 diabetes was significantly higher for children born with cesarean birth compared to children born with vaginal birth way. Samuelsson et al. (22) found an increased risk of type 1 DM for children born by cesarean in a study in Norway. However, there was no increased risk for children of non-diabetic mothers, whereas there was an increased risk for cesarean delivery in children born from diabetic mothers. They attribute that increased risk of diabetic mothers to diabetic mothers giving birth more frequently by cesarean. From our retrospective study, we found that the ascending population of type 1 diabetes in children borned by cesarean section continues when we exclude children with DM in their family (mother or father).

The increase of the risk of type 1 DM in children born with cesarean was observed in almost all studies conducted in this direction. However, other factors that cause diabetes type 1 in childhood (gestational age, birth weight, maternal age, breastfeeding and maternal diabetes) cause complexity to clarify relationship cesarean delivery and type 1 diabetes.

In a meta-analysis study conducted in 2010, type 1 diabetes was %10 risk increasement observed in children born with a birth weight over 4 kg compared to those born with 3-3.5 kg (23). The findings of the more population study holded by Khashan et al. (24) were consistent with synchronization results of this study. Another study published in 2014, the risk of occurs type 1 diabetes in childhood was found to be 18% in preterm infants (25). Additionally, Bingley et al. (26) found a strong relationship between high maternal age and an increased risk of developing diabetes in the child.

Cardwell et al. (15,23) found that high birth weight, short gestation period and high maternal age increased the risk of diabetes at child in a meta-analysis conducted over twenty studies. Moreover, when other risk factors (gestational age, birth weight, maternal age, breastfeeding of the baby and maternal diabetes) that were stated to exist for type 1 diabetes were added, a very small augmentation for risk of cesarean delivery was observed. On the other hand, without these factors, cesarean under elective conditions increased the risk of type 1 diabetes by approximately 20%. Compatible with Cardwell et al. research, when the presence of type 1 diabetes was excluded in the mother or father, we also found that the rate of cesarean delivery among children with type 1 diabetes was significantly higher than the normal vaginal delivery.

Many patients with type 1 diabetes have autoimmune damage mediated by T cells of pancreatic beta cells, some are idiopathic (27-29). Islet cell cytoplasmic antibodies (30), insulin autoantibodies (31), antibodies to glutamic acid decarboxylase (32), insulinoma-associated antigens (33), zinc transporter ZnT8 (34) are the best-defined autoantibodies associated with type 1 diabetes.

Although some hypothesis (intestinal microbiota, bacterial revelation during pregnancy, perinatal period stress and hygiene) have been suggested in increasing the risk of type 1 diabetes with cesarean delivery, it is not fully understood what the possible offender is. However, the most prominent of these hypotheses is the altered intestinal microbiata. It is thought that intestinal microbiota plays an important drive to stimulate the progress of immune system (35).

Novel studies have indicated that the intestinal microbiota of a child born by cesarean or normal vaginal birth is different (36). In a prospective study, the rate of bifidobacterium and lactobacillus-like bacterial colonization delayed in the cesarean babies of the mother who had antibiotic prophylaxis, and the rate of babies who gave birth in a normal vaginal way in 1 month and 10 days. This study also showed that changes on intestinal flora of a baby born by cesarean would not take less than 6 months (37).

Table 1. Demographic and obstetric characteristics

	Elective Cesarean Delivery (n=245)	Normal Vaginal Delivery (n=123)	p
Maternal age	29.50±4.15	29.3±1.07	0.599
Maternal BMI	24.21±3.77	23.68±1.94	0.144
HbA1c	8.52±2.15	8.14±1.86	0.096
Gender			
Male	102 (41.6%)	61 (49.6%)	0.147
Female	143 (58.4%)	62 (50.4%)	
Parents with DM			
Mother	22 (9.0%)	7 (5.7%)	
Father	13 (5.3%)	11 (8.9%)	0.248
None	210 (85.7%)	105 (85.4%)	

BMI: Body mass index, HbA1c: Hemoglobin A1c, DM: Diabetes Mellitus

Beside those in vivo experiments, normal intestinal flora has been shown to have an immunostimulating effect. Mucosal sort IgA cells are relatively less than immune-supressed animals (38). The fact that babies born with cesarean are relegated high risk of improving type 1 diabetes in childhood compared to children born with a normal vaginal route may be a late-activated immune system due to the difference in intestinal microbiota in these children, or another reason might be investigated (39). Further studies are needed to make etiopathogenic mechanism more clear about the frequency of diabetes and the way of birth.

Conflicts of Interest: The authors declared that no conflicts of interest.

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The Prediction of Allergic Proctocolitis by Using Hematological Parameters

Allerjik Proktokolit Öngörüsünde Hematolojik Parametrelerin Kullanımı

Sebahat ÇAM
0000-0001-7394-3569

İstanbul Medeniyet University School of Medicine Department of Pediatric Gastroenterology, İstanbul, Turkey

ABSTRACT

Aim: Allergic proctocolitis (AP) is a common cause of rectal bleeding in infants. There is no diagnostic tool specific to the disease. The aim of the study was to evaluate hemogram parameters as a marker of inflammation in patients with AP.
Material and Methods: The files of patients who were exclusively breastfed and diagnosed as food protein induced AP were examined retrospectively. A hundred and fifty patients diagnosed with AP were included in the study. The same number of healthy babies formed the control group. Parameters in complete blood count were compared between patient and control groups. Rectosigmoidoscopic examination was performed to confirm the diagnosis in patients who could not achieve complete improvement with diet therapy, and hemogram parameters were evaluated according to eosinophilic infiltration in biopsies.
Results: The mean platelet volume (MPV) values ($p<0.001$) and eosinophil percentages ($p=0.001$) of the AP group were higher than the control group. The mean hemoglobin values of the AP group were statistically significantly lower than the control group ($p<0.001$). No statistically significant difference was found between patient and control groups in terms of white blood cell (WBC) count, platelet count, platelet distribution width (PDW) and neutrophil/lymphocyte ratio (NLR). WBC count, platelet count, hemoglobin, MPV and C-reactive protein (CRP) values were similar between groups of patients according to the number of eosinophils in colon biopsies.
Conclusion: We observed a significantly higher MPV values and eosinophil percentages in patients with AP. These parameters maybe helpful in diagnosis of AP.
Keywords: Allergy; proctocolitis; hemogram.

ÖZ

Amaç: Alerjik proktokolit (AP), bebeklerde görülen rektal kanamanın yaygın bir nedenidir. Bu hastalığa özgü olan bir tanı aracı yoktur. Çalışmanın amacı, AP tanısı konmuş olan hastalarda inflamasyonun bir belirteci olarak hemogram parametrelerini değerlendirmektir.
Gereç ve Yöntemler: Sadece anne sütü ile beslenen ve gıda proteini kaynaklı AP tanısı konan hastaların dosyaları geriye dönük olarak incelendi. AP tanısı konulmuş olan 150 hasta çalışmaya dahil edildi. Aynı sayıda herhangi bir sağlık sorunu saptanmayan bebek ise kontrol grubunu oluşturdu. Tam kan sayımındaki parametreler hasta ve kontrol grupları arasında karşılaştırıldı. Diyet tedavisi ile tam iyileşme sağlayamayan hastalarda tanıyı doğrulamak için ilave rektosigmoidoskopik inceleme yapıldı ve hemogram parametreleri biyopsilerdeki eozinofil infiltrasyon düzeyleri dikkate alınarak değerlendirildi.
Bulgular: AP grubunun ortalama trombosit hacmi (mean platelet volume, MPV) değerleri ($p<0.001$) ve eozinofil yüzdeleri ($p=0.001$) kontrol grubundan daha yüksekti. AP grubunun ortalama hemoglobin değerleri kontrol grubundan istatistiksel olarak anlamlı derecede daha düşüktü ($p<0.001$). Hasta ve kontrol grupları arasında beyaz kan hücresi (white blood cell (WBC) sayısı, trombosit sayısı, trombosit dağılım genişliği (platelet distribution width, PDW) ve nötrofil/lenfosit oranı (NLR) açısından istatistiksel olarak anlamlı bir fark bulunmadı. WBC sayısı, trombosit sayısı, hemoglobin, MPV ve C-reaktif protein (CRP) değerleri, kolon biyopsilerindeki eozinofil sayısına göre hasta grupları arasında benzerdi.
Sonuç: AP'li hastalarda anlamlı olarak yüksek MPV değerleri ve eozinofil yüzdeleri gözlendi. Bu parametreler AP tanısında yardımcı olabilir.
Anahtar kelimeler: Allerji; proktokolit; hemogram.

Corresponding Author
Sorumlu Yazar
Sebahat ÇAM
sebahat.cam@medeniyet.edu.tr

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INTRODUCTION

Food protein-related allergic proctocolitis (AP) is a rather common cause of rectal bleeding in infants younger than 6 months old (1). It is mostly caused by cow's milk proteins, which pass into breast milk (2). Allergic reactions have been reported in approximately 0.5 to 1% of infants who exclusively breastfeed (2,3). The diagnosis is usually made by clinical history and in response to the elimination diet. Excluding other diagnoses is essential. In babies undergoing rectosigmoidoscopic examination, eosinophilic infiltration is observed in colon epithelium, lamina propria, and muscularis mucosa (4,5). Since there are no markers specific to the disease, it may be considered that complete blood count parameters such as white blood cell (WBC) count, platelet count, mean platelet volume (MPV) and eosinophil percentage can be used in the diagnosis and follow-up of inflammation. MPV has been recently studied as an inflammatory marker in various conditions, including ulcerative colitis, acute pancreatitis and myocardial infarction (6-8).

The aim of this study was to evaluate the hemoglobin, leukocyte and platelet parameters in the complete blood count in infants diagnosed with AP and also to investigate the relationship of these parameters with the severity of eosinophilic inflammation in patients undergoing rectosigmoidoscopic examination.

MATERIAL AND METHODS

The files of the patients who admitted to İstanbul Medeniyet University Faculty of Medicine Pediatric Gastroenterology Outpatient Clinic between January 2010 and January 2020, who were exclusively breastfed and diagnosed as food protein induced AP were examined retrospectively. Ethical approval was obtained from the İstanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee with the decision number 2020/0259 and dated 18.05.2020.

Study Groups

A hundred and fifty patients diagnosed with AP were included in the study. All of the babies were well, except for a history of bloody and/or mucous in stool. Patients who had no other infectious and inflammatory cause in etiology, and recovered in response to the elimination diet formed the study group. Patients older than 6 months, who had incomplete data in their files, who got complementary food or formulas, or who had another reason to explain rectal bleeding (infection, anal fissure, intussusception, volvulus, Hirschsprung's disease, necrotizing enterocolitis, inflammatory bowel disease, immune deficiency syndromes, hematological diseases), children with recent surgery and patients with signs of any chronic disease were excluded from the study. A hundred and fifty age and sex matched babies with nonspecific complaints who did not have any health problems in their physical and laboratory examinations or who admitted to a well-baby clinic for routine control and had a complete blood count were formed control group.

Clinical Evaluation

In our study, the diagnosis of AP was made with the history of the presence of blood and/or mucus in the stool, regression of symptoms with the removal of responsible foods from the mother’s diet, recurrence of symptoms after the provocation test and no other reason to explain it. All complete blood count analyzes were performed in our hospital's hematology laboratory with an automatic analyzer. Rectosigmoidoscopic examination was performed in patients who did not get an adequate response despite the oligoantigenic diet to confirm the diagnosis and exclude other causes of rectal bleeding. After the cases were evaluated endoscopically (Fujinon EG/450PE5), biopsies were taken from the sigmoid colon and rectum. Biopsy samples were taken into formaldehyde, sent to the pathology laboratory, stained with hematoxylin-eosin and evaluated by a pathologist. The diagnosis of AP was confirmed in all cases with endoscopic appearance and pathological examination. The procedure was performed without sedation or colon preparation.

Statistical Analysis

SPSS v.24 program was used for statistical analysis. Categorical data are given as numbers and percentages, and numerical data are given as mean±standard deviation. Kolmogorov-Smirnov test was used to assess data normality. In comparison of numerical variables, Student's t test was used to compare two groups, while ANOVA was used in more than two independent groups. Chi square test was used for categorical variables. A p value <0.05 was considered significant.

RESULTS

Of the babies who were diagnosed with AP, 72 (48.0%) were girls and 78 (52.0%) were boys. The mean ages of the patients and controls were 2.82±1.12 and 2.78±1.13 months, respectively. The demographic properties of the groups were similar (Table 1).

Time to see blood for the first time in stool was 9.3±1.7 (range, 4-18) weeks. All patients received breast milk only. The general condition of all patients was good and their systemic examinations and growth-development values were within normal limits. Coagulation tests and stool examinations were normal in all cases.

No statistically significant difference was found between patients and controls in terms of WBC count ($p=0.064$), platelet count ($p=0.230$), platelet distribution width (PDW) ($p=0.090$) and neutrophil/lypmhocyte ratio (NLR) ($p=0.778$). These hematological parameters are shown in Table 2. The MPV values ($p<0.001$) and eosinophil percentages ($p=0.001$) of the AP group were statistically significantly higher than the control group. The mean hemoglobin values of the AP group were statistically significantly lower than the control group ($p<0.001$).

When endoscopy findings were evaluated in babies with AP, erythema, friability, lymphonodular hyperplasia and/or milimetric ulcers were detected in 138 babies, and nearly normal appearance was present in 12 babies. Histopathologically, more than 10 eosinophils were counted in per high power field (HPF) in lamina propria in 8 patients, compared to 6-10 in 15 patients and below 6 in 7 patients. When patients were grouped according to the number of eosinophils in colon biopsies (<6, 6-10, >10 eosinophils/HPF), WBC count, platelet count, hemoglobin, MPV and C-reactive protein (CRP) values were not different between the groups. Distribution of hemogram parameters according to eosinophil numbers in biopsies are shown in Table 3.

DISCUSSION

Food protein-related AP is characterized by inflammatory changes in the colon and rectum triggered by the intake of certain nutrient proteins through the gastrointestinal tract. In AP, inflammation rich in eosinophils occurs in the rectum (9). In this study, inflammatory markers detected in peripheral blood in infants with AP were evaluated as a non-specific indicator of inflammation and compared with healthy children. At the same time, the relationship between eosinophil counts in the rectum mucosa and hemogram parameters was evaluated in patients with AP.

In the current trial, mean hemoglobin values were significantly lower in the AP group. Previous studies suggest that mild anemia may be present in patients with AP due to chronic blood loss (9-11). In this study, although the mean hemoglobin level in the AP group was lower than the control group, no severe anemia was detected in any case. This shows that in patients with AP, the loss of blood in the stool is very low, as observed in the clinic. Today, the easy-to-perform NLR is also used to measure systemic inflammation. The relationship of NLR with some diseases such as heart disease, chronic disease and Mediterranean fever has been shown in the literature (12-14). In their studies investigating NLR values in allergic rhinitis and asthma, Dogru et al. found higher NLR values compared to the control group (15,16). In our study, we did not find a difference between AP and control groups in terms of leukocyte counts and NLR values.

When it comes to platelet parameters, platelets have been shown to play a critical role not only in hemostasis, but also in the immune system and inflammation (17). Platelet indexes, including platelet count, MPV, PDW, and plateletcrit, are all markers of platelet activity. Increased MPV has been observed in many chronic, inflammatory and malignant diseases (17). Of particular concern to allergic inflammation is the observation that platelets express IgE receptors on their surface. Therefore, platelets from patients with allergic diseases can also respond to common allergens (18,19). There are few studies investigating the role of inflammatory patterns in allergic events. We thought that MPV values might be useful in diagnosis, because AP is a chronic inflammatory disorder. In our study, we found that the average MPV level in children with AP was higher than the control. Similarly, Nacaroglu et al. (20) reported that MPV and plateletcrit are higher in children with AP and can be used to determine prognosis. As far as we know, there is no other study in the literature on this subject.

In other studies evaluating platelet indices in allergic events (such as atopic dermatitis, asthma), these indices have been shown to be used in diagnosis and follow-up. However, data on platelet indices on allergic diseases are controversial. Dogru et al. (21) reported that the MPV increased in children with asthma in their study. However, there are also studies showing that MPV is not different from healthy children in allergic airway diseases (22,24). Topal et al. (25) showed that MPV values increased in patients with atopic eczema. In other studies, there was no significant difference between patients with atopic dermatitis and chronic urticaria and the control group in terms of MPV value (26).

In our study, we also evaluated the relationship between the severity of eosinophilic inflammation in mucosa and blood count parameters in patients undergoing rectoscopic examination. Although not an accepted standard criterion for the diagnosis of AP, eosinophilic infiltration is characteristic throughout the mucous layers, especially in lamina propria (27-30). Winter et al. (30) reported that more than 60 eosinophils per 10 HPF in lamina propria strongly suggest AP. Eosinophils interspersed in crypts or in the muscularis mucosa are also highly associated with AP (31,32). In our study when peripheral blood parameters were evaluated according to the number of eosinophils in the mucosa, there was no significant difference between the cases.

Table 1. Demographic characteristics of the groups

	AP (n=150)	Control (n=150)	p
Age (months), mean±SD	2.82±1.12	2.78±1.13	0.760
Gender, n (%)			
Girl	72 (48.0%)	74 (49.3%)	0.817
Boy	78 (52.0%)	76 (50.7%)	

AP: Allergic Proctocolitis, SD: Standard Deviation

Table 2. Comparison of hematological parameters between the control and study groups

	AP (n=150)	Control (n=150)	p
WBC count (×10³/µL)	9.76±2.86	9.59±3.02	0.064
NLR	0.45±0.16	0.45±0.17	0.778
Hemoglobin (g/dl)	10.97±1.18	11.13±1.30	**<0.001**
Platelet count (×10³/µL)	365.71±86.23	360.12±78.46	0.230
MPV (fl)	9.07±0.78	8.61±0.62	**<0.001**
PDW%	15.89±0.55	15.72±0.51	0.090
Eosinophil percentage	5.58±2.46	5.18±1.90	**0.001**

AP: Allergic Proctocolitis, WBC: White Blood Cell, NLR: Neutrophil/Lymphocyte Ratio, MPV: Mean platelet volume, PDW: Platelet distribution width

Table 3. Hemogram parameters according to eosinophil numbers in biopsies

	<6/HPF (n=7)	6-10/HPF (n=15)	>10/HPF (n=8)	p
WBC count (×10³/µL)	8.58±2.96	10.36±3.04	8.86±2.85	0.333
Hemoglobin (g/dl)	11.41±1.04	10.87±1.53	11.21±0.85	0.543
Platelet count (×10³/µL)	329.28±83.92	395.80±92.26	349.87±97.10	0.248
MPV (fl)	8.37±0.82	8.89±1.06	9.4±0.83	0.096
CRP (mg/L)	0.31±0.07	0.28±0.06	0.34±0.05	0.501

HPF: High Power Field, WBC: White Blood Cell, MPV: Mean Platelet Volume, CRP: C-Reactive Protein

In the literature, there is no study on the relationship between severity of inflammation in the colon and platelet parameters in patients with AP. When evaluating other diseases that cause inflammation in the colon, studies of inflammatory bowel disease generally found low MPV and high platelet counts. In some studies, platelet parameters have been associated with disease activity (33,34). This adverse condition may be related to the severity of inflammation. Gasparyan et al. (35) assumed that, high-grade inflammatory diseases result in low levels of MPV, while low-grade inflammatory diseases have an adverse effect on MPV. In our study, when colon biopsies of patients undergoing rectosigmoidoscopy were evaluated according to eosinophilic infiltration, there was no significant relationship between eosinophil count and hemoglobin value, leukocyte and platelet parameters in biopsy samples.

The limitations of the study are that it is retrospective. Also, other colon inflammation markers such as calprotectin have not been studied.

CONCLUSION

In this retrospective clinical trial, we observed a significantly higher MPV values and eosinophil percentages in patients with AP. These hematological parameters may be used to predict AP. Further clinical trials with larger number of patients are required to reach a definitive result.

Conflict of Interest: There are no conflicts of interest.

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Protective Effects of Ellagic Acid Against Chemotherapy-Induced Hepatotoxicity

Kemoterapi Kaynaklı Hepatotoksisiteye Karşı Ellajik Asitin Koruyucu Etkileri

Alper YALÇIN[1]
0000-0002-8975-1008
Hikmet KELEŞ[2]
0000-0002-2308-0811
Tahir KAHRAMAN[3]
0000-0003-4238-8528
Mehmet Fatih BOZKURT[2]
0000-0002-1669-0988
Hasan AYDIN[4]
0000-0002-1955-6178

[1]Sutcu Imam University Faculty of Medicine Department of Histology and Embryology, Kahramanmaras, Turkey

[2]Afyon Kocatepe University Faculty of Veterinary Medicine Department of Pathology, Afyonkarahisar, Turkey

[3]Karabuk University Faculty of Medicine Department of Medical Biochemistry, Karabuk, Turkey

[4]Adiyaman University Faculty of Pharmacy Department of Pharmaceutical Toxicology, Adiyaman, Turkey

Corresponding Author
Sorumlu Yazar
Alper YALÇIN
alperyalcin0171@hotmail.com

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ABSTRACT

Aim: Cyclophosphamide (CP) is a commonly used chemotherapeutic agent despite its toxic adverse effects, including hepatotoxicity. Ellagic acid (EA) is an antioxidant agent and exhibits free radical scavenging activities. In this experimental study, the effects of EA on CP-induced liver injury were investigated.

Material and Methods: Twenty-four Sprague-Dawley rats (180-220 gr) were separated into four equal groups. A single dose of 150 mg/kg CP was given intraperitoneally to generate hepatotoxicity. Different doses (50 and 75 mg/kg) of EA were administered orally 20 minutes before, 4 and 8 hours after CP administration. The histopathological evaluation of kidney tissues and immunohistochemical evaluation for caspase-3 were conducted as well as the serum biochemical analyses.

Results: CP treated group exhibited a significant increase in serum hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), compared to the control group. Similarly, the total triglycerides (TG) and very-low-density lipoprotein cholesterol (VLDL-C) levels increased significantly. Additionally, the high-density lipoprotein cholesterol (HDL-C) levels decreased, which was not significant, compared to the control group. At both EA doses, VLDL-C, AST, ALT levels decreased significantly while HDL-C level revealed a significant increase. 75 mg/kg EA treatment caused a non-significant elevation in total cholesterol (TC) concentration. Microscopic analysis showed a significant congestion, edema, degeneration and necrosis in the livers of CP administered group. However, edema, degeneration, and necrosis were significantly reduced in animals treated with EA-75. In addition, caspase-3 expression significantly decreased in EA-75 group.

Conclusion: These results indicate the protective effects of EA in CP-induced hepatotoxicity in rats.

Keywords: Caspase-3; cyclophosphamide; ellagic acid; hepatotoxicity; lipids.

ÖZ

Amaç: Siklofosfamid (CP), hepatotoksisite dahil olmak üzere, toksik yan etkilerine rağmen yaygın olarak kullanılan kemoterapötik bir ajandır. Ellajik asit (EA) antioksidan bir ajandır ve serbest radikal süpürücü aktiviteler sergilemektedir. Bu deneysel çalışmada, EA'nın, CP'ye bağlı karaciğer hasarı üzerindeki etkileri araştırılmıştır.

Gereç ve Yöntemler: Yirmi dört adet Sprague-Dawley türü sıçan (180-220 gr) dört eşit gruba ayrıldı. Hepatotoksisite oluşturmak için intraperitonal olarak tek doz 150 mg/kg CP verildi. CP uygulamasından 20 dakika önce ve 4 ila 8 saat sonra oral yolla farklı dozlarda (50 ve 75 mg/kg) EA uygulandı. Serumun biyokimyasal analizlerinin yanı sıra böbrek dokularının histopatolojik değerlendirmesi ve kaspaz-3 için immünohistokimyasal değerlendirme yapıldı.

Bulgular: CP uygulanan grup, kontrol grubuna kıyasla, serum hepatik enzimleri olan aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT)'da önemli bir artış gösterdi. Benzer şekilde, total trigliserit (TG) ve çok düşük yoğunluklu lipoprotein kolesterol (VLDL-C) seviyeleri önemli ölçüde arttı. Ayrıca, yüksek yoğunluklu lipoprotein kolesterol (HDL-C) seviyeleri, kontrol grubuna kıyasla anlamsız olarak azaldı. Her iki EA dozunda da VLDL-C, AST, ALT seviyeleri önemli ölçüde azalırken, HDL-C seviyesi önemli bir artış gösterdi. 75 mg/kg EA tedavisi, total kolesterol (TC) konsantrasyonunda önemsiz bir artışa neden oldu. CP uygulanan grubun karaciğerlerinde mikroskobik olarak önemli derecede konjesyon, ödem, dejenerasyon ve nekroz gözlendi. Bununla beraber EA-75 grubundaki hayvanlarda ödem, dejenerasyon ve nekroz önemli ölçüde azaldı. Ayrıca kaspaz-3 ekspresyonu EA-75 grubunda anlamlı şekilde azaldı.

Sonuç: Bu sonuçlar sıçanlarda CP'nin neden olduğu hepatotoksisitede EA'nın koruyucu etkisi olduğunu göstermiştir.

Anahtar kelimeler: Kaspaz-3; siklofosfamid; ellajik asit; hepatotoksisite; lipidler.

INTRODUCTION

Cyclophosphamide (CP) is a widely used alkylating chemotherapic agent and has myelosuppressive, immunosuppressive, and cytotoxic effects (1). However, excessive toxic side effects often limit the therapeutic uses of this drug (2). The most common adverse effects of CP include hemorrhagic cystitis, hepatotoxicity (3), lung injury, nephrotoxicity (4), testicular toxicity (5), cardiomyopathy (6) as well as damage to the islets of the pancreas (7). Treatment with CP causes toxicity through over production of reactive oxygen species (ROS) leading to increased oxidative stress (8).

As it is the most important detoxification organ (9), liver is frequently exposed to the adverse effects of CP (10). This drug triggers hepatotoxicity and lead damage (11) as liver is an easier target for the effects of oxidative stress (12). Phosphoramide mustard and acrolein constitute two main active metabolites of CP. Phosphoramide mustard has been reported to have antineoplastic property, while acrolein is responsible for CP induced liver injury (13) as it interrupts tissue antioxidant system and forms very reactive oxygen free-radicals (14). Preventive strategies for CP-induced hepatotoxicity are limited and new strategies should be developed to preserve healthy organs and cells against detrimental effects of CP metabolites (15). It has been reported that antioxidants may control the response of tissues to chemotherapy and reduce the adverse effects of antineoplastic agents (16).

Herbal cures have gained importance due their efficiency, safety and lower costs (17). Ellagic acid (EA), a phenolic compound, has an important value among antioxidant substances of vegetable origin and is found in high concentrations in fruits such as raspberries, strawberries, cloudberry, rose hip, and sea buckthorn (18). More recently EA is of interest against liver toxicity due to its pharmacological properties (19). EA has been reported to be effective in protecting cells against oxidative damage (20) and increasing the activity of the antioxidant defense system (21) as it has strong antioxidant potential (22,23).

In the current study, possible protective effects of EA on CP-induced hepatotoxicity were investigated by evaluating histopathological, immunohistochemical and biochemical parameters.

MATERIAL AND METHODS

Chemicals

EA (≥95%, CAS Number 476-66-4) was purchased from Sigma-Aldrich. CP was purchased as a commercial preparation (Endoxan®, Eczacibasi-Turkey).

Animals, Diets and Experimental Protocols

The ethical guidelines for the care of laboratory animals (Afyon Kocatepe University, Animal Experiments Local Ethics Committee, protocol no: 38-19 and date: 19.02.2019) were followed throughout the experiments. Twenty-four Sprague-Dawley rats (180-220 gr) were separated into four groups (n=6). The experiment was initiated after two days of acclimatization in ambient conditions, ensuring 12 h light/dark cycle with ad libitum standard rodent pellet diet and water at room temperature (25±3 °C). The first group was named as control group and the animals were not treated with anything other than isotonic saline by intragastric gavage (i.g.). The second group was named the CP group and the animals received a single dose of 150 mg/kg CP at the beginning of the study via intraperitoneal (i.p.) route in accordance with the previous studies (24) to induce hepatotoxicity. The animals in the other two experimental groups, named the EA50 and the EA75 groups, received a single dose of 150 mg/kg CP by i.p. and a total of three EA treatments which were administered 20 minutes before CP, and 4 and 8 hours after the CP administration with 50 mg/kg and 75 mg/kg doses, respectively by i.g. route of administration. The concentrations of EA were selected in accordance with previous studies (20,25). The animals were fasted 12 hours before anesthesia.

Biochemical Analysis

After 48 hours of treatments, blood samples were collected from the animals under anesthesia (ketamine HCl, 80 mg/kg, i.p. and xylazine HCl, 10 mg/kg, i.p.) by intracardiac puncture and then centrifuged at 3500 rpm to separate sera. The obtained sera were stored at -20 °C until examination. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) levels were measured to evaluate the degree of hepatocellular damage, using an auto analyzer (Cobas 6000, Roche, Switzerland).

Tissue Preparation and Histopathologic Evaluation

The animals were euthanized and quickly necropsied after blood sampling. Liver tissues were taken and a sufficient portion was fixed in buffered 10% formalin solution. After routine processing, samples were placed in paraffin and sectioned. Following stained with hematoxylin-eosin (HE), sections were blindly analyzed. Passive hyperemia, periportal edema, degenerative and/or necrobiotic changes in hepatocyte and possible mononuclear cell clusters in the sinusoids and/or periportal areas in the liver sections were scanned under a light microscope (CX41 attached Kameram® Digital Image Analyze System; Olympus, Tokyo, Japan).

Immunohistochemical Examination

For antigen retrieval, following rehydrating procedure, sections were boiled for 5 min in a microwave oven (750 W) seven times in citrate buffer solution, pH 6. Sections were cooled at room temperature for 20 min, washed three times with phosphate-buffered saline (PBS) (P4417; Sigma Chemical Co.) for 5 min. Next sections were incubated for 5 min with hydrogen peroxide block solution (TA-125-HP; Lab Vision Corp. USA) to block endogenous peroxidase activity. The sections, then, were washed with PBS three times for 5 min. After applying Ultra V Block (TA-125-UB; Lab Vision Corp.) for 5 min, in a humid environment, tissue sections were incubated with primary antibodies for caspase-3 (rabbit polyclonal IgG, ab2302; Abcam, London, UK), diluted 1:200, at room temperature for 60 min. The sections then were incubated at room temperature for 30 min in a humid environment with secondary antibody (biotinylated goat anti-mouse/rabbit IgG, TP-125-BN; Lab Vision Corp.) after washing with PBS three times for 5 min each. Afterwards, sections were washed with PBS three times for 5 min and incubated at room temperature for 30 min in a humid environment with streptavidin peroxidase (TS-125-HR;

Lab Vision Corp.), then placed in PBS. 3-Amino-9-ethylcarbazole (AEC) substrate + AEC chromogen (AEC substrate, TA-015 and HAS, AEC Chromogen, TA-002-HAC; Lab Vision Corp.) solution was dripped on the sections. The sections were washed with PBS. For counterstaining, Mayer's hematoxylin was applied, then, sections were passed through PBS, distilled water and mounted with Large Volume Vision Mount (TA-125-UG; Lab Vision Corp). Sections were evaluated and images were taken by using a Leica DM500 microscope (Leica DFC295). The histoscore, which reflects the prevalence of immunoreactivity of caspase-3 on the liver tissues, was calculated according to the rating scale as: 0.1, <25%; 0.4, 26-50%; 0.6, 51-75%; 0.9, 76-100%, and intensity of immunoreactivity: 0, unstained; 0.5, little staining; 1, some staining; 2, moderate staining; 3, strong staining. The histoscore = prevalence x intensity.

Statistical Analysis

Statistical analyses were performed using the SPSS 15.0 version (SPSS Inc., Chicago, IL). The normal distributions of numerical variables were assessed by the Shapiro Wilk test. Levene test was used for the homogeneity test of variances. One-way analysis of variance (ANOVA) was used for the assessment of group comparisons of hepatic enzyme activities and serum lipid profiles. Tukey's multiple comparison test was used to determine the differences between the groups of significant variables. Kruskal-Wallis H test was used for group comparisons of histopathological findings. Mann Whitney U test was used to determine the differences between the groups of significant variables. Bonferroni correction was applied for which p values <0.008. The results were given as mean±SD and median (min-max). The level of significance was considered to be at least $p<0.05$.

RESULTS

Hepatic Enzyme Activities

To establish the therapeutic utility of EA in CP-induced hepatotoxicity, we performed biochemical analyses of liver tissues from animals that were treated with CP and control alone or in combination with EA. There were significant differences between groups both for AST and ALT levels (both p values were <0.001). According to the post hoc test results, a significant increase in the AST and ALT levels were observed in CP group in comparison with saline-treated control group. However, significant decreases were seen of AST and ALT levels in the EA50 and EA75 groups compared to the CP-treatment group (Table 1).

Serum Lipid Profile

TG levels was shown a statistically significant difference in four groups (p=0.033). Post hoc test revealed that TG levels increased in a significant manner in CP group in comparison with the control group. In the EA50 and EA75 groups, the decrease in the levels of TG was not significant compared to the CP group. In terms of TC levels, no significant difference was observed between control, CP, EA50 groups and EA75 groups (p=0.137). A statistically significant difference was found in HDL-C levels of groups ($p<0.001$). EA-treatment at both concentrations increased HDL-C levels compared to CP group, whereas a higher level was detected in the EA75 group. While LDL-C levels were similar in the control, CP, and, EA 50 group, EA75 group exhibited a non-significant increase (p=0.069). There was a significant difference between the groups in terms of VLDL-C levels ($p<0.001$). CP treatment increased the VLDL-C level, which was significantly decreased in the animals treated with EA50 and EA75 mg doses (Table 2).

Liver Histopathology

In terms of histopathological findings, there were statistically significant differences between groups for congestion, edema, degeneration and necrosis (all p values were <0.001). Significant congestion and edema were seen in the CP applied groups compared to control group. Congestion and edema were reduced in the EA75 group, and to a lesser extent, in the EA50 group compared to the CP group. EA treatment at a dose of 75 mg/kg gave very good results against CP-induced degeneration and necrosis; however, administration of 50 mg/kg was insufficient. The success of 75 mg/kg EA in preventing necrosis has been particularly significant. In the CP and EA50 groups, severe degeneration and necrosis in the hepatocytes around the vena centralis was observed in comparison with other groups (Figure 1, Table 3). These results clearly demonstrate that EA significantly minimizes the cytotoxic effects of CP in liver tissue similar to the biochemical findings.

Caspase-3 Immunohistochemistry of Liver

For evaluating the effects of EA on CP-induced apoptosis in liver, caspase-3 antibody was used as a marker. Caspase-3 expression was determined in the cytoplasm of hepatocytes as shown in Figure 2. There was a significant difference in caspase-3 immunoreactivity of the groups (<0.001), and according to the post hoc test results, immunoreactivity of caspase-3 significantly increased in CP-treated group in comparison with those in the control group. However, contrary to CP-treated group, immunoreactivity of caspase-3 decreased in the CP+EA50 and CP+EA75 groups in a significant manner (Table 4). These findings provide evidence that EA significantly reduces the apoptotic effects of CP in liver tissue at both doses.

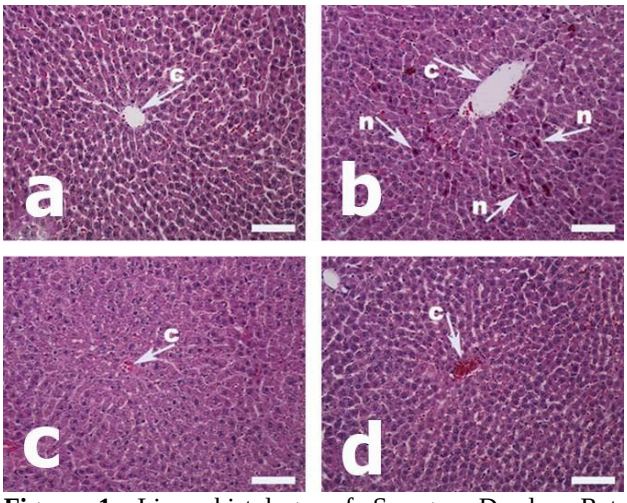

Figure 1. Liver histology of Sprague Dawley Rat. Representative figures were stained with HE. The scale bars represent 100 µm. Arrow pointed events; c: vena centralis, n: necrotic hepatocytes. **a)** Control group (standard rodent diet) **b)** CP group (150 mg/kg CP) **c)** EA50 group (150 mg/kg CP and 50 mg/kg EA) **d)** EA75 group (150 mg/kg CP and 75 mg/kg EA)

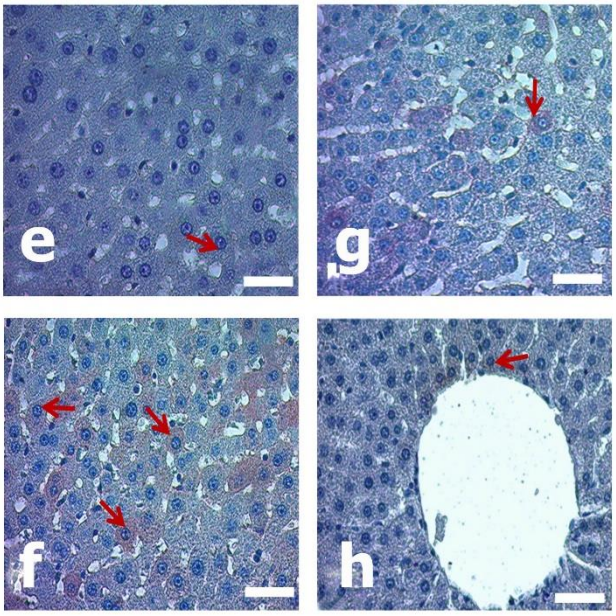

Figure 2. Immunohistochemical staining for caspase-3 (arrow) in liver tissue. The scale bars represent 100 µm. **e)** Control group **f)** Increased caspase-3 immunoreactivity of CP group **g)** Decreased caspase-3 immunoreactivity of EA50 group **h)** Decreased caspase-3 immunoreactivity of EA75 group

DISCUSSION

CP is a widely used chemotherapeutic and immunosuppressive agent for various cancers and autoimmune diseases (26). Although CP exhibit effective cytotoxic activity on malignant cells, it can also cause significant damage in normal tissues (4,5). The liver is one of the mostly affected organs by CP-induced toxicity (3) that leads to significant functional impairments (27). In experimental studies, CP was shown to cause deterioration of the oxidant-antioxidant balance (28) and increase in lipid peroxidation (29) in addition to the macroscopic and microscopic deformities in different tissues. CP applications may alter the serum lipid profile. In CP-treated rats, LDL-C and VLDL-C increased and HDL-C decreased along with changes in lipid metabolizing enzymes (30). Moreover, plasma TG, TC, and LDL-C levels were also increased in rats treated with CP (8). The TC level increased, but the HDL-C level decreased in CP-administered rabbits (31).

Chemotherapeutic agents used currently in cancer treatment usually have some side effects. In this respect, supportive applications that can minimize the side effects of those agents used in cancer patients are needed.

Some fruits and vegetables are a potential candidate in this context through their numerous bioactive components,

Table 1. Hepatic enzyme activities (AST, ALT) of the groups

	Control	CP	EA50	EA75	p
AST	68.28±6.09^{a}	133.58±13.65^{c}	97.28±8.46^{b}	81.45±8.90^{a}	**<0.001**
ALT	22.82±1.23^{a}	43.12±9.57^{b}	25.90±3.79^{a}	23.93±1.21^{a}	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ^{abc}: Different superscripts denote significant difference between groups (p<0.05, Tukey's test)

Table 2. Serum lipid profiles (TG, TC, VLDL-C, LDL-C, HDL-C) of the groups

	Control	CP	EA50	EA75	p
TG	33.88±3.73^{a}	50.67±17.06^{b}	47.80±9.25^{ab}	38.75±4.80^{ab}	**0.033**
TC	70.15±12.45	76.05±6.53	78.70±19.59	90.62±16.68	0.137
HDL-C	52.37±8.94^{ab}	43.42±5.30^{a}	62.40±10.19^{b}	85.55±11.67^{c}	**<0.001**
LDL-C	24.38±4.91	28.77±7.13	28.87±8.92	39.12±13.62	0.069
VLDL-C	6.78±0.75^{a}	12.63±1.75^{b}	8.73±1.01^{a}	8.42±1,51^{a}	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, TG: Triglyceride, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, ^{abc}: Different superscripts denote significant difference between groups (p<0.05, Tukey's test)

Table 3. Histopathological findings of the groups

	Control		CP		EA50		EA75		p
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
Congestion	0.17±0.41^{a}	0 (0-1)	2.50±0.53^{c}	2.5 (2-3)	2.33±0.52^{c}	2 (2-3)	2.00±0.00^{b}	1 (1-2)	**<0.001**
Edema	0.33±0.52^{a}	0 (0-1)	2.50±0.55^{c}	2.5 (2-3)	2.17±0.42^{c}	2 (2-3)	1.83±0.42^{b}	1 (1-2)	**<0.001**
Degeneration	0.17±0.41^{a}	0 (0-1)	3.83±0.41^{b}	4 (3-4)	3.17±0.75^{b}	3 (2-4)	0.50±0.84^{a}	0 (0-2)	**<0.001**
Necrosis	0.00±0.00^{a}	0 (0-0)	3.33±0.52^{c}	3 (3-4)	2.17±1.17^{b}	2 (0-3)	0±0.00^{a}	0 (0-0)	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, Descriptive statistics were given as mean±SD and median (min-max), ^{abc}: Different superscripts denote significant difference between groups (p<0,008 was considered significant because of multiple tests, Mann Whitney U test was applied with Bonferroni adjustment), SD: Standard deviation, Congestion; 1 vs 4: p=0.005, 1 vs 3: p=0.003, 1 vs 2: p=0.002, 4 vs 3: p=0.003, 4 vs 2: p=0.002 and 3 vs 2: p=0.118, Edema; 1 vs 4: p=0.005, 1 vs 3: p=0.003, 1 vs 2: p=0.002, 4 vs 3: p=0.003, 4 vs 2: p=0.002 and 3 vs 2: p=0.241, Degeneration; 1 vs 4: p=0.461, 1 vs 3: p=0.003, 1 vs 2: p=0.002, 4 vs 3: p=0.002, 4 vs 2: p=0.002 and 3 vs 2: p=0.083, Degeneration; 1 vs 4: p=1.000, 1 vs 3: p=0.007, 1 vs 2: p=0.002, 4 vs 3: p=0.006, 4 vs 2: p=0.002 and 3 vs 2: p=0.007

Table 4. Caspase-3 immunoreactivity of the groups

	Control	CP	EA50	EA75	p
Caspase-3	0.486±0.186^{a}	1.200±0.300^{b}	0.729±0.198^{a}	0.429±0.180^{a}	**<0.001**

CP: Cyclophosphamide, EA50: Ellagic acid 50 mg/kg, EA75: Ellagic acid 75 mg/kg, ^{ab}: Different superscripts denote significant difference between groups (p<0.05, Tukey's test)

including fiber, vitamins, minerals, and especially polyphenols. Dietary polyphenols could modulate lipid metabolism as well as other positive effects (32). EA is a polyphenol found abundant in fruits and vegetables. In natural conditions, EA is largely taken as free EA or ellagitannins (ETs) by consumption of fruits and vegetables (33).

In the current study liver injury induced by CP was evidenced by severe congestion, edema, degeneration, and necrosis. The mechanism of CP induced cellular injury has been reported to be related with the induction of oxidative stress by the formation of free radicals and ROS (34). Histopathologic abnormalities were ameliorated by EA and decreased to the control group levels in the EA75 group, except congestion. This reveals the hepatoprotective potential of EA against liver toxicity of CP through its antioxidant potential. Similar to our findings, EA supplementation improved hepatic steatosis (35), degenerative, necrotic and inflammatory changes (36) sinusoidal dilatation, degeneration, edema, and lymphocyte infiltration in the liver (37).

Apoptosis has a significant role in the pathogenesis of CP induced hepatotoxicity (38). In this study, CP treatment increased caspase-3 expression in liver tissue in accordance with previous publications (23,39). This increase could be attributed to the inductive role of CP on apoptosis in liver (40). On the other hand, EA at the concentration of both 50 mg/kg and 75 mg/kg were able to reduce caspase-3 reactivity significantly due to its anti-apoptotic effects (41) in line with the previous studies (37,41,42). CP treatment causes significant elevations of liver enzymes such as ALT and AST in blood of rats (8). In the current study, serum ALT and AST levels increased in the CP-administered rats. Increased enzyme levels are a marker of liver damage and they indicate CP caused formation of toxic metabolites that result in liver damage. However, in the EA treated groups, especially in the EA75 group, these values returned to normal level as it decreased the leakage of the enzymes. This restoration might be attributed to the protective and free radical scavenging property of EA (35) against CP induced liver toxicity. These findings were consistent with the effects of EA on alcohol-induced liver damage reported by Devipriya et al (25). Moreover, sodium arsenide-induced changes in serum AST and ALT concentrations returned to normal levels with EA supplementation in rats (36).

The regulatory effects of EA on the lipid metabolism are known, and a wide range of positive effects of EA and EA-containing products on chronic metabolic diseases such as type 2 diabetes, dyslipidemia, insulin resistance, and non-alcoholic fatty liver disease have been reported (33). For example, the lipid level (TC, TG, free fatty acids, and phospholipids), which is negatively affected by alcohol consumption in rats, was normalized by EA (24). EA supplementation improved the serum lipid profile in high-fat diet fed mice (35) and in cholesterol fed hyperlipidemic rats (43). Moreover, Kang et al. (44) reported that high-fat and high sugar-induced dyslipidemia was reversed in mice given raspberry seed powder. Ahad et al. (45) showed that oral EA intake significantly inhibited dyslipidemia in high-fat diet/low dose in type 2 diabetic rats. However, the evaluation of serum lipid parameters revealed that EA treatment at both concentrations raised HDL-C level compared to the CP group and control group in this study. EA treatment reduced CP-induced elevations of VLDL-C to a significant level as well as TG levels but not to a significant level. The effects of EA on VLDL-C and TG found in this study are in line with the previous reports (45,46).

Kang et al. (33) reviewed the effects of EA or EA-enriched fraction on obesity and metabolic complications and they reported that EA administration at a dose of 7.5-800 mg/kg for 10 days-16 weeks showed significant improvement in the lipid profiles of mice and rats. As demonstrated in this review, chronic supplementation of EA/ET enriched extracts or pure EA was effective in alleviating metabolic syndrome-associated disorders in rodents.

Studies showed that EA significantly decreases TC and LDL-C in rodents (45,46). However, in the present study EA increased both LDL-C and TC levels, although it was not significant. This inconsistency of results with the previous reports could be due to the difference of disease models and chemical agents used.

EA markedly increased HDL-C levels in dose dependent manner. HDL is a key molecule to remove excess cholesterol from peripheral tissues through a process defined as reversed cholesterol transport (47,48). Beneficial effects of EA on HDL levels have been previously reported (43,49), an important way to eliminate cholesterol.

CONCLUSION

Alterations in the lipid profile observed in this study due to the use of EA showed some consistency and also some inconsistency when compared to other studies. However, most of the studies in literature are designed to evaluate the long-term effect of EA, whereas the present study has established short term activity of EA (48-hour), and is an acute examination compared to other chronic applications. In this context, when the literature is reviewed again, it is seen that the metabolic rate increases due to the EA, and lipid efflux occur shortly after ingestion of EA and EA-containing products. In our study, high lipid level in serum samples may be related to the rearrangement of the lipid balance in the body and the efflux of peripheral lipid deposits compared to other studies. Despite the instability of biochemical lipid results, no cytoplasmic accumulation, degeneration or necrosis of liver hepatocytes in histopathological examination and reduced apoptotic hepatocytes in immunohistochemical examinations demonstrate the protective effect of the EA administration. Moreover, the protective effects of EA on the liver were confirmed by serum liver enzymes which were elevated by CP applications. Our study has demonstrated a significant reduction of these enzymes by 75 mg/kg EA administration. Time-dependent experiments are needed to elucidate this issue. Currently, the cases of liver damage related to polluted water, food, drugs, and other environmental factors continue to rise in the world. Therefore, the consumption of fruits and vegetables containing EA, which have positive effects on health, may be supportive for the liver.

Declaration of Conflict of Interest

The authors report no conflict of interest.

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An Eligible Surgical Repair Technique for Bland-White-Garland Syndrome

Bland-White-Garland Sendromlu Olguda Seçilebilir Bir Cerrahi Teknik

Ata Niyazi ECEVİT[1]
0000-0002-8820-9305
Nur DİKMEN YAMAN[2]
0000-0002-7515-8465
Burcu ARICI[3]
0000-0002-9824-9220
Zeynep EYİLETEN[4]
0000-0003-3405-7139
Bülent KAYA[4]
0000-0002-7943-8683
Mustafa Adnan UYSALEL[4]
0000-0001-5395-7111

[1]Ankara City Hospital Cardiovascular Surgery Clinic, Ankara, Turkey

[2]Dr. Sami Ulus Obstetrics, Gynecology and Children's Research and Training Hospital Cardiovascular Surgery Clinic, Ankara, Turkey

[3]Cengiz Gökçek Obstetrics, Gynecology and Children's Hospital Cardiovascular Surgery Clinic, Gaziantep, Turkey

[4]Ankara University Faculty of Medicine Department of Cardiovascular Surgery, Ankara, Turkey

ABSTRACT

Anomalous origin of left coronary artery from pulmonary artery (ALCAPA) is an uncommon but major congenital cardiac anomaly, which was firstly defined in 1866. Multiple surgical techniques intend to compose 2-coronary artery system, including direct reimplantation of left coronary artery to aorta and coronary artery bypass grafting procedures. Left coronary artery seldomly rises from non-facing pulmonary sinus, where direct reimplantation is technically quite difficult for surgical correction because of long distance from native aorta. In this case report, a surgical technique option of reconstructing left coronary artery, arising from non-facing sinus which we turned to aorta with a tunnel created from autologous pulmonary artery wall, was presented.
Keywords: ALCAPA; coronary artery anomaly; congenital heart disease.

ÖZ

Sol koroner arterin pulmoner arterden orijin alması (ALCAPA) nadir olmakla birlikte, ilk olarak 1866'da tarif edilen bir major konjenital kardiyak anomalidir. Sol koroner arterin direkt aortaya reimplantasyonu ve koroner arter baypas greftleme prosedürleri de dahil olmak üzere çok sayıda cerrahi teknik ikili koroner arteriyel sistem oluşturmayı amaçlamaktadır. Sol koroner arter nadir olarak non-facing pulmoner sinüsten köken alır ve bu nativ aortaya uzak mesafede bir bölge olduğu için, direkt reimplantasyonun teknik olarak oldukça zor olduğu cerrahi onarımdır. Bu vaka sunumunda, non-facing pulmoner sinus kökenli sol koroner arterin, otolog pulmoner arter duvarından tünel oluşturularak aortaya rekonstrüksiyonu cerrahi tekniği bir seçenek olarak sunulmuştur.
Anahtar kelimeler: ALCAPA; koroner arter anomalisi; konjenital kalp hastalığı.

INTRODUCTION

Anomalous origin of left coronary artery from pulmonary artery (ALCAPA), also known as Bland-White-Garland syndrome, is an uncommon congenital abnormality that affects 300000 live births per year and approximately 0.25-0.5% of all congenital heart defects (1). There are two varieties of ALCAPA: The infant and the adult type. They have different appearance and outcomes. If not interfered, approximately 90% of infant type patients die in the first year (2). Although, certain number of the patients with adult type do not have symptoms at early periods in life. Elder patients with ALCAPA generally have symptoms as mitral insufficiency, cardiomyopathy, dysrhythmias, and moreover death (3). As a result, presumptive identification of ALCAPA indicates prompt certain diagnosis and early surgical intervention regardless of the age except for the newborn.

Corresponding Author
Sorumlu Yazar
Nur DİKMEN YAMAN
ndikmenyaman@gmail.com

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CASE REPORT

A 4 month-old and 5 kilograms weighing infant presented with paroxysmal tachycardia, pulmonary infection and mild dyspnea with feeding. Transthoracic echocardiography demonstrated a dilated left ventricle and significantly reduced ejection fraction with measurements under 20%. Angiography determined the way that ALCAPA arises from non-facing pulmonary sinus. Patient was identified as ALCAPA and taken to surgical procedure.

A median sternotomy incision was employed, autologous pericardium was harvested, and glutaraldehyde preserved autologous pericardium was prepared for reconstruction. Bicaval cannulation was used for venous drainage, and distal aortic cannulation was performed. Antegrade crystalloid cardioplegia (30 mL/kg body weight) was introduced under moderate hypothermia of 28°C. After horizontal incision of main pulmonary artery, left coronary artery (LCA) was identified in the non-facing sinus of valsalva.

Anomalous coronary artery's direct reimplantation into aorta was technically challenging due to its remote site. So, coronary artery reimplantation was performed by method of transecting pulmonary artery wall and releasing LCA by means of an extended incision to the coronary ostium in the non-facing pulmonary sinus. We diverted LCA and nonfacing sinus with a tunnel created from autologous pulmonary artery wall (Figure 1). We think that this technique provides the most well agreed and adapted anastomosis with proper linear measure, appropriate angling, and the best course of the coronary artery. Pulmonary artery reconstruction was performed with autologous pericardium which was trimmed to the appropriate size. After de-airing and aortic unclamping, inotropic support was achieved with infusions of adrenalin by dosing of 0.05 mcg/kg/min and milrinone by 0.5 mcg/kg/min before weaning from cardiopulmonary bypass (CPB). The patient was transferred to the intensive care unit in an uneventful manner.

Figure 1. Coronary artery reimplantation was performed by method of transecting the pulmonary artery and releasing the left coronary artery through an extended incision to the coronary ostium in the non-facing pulmonary sinus and with a tunnel created from autologous pulmonary artery wall.

DISCUSSION

ALCAPA is a rare condition that requires early surgical repair. Even so, nearly 15% of patients reach adulthood (4). In adults, this can be a cause of sudden death. Sudden cardiac death occurs in most of the cases due to exercise, mostly in males and young athletes (5).

Surgical techniques necessitate qualifications for ALCAPA. Direct reimplantation of the anomalous coronary artery to aorta is the most commonly accepted repair modality that has provided the most successful results at long-term follow-up. Other techniques are subclavian and LCA anastomosis, Takeuchi repair, LCA with autologous pulmonary artery wall tubulary extension and coronary artery bypass grafting with vein grafts or left internal thoracic artery (1).

In our patient, direct implantation of ALCAPA was not convenient, due to the non-facing sinus origin of coronary artery. We believe that, our technique of diversion of LCA and non-facing sinus with a tunnel created from autologous pulmonary artery wall, verified a new point of view. Also in this technique, we have preferred autologous pericardium for pulmonary arterial repair because of advantages: it provides sufficient integrity and strength, it has a potency of growth and also remodeling, it is free of cost, it has more biocompatibility than bovine pericardium, the tissue is adequate for procedure and we do not need a separate operation for harvesting.

If compared with Takeuchi procedure which we can identify as an intrapulmonary baffle from LCA to ascending aorta, this procedure has best patency rates initially, but it has long-term complications, such as pulmonary artery and baffle obstruction, leak, and aortic valvar insufficiency. Reintervention was necessary in 30% of these patients to resolve the complications (6).

As conclusion, anomalous coronary artery that rises from non-facing sinus of valsalva of pulmonary artery or from a greater distance from the ascending aorta may necessitate different approaches. We describe our technique to be instructive for this challenging group of patients. However, further postoperative outcomes and follow-up parameters is necessary to evaluate whether this technique can contribute to the improvement of current surgical management of ALCAPA.

Informed consent: Written informed consent was obtained from the patient's parents.

Conflict of interest: All the authors declare that there is no conflict of interest.

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Medicolegal Evaluation of the Case of Implantable Cardioverter Defibrillator (ICD) Lead Fracture Due to Domestic Violence

Aile İçi Şiddet Sonrası İmplante Cardioverter Defibrillator (ICD) Lead Fraktürü Meydana Gelen Olgunun Medikolegal Değerlendirmesi

Ahsen KAYA
0000-0002-6969-1562
Cemil ÇELİK
0000-0002-8103-459X
Hülya GÜLER
0000-0002-3205-4113
Ender ŞENOL
0000-0001-9018-2466
Ekin Özgür AKTAŞ
0000-0003-0934-3731

Ege University Faculty of Medicine
Department of Forensic Medicine,
İzmir, Turkey

ABSTRACT

There has been an increase in sudden cardiac death in recent years. Lately, implantable cardioverter defibrillator (ICD) is a device which is used to prevent sudden cardiac death. Some problems may occur in the device as a result of trauma. Lead fracture constitutes one of these problems. In the literature, blunt trauma-related ICD complications, particularly lead fracture, seem to be very rare. In this case report, we evaluated a female patient who was exposed to a blunt trauma while getting pulled her left arm by her ex-husband and to some inappropriate shocks due to lead fracture, in medicolegal aspect. In case, a lead fracture occurred by pulling her left arm without chest trauma. It is aimed to take importance that even a mild violence might cause life-threating, severe clinical outcome.
Keywords: Partner violence; blunt trauma; sudden death; medicolegal evaluation; implantable cardioverter defibrillator.

ÖZ

Son yıllarda ani kardiyak ölüm oranlarında artış görülmektedir. Günümüzde, implante edilebilir kardiyoverter defibrilatör (implantable cardioverter defibrilator, ICD) ani kardiyak ölümleri önlemede kullanılan bir cihazdır. Travma sonucunda cihazda birtakım problemler meydana gelebilmektedir. Kablo kırığı bu problemlerden biridir. Literatürde, künt travmaya bağlı ICD komplikasyonlarının, özellikle kablo kırığının oldukça nadir görüldüğü belirtilmektedir. Bu olgu sunumunda; eski eşi tarafından sol kolu çekilerek künt travmaya maruz kalan ve oluşan kablo kırığı nedeniyle uygunsuz şoklara maruz kalan kadın olgu medikolegal açıdan değerlendirildi. Olguda, göğse direkt bir travma olmadan, sol kolun çekilmesine bağlı olarak lead kırığı meydana gelmiştir. Hafif şiddetteki bir travmanın bile, yaşamsal tehlike oluşturabilecek, ciddi klinik sonuçlara neden olabileceğine dikkat çekmek amaçlandı.
Anahtar kelimeler: Partner şiddeti; künt travma; ani ölüm; adli-tıbbi değerlendirme; implante edilebilir kardiyoverter defibrilatör.

INTRODUCTION

Recently, there has been an increase in sudden cardiac death. Implantable cardioverter defibrillator (ICD) is a device used to prevent sudden cardiac death nowadays (1). Nevertheless, it is underlined that an appropriate patient selection is significant due to cost and potential complications (2). During the perioperative period, there are risks of various complications such as the removal of the lead wires of the ICD's to the heart, pneumothorax, infection, and bleeding, whereas, in the long term, inappropriate shocks (3). The complications that may lead to death related to ICD are rare. The rate of ICD complications that lead to the change of device

Corresponding Author
Sorumlu Yazar
Cemil ÇELİK
cemilcelik89@hotmail.com

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and surgical revision maintains its importance clinically despite remarkable developments in ICD technology (2,4). Though an inappropriate shock incidence, which is one of the ICD complications, shows alterations in different studies, it often occurs between 4-25%, and it is associated with the increase in morbidity (5-7). Inappropriate shocks, mostly get origin from supraventricular tachycardia, self-terminating ventricular tachycardia, sensing artifacts, myopotentials or T wave oversensing (2,6). In addition to these, trauma can also be a reason for these inappropriate shocks (2,4). From a medicolegal perspective, it's significant to identify what clinical consequences that the trauma causes. In patients with ICD, various problems may occur such as dislodgement of the lead, lead fracture, device header fracture as a result of mechanical chest trauma. Even though, lead fracture, constituting one of these problems, occurs rarely, it may result in inappropriate shocks, loss of pacing, failure of defibrillation or fatal proarrhythmia (8-11).

In this case report, a female patient, who was exposed to blunt trauma while getting pulled her left arm and to some inappropriate shocks resulting from lead fracture was evaluated in medicolegal aspect. It is aimed to take importance that even a mild violence might cause life-threating, severe clinical outcome.

CASE REPORT

A 50-year-old female case, who was battered by her ex-husband, appealed to our department for the preparation of her forensic report. She was examined after her informed consent was obtained.

The patient reported that she was diagnosed with dilated cardiomyopathy and left bundle branch block and planted a pacemaker, five years ago. She stated that, she was arguing with her ex-husband on the date of the incident. Then, he pulled her left arm and she felt a severe pain from her left arm to her chest. She had not been exposed to another trauma. She continued to feel the pain at that night, she heard a sound from her pacemaker during sleep, which is like a tick-tack sound coming from a clock, and she felt something like an electric shock on her body twice before applying to a health center the next day.

It was observed in her medical documents that the device was alerted and there were inappropriate shocks due to ICD lead fracture one day after the incident. It was also apparent in the documents that there was no clinical pathology and the device was shut down and then she was referred to a university hospital.

After her application to our department, a consultation was requested from the department of cardiology. Cardiology consultation note indicated that the patient had a Medtronic Cardiac Resynchronization Battery/Defibrillator (CRT-D) device and Medtronic 6944-A cable was used as a defibrillation cable. Impedance height (>3000 Ohms) and inappropriate shocks, which indicate the fact that the cable was broken, were detected in the device controls. It was also reported that the broken cable was changed with a new cable and the ICD battery life about to be out was replaced. The forensic report of the case was prepared in the light of the medical documents, the comprehensive medical anamnesis taken from the patient, her examination and cardiology consultation report.

DISCUSSION AND CONCLUSIONS

In the present case, a lead fracture occurred by pulling the case's left arm without chest trauma. This case has revealed that even a seemingly slight violence, such as pulling arm, can have serious life-threatening consequences. In the literature, blunt trauma-related ICD complications, particularly lead fracture, seem to be very rare (8). Furthermore, in the literature, there is a case of ICD complication (header fracture) resulting from a deceleration injury in a car accident without chest trauma (9). Therefore, it should be taken into consideration that ICD will fail even if there is no significant blunt chest trauma in patients with ICD. It is significant for the prevention of poor clinical outcomes. In addition to this, we realize the significance of the fact that patients with ICD are to be placed under remote device monitoring and the devices should be re-inspected after such traumas.

In this case, the device was shut down following inappropriate shocks triggered by trauma and it was replaced by the new one for some time later. Inappropriate shocks can lead to arrhythmias and the shortening of the battery life (2,4). In order to prevent the occurrence of ventricular tachycardia/fibrillation during the acute care of the patients with inappropriate shocks who applied to any health care provider, ICD should be immediately deactivated. During the time slot between the shutting down of a device emanating inappropriate shocks and the implantation of the new one, it is of significance to monitor and follow up patients in order to detect such complications as arrhythmia. It is also significant on the one hand for an early diagnosis and treatment; and, on the other, for a medicolegal evaluation in forensic cases.

All modern pacemakers/ICDs constitute an internal memory in order to retain information during interventions and to hide the long-term information (mostly 24 h). The whole information regarding the interventions is stored in this memory in order to enable post-traumatic diagnosis. Nevertheless, ICDs are unable to differentiate between a cardiac-related arrhythmia and the findings stemming from any fracture within the electrode system (12). The occurrence of arrhythmia is probable among the patients who are subjected to inappropriate shocks. From a medicolegal perspective, it is notable to detect this and to differentiate the shocks triggered by the device from those which are cardiac related. Therefore, particularly in forensic cases, it is highly significant to document and retain the ICD information of patients, which are legible. These documents are significant in that they enable to reply to potential allegations to be put forward particularly in forensic cases and to identify the cause of death in mortal cases.

According to Turkish Penal Code (TPC), it was evaluated that the event did not endanger her life, as it did not cause any cardiac disorders such as cardiac rhythm disturbances that could endanger a person's life and there were not any clinical signs which show affecting of circulatory, respiratory or central nervous system functions in her medical records. At the same time, it was concluded that the effect of this intentional injury upon a person was not minor and could not be cured by a simple medical treatment due to the fact that the person required a medical intervention (replacing the battery and the broken lead). Although the present event did not cause a life-threatening

situation by chance according to TPC, it is valuable to point out that without direct regional trauma, indirect trauma can cause damaging of instruments that were used for medical purposes, and this damage may lead to life-threatening conditions according to the properties and intended usage of the device.

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Malignant Epithelial Tumor in a Patient with Recurrent Axillary Abscess

Tekrarlayan Aksillar Apse ile Gelen Hastada Malign Epitelyal Tümör

Mehmet PATMANO[1]
0000-0002-1755-614X
Durmuş Ali ÇETİN[1]
0000-0003-3012-6774
Tufan GÜMÜŞ[1]
0000-0003-1861-8282
Özlem KARA[2]
0000-0001-6971-1298

[1]Şanlıurfa Education and Research Hospital General Surgery Clinic, Şanlıurfa, Turkey

[2]Şanlıurfa Education and Research Hospital Pathology Clinic, Şanlıurfa, Turkey

ABSTRACT

Skin infections are among the common diseases encountered in general surgery, dermatology and pediatrics outpatient clinics. Skin abscess occurs as a result of pus accumulation in the dermis or subcutaneous tissue; it is composed of swollen, red, tender, fluctuating mass and often accompanied by cellulite around them. The treatment is primarily antibiotic therapy according to the abscesses culture result after drainage with local anesthesia. Some of diseases such as diabetes, immunosuppressive conditions and cancer should be considered in adults, especially in recurrent abscesses. Skin metastasis in primary malignancies is very rare. Lung cancer is the most common one with skin metastasis. In this case report, we wanted to emphasize that the skin metastasis of primary malignancy should be kept in mind in cases with recurrent abscess which is resistant to treatment.
Keywords: Abscess; tumor; skin metastasis.

ÖZ

Deri enfeksiyonları genel cerrahi, dermatoloji ve pediatri polikliniklerinde sık karşılaşılan hastalıklar arasında yer almaktadır. Deri apsesi, dermiste veya subkutan dokuda püy birikmesi sonucunda meydana gelir; şiş, kızarık, hassas, fluktuasyon veren ve çoğunlukla çevresinde selülitin eşlik ettiği bir görünüme sahiptir. Tedavi lokal anestezi altında drenaj sonrasında apse kültürü sonucuna göre öncelikle antibiyotik tedavisidir. Erişkinlerde özellikle tekrarlayan apselerde diyabet, bağışıklık sistemini baskılayan durumlar ve kanser gibi bazı hastalıklar göz önünde bulundurulmalıdır. Primer malignitelerde cilt metastazı gelişimi çok nadirdir. Cilt metastazı nedeni olarak akciğer kanseri ilk sırada yer alır. Bu olgu sunumunda, tedaviye dirençli tekrarlayan apse olgularında primer malignitenin cilt metastazının akılda tutulması gerektiğini vurgulamak istedik.
Anahtar kelimeler: Apse; tümör; cilt metastazı.

INTRODUCTION

Skin is the largest organ and one of the important defense mechanisms for our body. The epidermis consists of dermis and subcutaneous tissue. Gram positive bacteria are the most common microorganisms in the skin flora. Predisposing factors for skin and soft tissue infections are; trauma, immune deficiency, venous and lymphatic drainage problems, local inflammatory disorder, foreign body, vascular insufficiency, obesity, poor hygiene (1).

Skin infections are common diseases in general surgery, dermatology and pediatrics outpatient clinics. Skin abscesses occur as a result of pus accumulation in the dermis or subcutaneous tissue. It has four cardinal signs of inflammation; redness, heat, swelling, and pain. The treatment is primarily antibiotic therapy according to the abscesses culture result after drainage (2,3). If recurrent abscesses occur in early childhood, patients should be examined for neutrophil disturbances. Some of systematic diseases

Corresponding Author
Sorumlu Yazar
Mehmet PATMANO
mpatmano@yahoo.com

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such as diabetes, immunosuppressive conditions and cancer should be considered in adults, especially in recurrent abscesses. Skin metastasis in primary malignancies is very rare. Lung cancer is the most common one with skin metastasis. Skin metastasis could be the first symptom of lung cancer (4). Pathological determination of skin metastasis is very important, because the asymptomatic patients could be diagnosed earlier (5,6). In this case report, we wanted to emphasize that the skin metastasis of primary malignancy should be kept in mind in cases of subcutaneous recurrent abscesses.

CASE REPORT

A 52-year-old woman presented to our hospital with complaints of pain, swelling and redness under the left axilla. The patient had diabetes mellitus as systematic disease and under the control with oral antidiabetics. In physical examination, there was swelling and redness in the left axilla. Breast examination was normal. Breast ultrasonography and mammography were performed to the patient. According to the ultrasonography; 50x34 mm, irregular bounded, dense, liquid locus (abscess?) was observed in the left axilla. No pathological findings were found in the breast tissue. Mammography revealed vascular wall calcifications in bilateral breast parenchyma. Laboratory results of the patient was as follows; WBC:12000 /µL, Hgb:12.4 g/dL, Plt:128000 /µL and CRP: 145.5 mg/L. The patient underwent abscess drainage with local anesthesia (jetosel^{R}, lidocaine and epinephrine, Osel) and the sample for abscess culture was taken. There was no growth in the culture of the patient. Ampicillin-sulbactam 1000 mg oral treatment for 7 days was given. The patient was followed with daily dressing. After 3 weeks from the first drainage, the patient's abscess was recurred in the same localization. After the drainage under local anesthesia, no growth in the culture was detected again. Ampicillin-sulbactam (1000 mg, 2x1) and metronidazole (3x500 mg) were ordered to the patient. The patient was followed with daily dressing and cavity washing procedures. During the follow-up, a fistulated abscess was detected at the same location which forms fistula to skin at different locations 1.5 months later (Figure 1). The patient underwent an abscess drainage again. Skin biopsy was performed to the patient due to granulomatous diseases and suspicion of malignancy. The patient's biopsy revealed as malignant epithelial tumor, primarily squamous cell carcinoma. Tumor cells have abundant eosinophilic cytoplasm and a large vesicular nucleus. There is focal keratinization, keratin pearls and moderate nuclear atypia. Squamous differentiation in these cytologically malignant cells, is manifest by squamous pearl and keratinization (Figure 2). The patient was examined with thoracic and abdominal computed tomography (CT). In thorax CT slight irregular bordered densities were observed in the upper and lower lobes of both lungs. The patient was referred to the medical oncology clinic in terms of primary pulmonary malignancy and skin metastasis.

DISCUSSION

In case of recurrence of subcutaneous abscesses which are among the reasons of frequent application to our clinic, systemic diseases and immunodeficiency conditions such as cancer should be kept in mind. Skin metastasis in primary malignancies is very rare. Lung cancer is the most common one with skin metastasis. As in our patient, skin metastasis may be the first finding in the patients (7). The pathological definition of skin metastasis in patients is important in two aspects. It enables us to reach the diagnosis in patients who are asymptomatic, and it is important in the follow-up of patients with cancer diagnosis in terms of treatment failure (5,6).

Skin metastasis as the first symptom is seen in 20% of cancer patients (5). First of all, skin metastasis in lung and kidney cancers could be seen as the first symptom (8). Skin metastasis have clinical different meanings. It could be the first symptom of a silent cancer. Brownstein and Helwig report that this is especially seen in lung and kidney cancers (9). Therefore in patients with a history of new developing skin lesions, especially in older patients, smokers and patients with a history of cancer in their families, histopathological diagnosis by taking a biopsy from the lesion is necessary to exclude the possibility of malignancy. Metastatic skin lesions are usually 1-3 cm in size and hard, painless, mobile lesions with normal skin color (7). Rarely, fruncle-like nodules, erosions, and ulcerated granuloma-like papules may occur (10-12). In

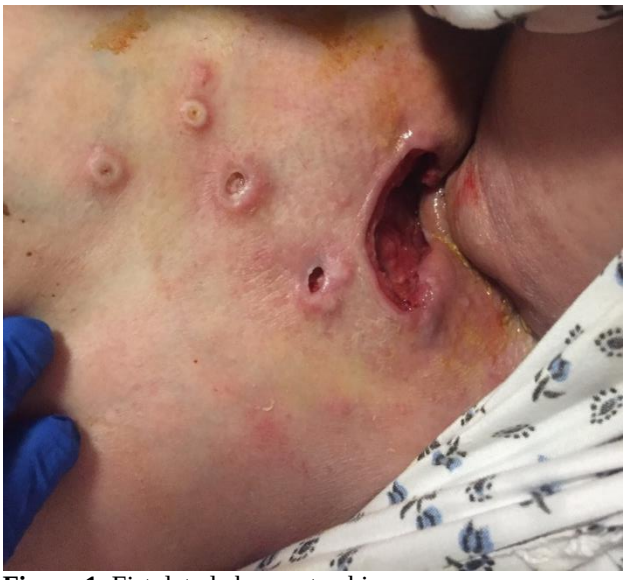

Figure 1. Fistulated abscess to skin

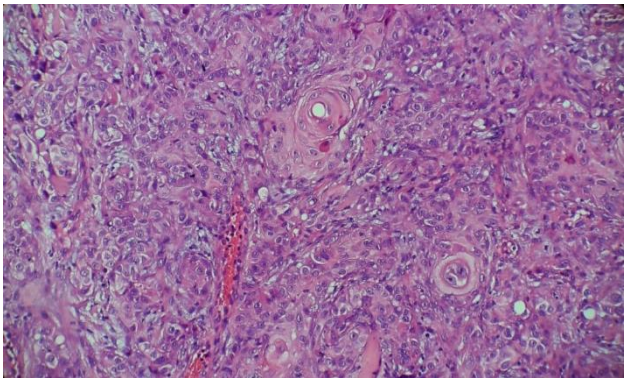

Figure 2. Histopathological image: focal keratinization, keratin pearls and moderate nuclear atypia (HEx100)

our case, fistulization in the form of papules was observed during the follow-up. Skin lesions are classified according to their appearance as follows; nodular, inflammatory and sclerodermoid metastatic lesions (5). Nodular type is the most common one, usually shows hematogenous spread and seems multiple (13-15). Skin metastasis could be seen in the region close to primary tumor (4,5,11). The most common site is the anterior chest wall (5,9,11,16). In our case, abscess extending from the anterior axillary line to the chest wall was detected.

In conclusion, malignancies should be kept in the mind in cases with recurrent abscess which is resistant to treatment. It should be kept in the mind that skin lesions may be the first sign of a silent cancer.

We believe that histopathological diagnosis after skin biopsies will be useful especially in the middle age and older patients with smoking history in order to rule-out malignancies.

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Interstitial Lung Disease due to Quetiapine: Case Report and Literature Review

Ketiapine Bağlı İnterstisyel Akciğer Hastalığı: Olgu Sunumu ve Literatür İncelemesi

Müge TOPCUOĞLU
0000-0002-7098-8806
Ali ERDOĞAN
0000-0003-0329-6778

Akdeniz University Medical Faculty
Department of Psychiatry, Antalya, Turkey

ABSTRACT

Quetiapine is a commonly used antipsychotic drug which has many common side effects such as dizziness, sedation, and metabolic side effects. In addition to them, the drug has also some rare side effects. Among them, little is known about drug-induced interstitial lung disease (DI-ILD). In this case report, we aimed to discuss the side effects of quetiapine use on the lung. We present a case of interstitial lung disease, which is rare side effect, that occurred following quetiapine use in a 54-year-old male schizophrenia patient. In our case, DI-ILD was examined due to the lung findings that occurred after quetiapine treatment started and regressed after quetiapine cessation. Clinicians should be careful about rare lung side effects such as DI-ILD that may develop as a result of quetiapine use.
Keywords: Interstitial lung disease; quetiapine; schizophrenia.

ÖZ

Ketiapin yaygın kullanılan, baş dönmesi, sedasyon ve metabolik yan etkiler gibi birçok yaygın yan etkisi olan bir antipsikotik ilaçtır. Bunlara ek olarak, ilacın bazı nadir yan etkileri de mevcuttur. Bunlar arasında ilaca bağlı interstisyel akciğer hastalığı (drug-induced interstitial lung disease, DI-ILD) çok az bilinmektedir. Bu olgu sunumunda ketiapin kullanımının akciğer yan etkilerini tartışmayı amaçladık. Elli dört yaşında erkek bir şizofreni hastasında ketiapin kullanımını takiben ortaya çıkan, nadir görülen bir yan etki olan interstisyel akciğer hastalığı olgusu sunulmaktadır. Olgumuzda, ketiapin tedavisinin başlamasından sonra ortaya çıkan ve ketiapin kesildikten sonra gerileyen akciğer bulguları nedeniyle DI-ILD düşünülmüştür. Klinisyenler, ketiapin kullanımının bir sonucu olarak ortaya çıkabilecek DI-ILD gibi nadir akciğer yan etkileri konusunda dikkatli olmalıdır.
Anahtar kelimeler: Interstisyel akciğer hastalığı; ketiapin; şizofreni.

INTRODUCTION

Quetiapine is an atypical antipsychotic that is used in the treatment of psychiatric disorders such as schizophrenia, bipolar disorder, and major depressive disorder (1). Quetiapine causes various side effects such as prolonged QT interval, metabolic disorder, hypothyroidism and hepatic dysfunction. However, side effects in the respiratory system are less common (2). As an example, drug-induced interstitial lung disease (DI-ILD) is a diagnostic problem due to its nonspecific clinical presentation. It is diagnosed by the use of drugs and the development of symptoms, the presence of infiltration sites in the lungs, the exclusion of other causes, and the regression of symptoms after discontinuation of the drug (3). In this article, we present a patient with DI-ILD due to quetiapine therapy and we aimed to contribute to the medical literature by our clinical experience and discussing the side effects of quetiapine use on the lung system.

Corresponding Author
Sorumlu Yazar
Ali ERDOĞAN
erdoganali006@hotmail.com

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CASE REPORT

A 54-year-old male patient was admitted to our outpatient clinic due to psychotic exacerbation and was referred to our clinic. The patient had a history of schizophrenia for 34 years, and aggression, paranoid delusions. Auditory hallucinations had begun again for the past 15 days. In addition, the patient had high fever, cough, sputum and shortness of breath. Besides, it was learned that his complaints started one week before the admission.

In the psychiatric examination, the patient had decreased self-care, his orientation was normal, his speech was normal, and his psychomotor activity was increased. He had paranoid delusions, auditory and visual hallucinations. Respiratory system examination revealed diffuse inspiratory rales in the bilateral middle and lower regions of the lungs. Other system examination findings were within normal limits.

He had a history of 50-year-smoking addict and had quit smoking one year ago. There was no history of alcohol or substance use. The patient had no previous history of lung disease, allergy, and medical history. The patient's blood pressure was 110/70 mm Hg, body temperature was 37.8°C (axillary), pulse rate was 112 beats/min and respiratory rate was 26 breaths/min. Laboratory tests had mild high C-reactive protein (7.4 mg/L, reference range: 0-5 mg/L) and mild leukocytosis (13×10^9 /L, reference range: 4.5-11 $\times10^9$ /L). All other laboratory parameters were within normal ranges. Hepatitis viruses and Human Immunodeficiency Virus (HIV) markers were negative. Arterial blood gas analysis revealed pH was 7.46, PaO_2 was 92.6 mm Hg, PCO_2 was 32.8 mm Hg, bicarbonate concentration was 22.9 mmol/L and oxygen saturation was 89%.

The patient has been using clozapine 350 mg/day for 10 years, aripiprazole 20 mg/day for 1.5 years, amisulpride 800 mg/day for 6 years and quetiapine 300 mg/day for 5 months. Although the patient had a history of treatment with various antipsychotic drugs, the important point was that he started to use quetiapine about 5 months before his admission to our clinic.

The patient's echocardiogram was normal. Infiltrated areas were seen on chest X-ray (Figure 1). In thorax computed tomography and high resolution computed tomography (HRCT), centrilobular nodular appearances of peribroncovascular diffuse ground glass density involving both lower lobes were observed (Figure 2). Significant progressive changes in the lung were observed when the lung radiographies were compared with the previous ones. Pulmonary function test showed restrictive pattern and decreased diffusion capacity (FEV1/FVC: 80% and DLCO: 60%).

His urine and blood culture results were sterile. First, the patient was diagnosed with interstitial pneumonia and oral moxifloxacin 400 mg/day treatment was initiated. Bronchoalveolar lavage and bronchoscopy were performed for diagnostic purposes. Tracheal aspirate culture was examined. Bronchoscopy revealed normal vocal cords and trachea. In both bronchial systems, all segments and subsegments were open and purulent secretions were present. There was no endobronchial lesion. No pathogen was seen in bronchoalveolar lavage culture. 20% alveolar-macrophage, 10% lymphocyte and 70% neutrophils were observed in bronchoalveolar lavage, but no eosinophils were detected. Tracheal aspirate culture showed polymorphonuclear lymphocytes (PNL) and labile cocci. Viral and mycobacterial cultures prepared from aspirates were negative. The patient and his relatives refused thoracoscopic and trans-bronchial lung biopsy.

In clinical and laboratory findings, the patient did not respond adequately to oral medical therapy. Therefore, intravenous piperacillin/tazobactam combination (4,5 gr, once daily) was used instead of the current oral treatment. Although there was no histopathological evidence, we suspected the patient to be DI-ILD, because the patient did not respond to antibiotic therapy, and the existing HRCT findings overlapped DI-ILD. We did not detect any environmental exposure, infection, or rheumatologic process in the patient. He had been using other antipsychotics for a very long time and quetiapine was the last drug exposure. So we thought DI-ILD might be related to quetiapine and, quetiapine was discontinued. Adequate response was obtained in clinical and laboratory parameters within approximately 10 days. The patient's cough decreased and lung auscultation was normal. The pre-discharge control chest X-ray and HRCT showed regression in alveolar infiltration. Based on all these data, the possible agent causing the disease appears to be quetiapine.

DISCUSSION

In our case report, we presented an interstitial lung disease case due to the quetiapine administration. Based on clinical symptoms, laboratory, and radiological findings, we suspected that the patient had pneumonia. Therefore, in the first step, we applied empirical antibiotic therapy. Since there was no improvement in the patient's clinical symptoms, DI-ILD was diagnosed after reassessment of the case.

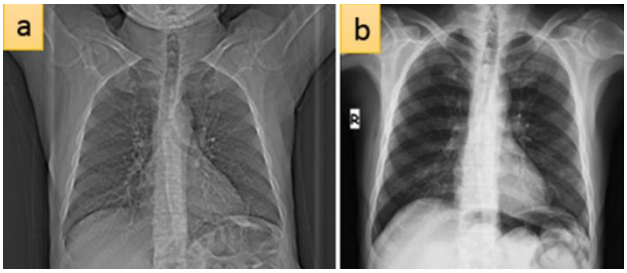

Figure 1. a) Chest X-ray of patient when using quetiapine. **b)** Infiltrations regress almost completely on chest X-ray one month after cessation of quetiapine treatment.

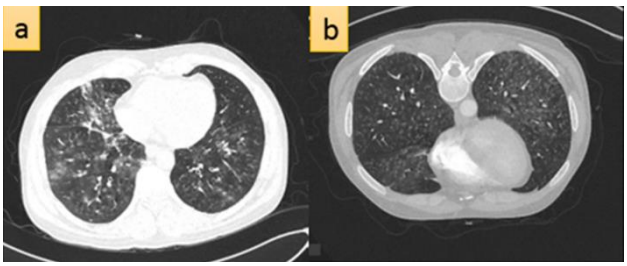

Figure 2. a) High resolution computed tomography (HRCT) of the patient with bilateral micronodular and diffuse ground glass opacities when using quetiapine. **b)** One month after cessation of quetiapine treatment, the infiltrations in HRCT almost completely regressed

In case reports, it has been reported that DI-ILD develops with psychiatric drugs such as paroxetine, sertraline, clozapine and risperidone (4). Rarely, DI-ILD has been reported with some antipsychotics, such as clozapine (3). Quetiapine which is an atypical antipsychotic drug has many known side effects. However, DI-ILD which is one of the respiratory system side effects of quetiapine is rare (5). In a new study, in critically injured trauma patients, quetiapine exposure was associated with increased pulmonary complications (PC) risk. Patients administered quetiapine were more likely to develop PC and acquire PC earlier than those without quetiapine. Quetiapine has reported a positive risk factor for PC (6). Diagnosis of DI-ILD usually depends on the association between a history of medication and the onset and progression of respiratory complaints. The most important factor in making the accurate diagnosis is the exclusion of other causes of lung damage such as infections. Laboratory findings and non-specific clinical symptoms of DI-ILD such as fever, cough, dyspnea and hypoxemia make the diagnosis difficult. Studies have suggested that cytotoxic lung injury and immune-mediated damage are mechanisms of DI-ILD (7). Some drugs may disrupt the blood-alveolar barrier by making a direct toxic effect on endothelial cells in the lungs. Thus, proteins that pass through the alveoli form hyaline membranes and exudate type fluid causes hypoxia. If exposure to the drug continues during this period, nonspecific interstitial pneumonia or lymphocytic interstitial pneumonia patterns may occur. Immunity-mediated damage is usually T-cell mediated (8). In addition to these mechanisms, Wijnen et al. (9) found that various cytochrome-P450 (CYP) genotypes posed a risk of susceptibility to the development of DI-ILD in individuals. CYP2D6, a high-affinity-low-capacity enzyme, prefers to metabolize drugs at lower concentrations. CYP3A4 activity decreases with hepatic dysfunction and CYP2D6 plays an important role in metabolizing quetiapine, especially at a lower concentration. Since the lungs contain CYP2D6 enzyme systems, quetiapine is thought to contribute to CYP-mediated damage.

In our case, we do not know the histopathological diagnosis. The patient had no environmental exposure, occupational disease, or rheumatologic process that explain the symptoms and signs. Eosinophilic lung diseases such as eosinophilic pneumonia, Churg Strauss Syndrome and allergic bronchopulmonary aspergillosis were excluded due to a lack of normal peripheric eosinophil counts and radiological pattern. Our patient did not respond to various antibiotic treatments. Quetiapine was the last drug exposure to the patient. In addition, after discontinuation of quetiapine, chest radiography findings and the patient's symptoms improved rapidly. With all these findings, we diagnosed the interstitial lung disease that developed as a result of the patient’s quetiapine use. This case highlights that quetiapine may be one of the possible causes of DI-ILD. We recommend that clinicians should be careful about the rare side effects of quetiapine, such as interstitial lung disease, other than the common side effects.

Informed Consent

Written informed consent was obtained from the patient for publication.

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Rectum Perforation Secondary to the Enema with Garden Hose: A Case Report

Bahçe Hortumu ile Lavmana Sekonder Rektum Perforasyonu: Olgu Sunumu

Mehmet Emin GÖNÜLLÜ
0000-0001-9126-2396
İsmet ÖZAYDIN
0000-0001-9661-328X

Düzce University Faculty of Medicine
Department of General Surgery,
Düzce, Turkey

ABSTRACT

A 73-year-old male patient was admitted to the emergency department with severe abdominal pain. It was thought that the patient might have colon perforation with the patient's history, physical and radiological examination. In the patient's history; since his youth, it was understood that when he has been constipated, he has made an enema with tap water by attaching the garden hose into the rectum and connects the other end to the fountain. In this case report, we wanted to present the colon perforation during the hose penetration, and peritonitis of the patient who had foreign body penetration chronically into the rectum with the purpose of making enema periodically, and the operation performed after. Since our patient was a delayed case, we quickly initiated resuscitation, prophylactic antibiotherapy, and underwent protective colostomy in our operation. We think that colostomy reduces the mortality and morbidity in delayed cases.
Keywords: Rectum perforation; enema with garden hose.

ÖZ

Yetmiş üç yaşındaki bir erkek hasta acil servise şiddetli karın ağrısı şikayetiyle başvurdu. Hastanın anamnezi, fizik muayene ve radyolojik tetkikleri sonucunda kolon perforasyonu olduğu düşünüldü. Hastanın anamnezinde; gençliğinden beri, kabız kaldığında rektuma bahçe hortumu sokup hortumun diğer ucunu da çeşmeye bağlayarak çeşme suyu ile lavman yaptığı anlaşıldı. Bu vaka sunumunda, kronik olarak rektuma dönem dönem lavman yapmak amacı ile yabancı cisim penetrasyonu olan hastanın, hortumun penetrasyonu esnasında kolon perforasyonu, peritonit ve sonrasında yaptığımız operasyonu sunmak istedik. Hastamız gecikmiş bir vaka olduğu için hastaya hızlı bir şekilde resüsitasyon, proflaktik antibiyoterapi başladık ve hastamıza operasyonda koruyucu kolostomi açtık. Kolostominin gecikmiş vakalarda mortalite ve morbiditeyi azalttığını düşünmekteyiz.
Anahtar kelimeler: Rektum perforasyonu; bahçe hortumu ile lavman.

Corresponding Author
Sorumlu Yazar
Mehmet Emin GÖNÜLLÜ
dr.m.emin.gonullu@gmail.com

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INTRODUCTION

Injury of the rectum with a foreign body is frequently reported in the literature (1). Injury of the anal canal, rectum and sigmoid colon with foreign bodies has been increasing in recent years, and complications associated with some of the major emergency surgical problems (2). It is reported that patients are used foreign bodies in the rectum; for diagnostic or therapeutic purposes, and most often for sexual purposes in constipation or anorectal diseases (3). In this case report, we present a 73-year-old male patient who applied to the emergency department and had emergency intervention with the perforated rectum as a result of the pressurized water applied to the rectum for enema.

CASE REPORT

A 73-year-old male patient applied to our emergency department with complaints of severe abdominal pain and fever. In the anamnesis of the patient; it was learned that approximately 10 hours before applying to the emergency department, he placed the garden hose for the enema he made periodically in the anus and opened the fountain and provided pressurized water. When the patient experienced constipation in the military, someone told him that he made an enema with tap water with a hose. His medical history revealed that he had chronic arterial disease and hypothyroidism and that he was receiving treatment for it. Also he had appendicitis surgery, inguinal hernia surgery, and prosthetic surgery in the knees. His physical examination was conscious-open and cooperative-orientated. Vital findings were tension arterial: 140/85 mmHg, fever: 37.8°C, pulse: 108/min-rhythmic, respiratory rate: 17/min-regular, oxygen saturation: 93%. In the examination of the abdomen; especially prevalent mainly in the lower quadrant taken tenderness, defense and rebounds. In the rectal examination; there was blood smear on the stool, the mucosa was hyperemic, the tonus was weakened. On the chest x-ray taken in the emergency department, free air was seen under the diaphragm (Figure 1).

In our patient's laboratory examination, a slight neutrophil increase ($7.8x10^3$/uL) and CRP increase (5.5 mg/dL) were observed. Apart from these, we look at the biochemical and hematological parameters and that were normal. In computed tomography (CT), perforation area and fluid density were seen adjacent to the bladder of the rectum (Figure 2). After that, the informed consent form was signed by the patient and was operated and diagnostic laparotomy was performed under general anesthesia. In the rectum, about 6 cm proximal to the anal canal, a perforated area of about 6 cm and widespread fluid (tap water) were seen in the abdomen. Rectum was repaired with primary suture. A loop colostomy was created from the sigmoid colon. On the second day after the operation, the regime was started for the patient who had gas and fecal discharge. The patient was discharged on the 8th day of his hospitalization, since there were no problems in his clinical follow-up.

DISCUSSION

Regardless of the etiology, foreign bodies reaching the rectum orally or anally may lead to varying degrees of ano-rectal injury depending on the anatomical and physiological features of this region and the physical properties of the foreign body. Especially, the attempt to manually push and remove the long foreign bodies into the rectum unconsciously causes the ano-rectal region to be more traumatized (4). Among the common causes of transanal injury are; medical procedures for diagnostic or therapeutic purposes, transanal enemas, foreign bodies applied for autoeroticism, sexual assaults or falling over a sharp object (5). While foreign bodies inserted from the rectum at a young age are used for sexual purposes, they are used for fecal stoning and prostate massage in later ages (2). Most cases with rectal foreign body are men including homosexuals, elders, and patients with mental disability or victims of sexual assault in particular (7). Additionally, in drug trafficking, illicit drugs (cocaine, amphetamine and marijuana) are packed in small plastic

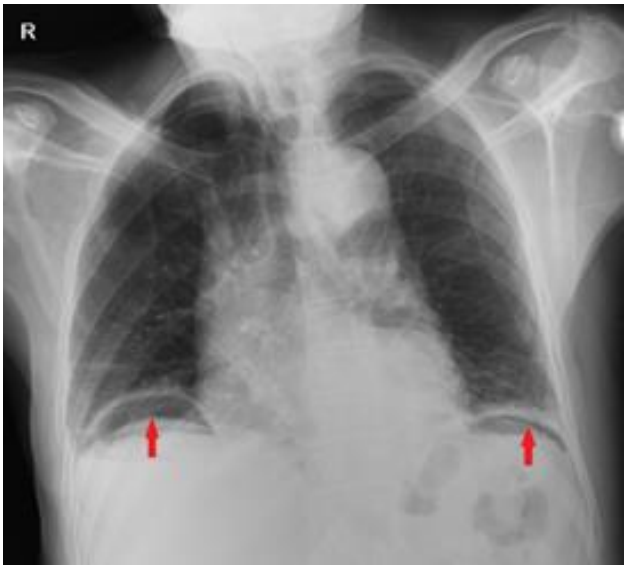
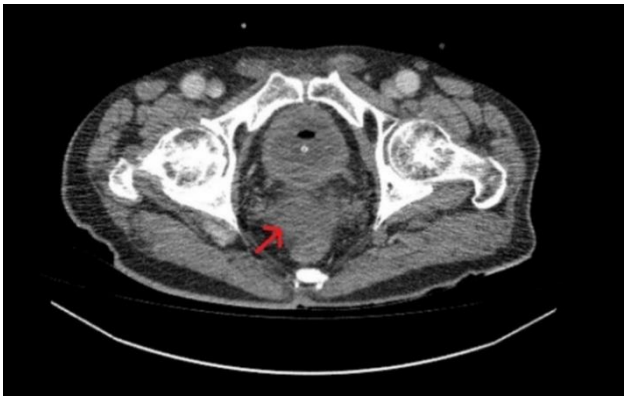


Figure 1. Free air under the diaphragm in chest X-ray



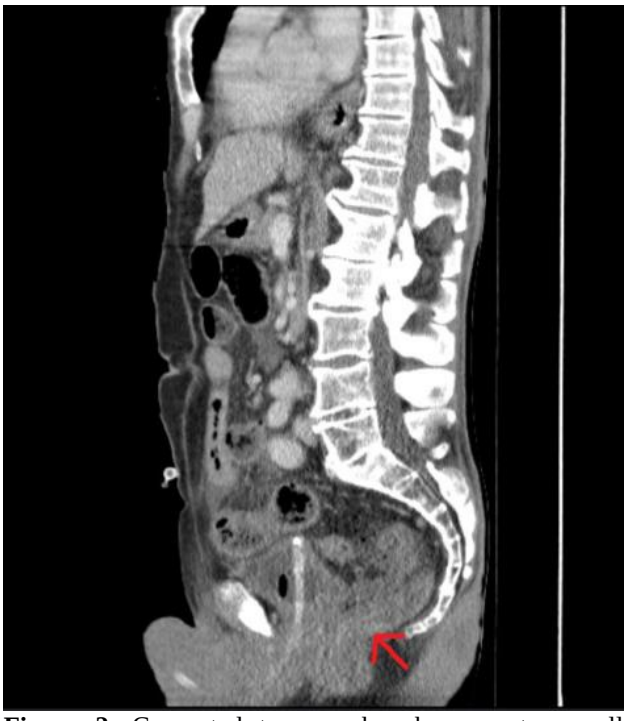


Figure 2. Computed tomography shows rectum wall irregularity and fluid density out of rectum (vertical and sagittal images)

bags and placed in the rectum, which can be encountered. Uncommonly, objects could be lodged in the colon from perioral ingestion. Various retained objects recorded in the literature include sex toys, tools and instruments, bottles, cans, jars, poles, pipes and tubing, fruits and vegetables, stones, balls, balloons, umbrellas, light bulbs, and flashlights (8). In our patient, the etiology of the anal foreign body is the injury of the garden hose to the rectum for the purpose of enema for self-treatment.

Most of the time, a simple rectal examination is sufficient for diagnosis if suspected injury to the anal canal rectum converge. The rectal examination of blood taint or injury can be diagnosed by palpation. While intraabdominal free air seen in intraperitoneal injuries away from the anal canal makes diagnosis easier, free air may not be detected in the extraperitoneal region of the rectum. However, it should be kept in mind that in extraperitoneal rectum injuries, air can pass through the peritoneal cavity by diffusion and free air can be detected on the radiographs (5). Other organ injuries should be investigated preoperatively because of their close neighborhood in rectal foreign body injuries. CT is an effective method to demonstrate intra-abdominal extra organ injuries. With the CT of the patient, we saw wall irregularity in the rectum and fluid density out of the rectum and free air under the diaphragm.

The first step in the treatment of ano-rectal injuries is to provide hemodynamics and start broad-spectrum antibiotics. In our patient, we quickly start ceftriaxone 2x1 gr and metronidazole 3x500 mg after the patient's hemodynamics was corrected quickly. Then, we performed a diagnostic laparotomy immediately.

Colostomy is recommended for transanal or perianal injuries. Especially after eight hours, colostomy is of high importance for intraperitoneal rectum injuries (6). We created a colostomy as our patient was a delayed case and had extensive peritonitis.

CONCLUSION

Early and accurate diagnosis in anorectal injuries is required to reduce morbidity and mortality. For this purpose, we think that rare cases should be known and in the presence of peritoneal contamination, colostomy should not be avoided in delayed cases. It is certain that rapid diagnostic laparotomy will decrease morbidity and mortality immediately after hemodynamics has improved and broad spectrum antibiotherapy has started before surgery. In addition, we think that CT imaging has an effect on the planning of the surgery in order to separate between any other organ injuries before the surgery and to show the extent of the fluid in the abdomen.

Written informed consent form was obtained from the patient.

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Airway Obstruction and Patchy Parenchymal Infiltrations Related to Tear Gas Exposure

Göz Yaşartıcı Gaz Maruziyetine Bağlı Hava Yolu Obstrüksiyonu ve Bilateral Yamalı Parankimal İnfiltrasyonlar

Nilüfer AYKAÇ
0000-0003-1407-8258

Gayrettepe Florence Nightingale Hospital Chest Diseases Clinic, İstanbul, Turkey

ABSTRACT

A 50 year old nonsmoker female patient with respiratory complaints including phlegm and effort-related dyspnea admitted to the hospital. Anamnesis pointed out neither occupational nor hobby-related exposures. However, she mentioned several intense tear gas exposures, and describe dyspnea and wheezing right after these exposures. Computed tomography of the lung showed patchy areas of consolidation and ground glass opacity. Pulmonary function tests revealed an FEV1/FVC ratio of 67%. Chlorobenzylidene malononitrile (CS) gas related pulmonary involvement was evident due to the lack of lesions on previous radiographs, improvement of her complaints and respiratory function test, and regression of the parenchymal lesions after ceasing the exposure. This case is presented to emphasize the importance of taking detailed anamnesis of the patient and to demonstrate the negative impacts of physical and social environment on human health, and more specifically to suggest that the riot control gases used are not as harmless as believed.
Keywords: Toxic gas inhalation; inhalation injury; tear gas.

ÖZ

Elli yaşında hiç sigara içmemiş kadın hasta, balgam ve eforla artan nefes darlığı gibi solunum yakınmaları ile hastaneye başvurdu. Anamnezde bu semptomlara yol açabilecek ne mesleki ne de hobileri ile ilişkili bir özellik yoktu. Bununla birlikte, birkaç kez yoğun göz yaşartıcı gaza maruz kaldığından bahsetti ve bu maruziyetlerden hemen sonra nefes darlığı ve hırıltı tarif etti. Bilgisayarlı tomografisinde akciğerde iki taraflı periferik dağılımlı yamalı infiltrasyonları ve buzlu cam opasiteleri mevcuttu. Solunum fonksiyon testinde FEV1/FVC oranı %67 idi. Daha önceki radyografilerde lezyon olmaması, maruziyet kesildikten sonra yakınmalarında ve solunum fonksiyon testinde düzelme ve parankim lezyonlarında gerileme olması chlorobenzylidene malononitrile (CS) gazı maruziyetine bağlı pulmoner tutulumun kanıtlarıydı. Bu olgu özellikle hastadan ayrıntılı anamnez almanın öneminin altını çizmek, çevresel ve sosyal ortamların insan sağlığı üzerine olumsuz etkisini göstermek ve kullanılan gazların düşünüldüğü kadar masum olmadığını vurgulamak için sunulmuştur.
Anahtar kelimeler: Toksik gaz inhalasyonu; inhalasyon hasarı; göz yaşartıcı gaz.

Corresponding Author
Sorumlu Yazar
Nilüfer AYKAÇ
niluferkongar@gmail.com

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INTRODUCTION

Aerosols used as riot control agents mainly include chlorobenzylidene malononitrile (CS), chorobenzoxazepine (CN) and oleorensincapsicum. These agents are taken via inhalation, skin contact and digestive system. They may cause rhinorrhea, sneeze, cough, dyspnea, bronchoconstriction and lung edema (1). Due to the lack of post-exposure studies, adverse health effects of tear gas are not well documented. This report is about a case with airway obstruction and bilateral patchy parenchymal infiltrations due to tear gas exposure.

CASE REPORT

A 50 year old nonsmoker female with respiratory complaints including phlegm and effort related dyspnea with no prior history of similar problems (including asthma) admitted to the hospital. Anamnesis pointed out neither occupational nor hobby-related exposures. However she mentioned several intense tear gas exposures 14, 13 and 11 days ago. Right after these exposures, the patient described dyspnea and wheezing. She also complained about effort-related dyspnea and dry cough, sometimes in periods of about 10 days.

The patient's medical history includes left partial mastectomy caused by invasive ductal carcinoma, lymph node dissection, radiotherapy and chemotherapy two years ago. The patient was stable and taking no medical care for breast cancer. She also had a history of escitalopram use for two years.

Examination results were as follows: vital respiratory rate was 18/min, SaO_2 was 97% in room air, expiratory wheezing and widespread rhonchi were present. Routine laboratory tests, including white blood cell count and differential red blood cell count, liver and renal functions, and serum C-reactive protein levels were normal.

Computed tomography (CT) of the lung showed patchy areas of consolidation and ground glass opacity with a predominantly peripheral distribution on upper lobe of right lung and lower lobe on left lung (Figure 1).

Pulmonary function tests revealed FEV1 as 70% and FEV1/FVC ratio of 67%. The patient, with preliminary diagnosis of toxic gas inhalation injury was given maintenance therapy with an inhaled corticosteroid twice daily and, when needed, an inhaled short-acting beta2 sympathomimetic agent and macrolide antibiotic. She was also informed about the potential adverse effects of tear gas exposure. One month later, tear gas exposures was ceased and the patient was asymptomatic, the CT scan and pulmonary function tests were almost normal (FEV1 %87, FEV1/FVC ratio of %85).

Informed consent was obtained from patient who participated in this case.

DISCUSSION

Numerous public protests took place among various countries during 2013. Local police forces utilized riot control agents, mainly tear gas, excessively and systematically. Due to the nature of events, the probability of being exposed to high levels of tear gas, more than once, was increased among participants. Aforementioned case had a history of multiple tear gas exposures.

Respiratory problems caused by the exposure to CS include burning sensation in the throat, cough, wheezing, dyspnea and laryngospasm. CS exposure basically leads to irritable effect on mucous membranes. Common cause of death is due to asphyxia and circulatory failure (1). Animal experiments proved that high levels of CS exposure may cause chemical pneumonitis and fatal pulmonary edema (2). Severe pneumonitis that required the use of oxygen, steroids and antibiotics, was observed on an infant after excessive exposure to CS (3). Irritant induced asthma was reported when a gas canister exploded 2-3 meters away from a healthy female (4). In case of environmental ventilation is limited, exposure to high levels of CS caused death in 12-48 hours among rabbits and guinea pigs. Cause of death in animal experiments is lung damage (5).

Moreover, in postmortem examination of 10 cases that resulted in death after the use of riot control agents support the experimental research findings; Toprak et al. (6) detected edema accompanied by intra-alveolar hemorrhage in the posterior-basal areas of the lung, diffuse superficial acute necrosis in the mucosa, acute inflammatory cell infiltration, epithelial desquamation, bronchopneumonia, hyaline membranes and "asthmatic changes" in alveoli. In the long-term follow-up of 93 cases exposed to similar agents, Arbak et al. (7) showed that the rate of % predicted maximal mid expiratory flow rate (MMFR) in people exposed to gas with no smoking history is significantly less in comparison to the mean FEV1/FVC and % predicted MMFR in people exposed to gas that are tobacco users (7). Finally, in the examination of 86 patients exposed to gas where Ilgaz et al. (8) reported that the respiratory function test findings of people exposed to gas were more adversely affected if they were exposed to gas or used tobacco in the indoor environment.

In the Turkish and English literature, there is no patient with lung parenchymal pathology similar to our case after exposure to tear gases. In our opinion, the most important reason for this is that the research on patients exposed to similar agents are based on questionnaire inquiry and/or respiratory function test. Therefore, our case is the first stimulating example to detect lung parenchymal pathologies caused by tear gases.

With no contrary evidence, the airway spasm findings and infiltrative images seen in lung parenchyma were associated with the exposure to excessive tear gas (CS).

To sum it up, experimental studies which demonstrated death and lung damage, and real life findings about the exposure to tear gases suggests that they may not be as

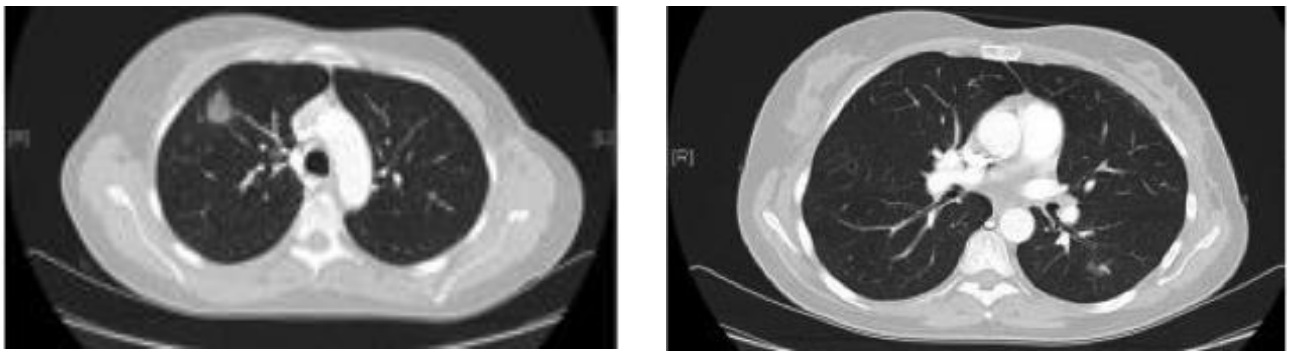

Figure 1. Computed thorax tomography (patchy areas of consolidation and ground glass opacity)

innocent as it is generally believed. Mid and long term health effects of exposure to these should be (re)evaluated more seriously.

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AUTHOR GUIDELINES

SCIENTIFIC RESPONSIBILITY

In terms of scientific publishing standards, articles to be submitted should be prepared in accordance with the criteria of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME) and the Committee of Publication Ethics (COPE).

- All articles must be complied with the research and publication ethics. The responsibility of the articles belongs to the authors.
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- Articles must be submitted with the Copyright Transfer Form signed by all authors to begin the evaluation process. For authors' order, the signature order in the Copyright Transfer Form is based on.
- The corresponding author is responsible for the final version of the article on behalf of all authors.

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- Compliance with The Principles of Helsinki Declaration (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) is required in all studies including "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken the approval from ethics committee of their institution and the "informed consent" from people participating the study, in the MATERIAL AND METHODS section.
- If "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and they have taken the approval from ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information of the ethics committee approval should be indicated together with the name of the committee, approval date and number, in the MATERIAL AND METHODS section.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
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Articles must be uploaded to the system as separate files as described below.

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SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

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- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
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ACKNOWLEDGEMENT

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Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

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Book Chapter:

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YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

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